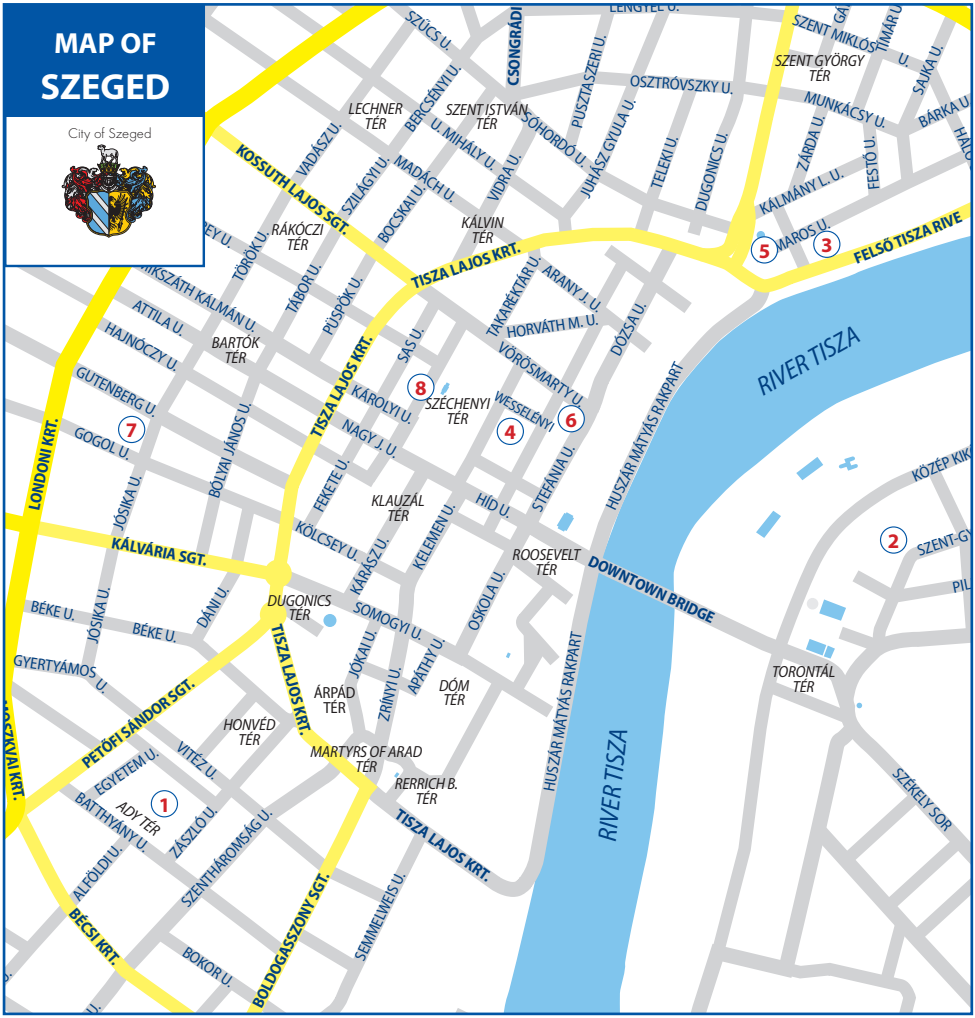
75TH ANNIVERSARY OF ALBERT SZENT-GYÖRGYI'S NOBEL PRIZE AWARD

FINAL PROGRAMME

Szeged 22-25 March, 2012

NEW SZÉCHENYI PLAN



1. **TIK** *(6722 Szeged, Ady tér 10.)*
2. **HOTEL FORRÁS** *(6726 Szeged, Szent-Györgyi Albert u. 16-24.)*
3. **HOTEL NOVOTEL** *(6721 Szeged, Maros u. 1.)*
4. **HOTEL TISZA** *(6720 Szeged, Széchenyi tér 3.)*
5. **IH EVENT CENTRE** *(6721 Szeged, Felso Tisza-part 2.)*
6. **NATIONAL THEATRE** *(6720 Szeged, Vaszy Viktor tér 1.)*
7. **NEW SYNAGOGUE** *(6722 Szeged, Gutenberg u. 20.)*
8. **CITY HALL** *(6720 Szeged, Széchenyi tér 10-11.)*

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WELCOME

Dear Colleagues,

We would like to welcome you to the Conference series commemorating the 75th Anniversary of Albert-Szent Györgyi's Nobel Prize award. We are all proud of Albert Szent-Györgyi, a former professor and dean of the Faculty of Medicine and the former rector of the University of Szeged. He is an idol both for lecturers and students, presenting the idea that world famous results can be achieved in Hungary and Szeged.

We are delighted to have around 1000 participants from 30 countries and are looking forward to a stimulating and interesting conference. We will have nine state-of-the-art lectures delivered by Nobel Laureates, an a further 182 state-of-the-art lectures delivered by other top experts from all over the world. The oral and poster sessions are all designed to facilitate interaction with other basic and clinical scientists interested in cardiology, gastroenterology, immunology & inflammation, molecular biology & genetics, neuroscience and tuberculosis evolution. Of the 231 submitted abstracts, 83 will be delivered as oral presentations. Hopefully, you will also enjoy the Social Events and an introduction to Hungarian Gastronomy during the Conference. The host of the Conference is the University of Szeged.

The **history of the University of Szeged** dates back to 1581 when István Báthory, the Prince of Transylvania, founded a higher educational institution in the city of Kolozsvár (Cluj-Napoca), which became highly prestigious within a very short period of time. Due to its professors well-known all around Europe it provided a high standard of education and also had the right to confer bachelor and master's degrees. Moreover, it was the only institute for higher education at the end of the 16th century in Hungary. Later Mary Theresa entrusted the Piarists to reorganize the institution, as a result of which the Faculty of Medicine and Surgery was established in 1775. Later on, these served as the basis for the Hungarian Royal University of Kolozsvár, founded by Francis Joseph I in 1872. It was renamed after the king in 1881 and bore his name until 1940. The institution moved to Szeged in 1921.

Szeged is a beautiful and spectacular city in Hungary, situated on the banks of River Tisza with a population of almost 170,000. It lies along Hungary's south-eastern border, just to the south of the estuary of River Maros. Szeged is the capital city of Csongrád county and serves as a commercial and cultural centre of the region. The climate is very pleasant and the area is well known for its beautiful spring. Szeged gets an average of 2,000 hours of sunshine each year (that's 83.5 days of daylight), no wonder it's often called the City of

Sunshine. Local industry is reputed for food processing and production. Szeged is especially famous for its two most prominent agricultural products: Pick salami and one of the most distinctive Hungarian spices: red pepper. Red pepper gets its red colour and divine flavour from an extended exposure to sunlight, which makes Szeged an ideal place to grow this spice. Szeged is also famous for Székelygulyás, a goulash made of pork, pickled cabbage and sour cream, and Halaszlé, fish soup made of carp and catfish. Textile and clothes industry, as well as oil and natural gas processing are also of importance to the region. Theatres, cinemas, clubs, riverside swimming pools and sports grounds provide plenty of opportunities for recreation. There are several large parks and a famous botanical garden to stroll through. Fine restaurants are situated all over Szeged offering various domestic and international cuisines (including Greek, Chinese, Italian, German, Indian, Transylvanian and Arabic). Public transportation provides a wide variety of vehicles with tram, trolleybus and bus services being so extensive that you can virtually reach every corner of the city.

It goes without saying that the organizers intend to provide a friendly atmosphere by preparing a variety of cultural and recreational programmes to accompany the conference series itself.

***We hope you will enjoy the* conference series on the occasion of the 75th Anniversary of Albert Szent-Györgyi's Nobel Prize Award.**

On behalf of the organizing committee, With kind regards,

Gábor Szabó
Rector

László Vécsei
Dean of the Faculty of Medicine

University of Szeged

STRUCTURE OF THE CONFERENCE(S)

(I) Main Anniversary part – Nobel Conference

There will be **nine** state-of-the-art lectures delivered by **Nobel Laureates**.
We are very grateful to the **Nobel Laureates** for accepting our invitation to this anniversary meeting:

Andrew V. SCHALLY
Nobel Prize in Physiology or Medicine
1977

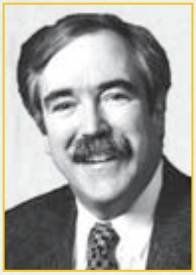
Prize motivation:
"for their discoveries concerning the peptide hormone production of the brain"

Bert SAKMANN
Nobel Prize in Physiology or Medicine
1991

Prize motivation:
"for their discoveries concerning the function of single ion channels in cells"

Robert HUBER
Nobel Prize in Chemistry 1988

Prize motivation:
"for the determination of the three-dimensional structure of a photosynthetic reaction centre"



Eric WIESCHAUS
Nobel Prize in Physiology or Medicine
1995

Prize motivation:
"for their discoveries concerning the genetic control of early embryonic development"

Peter C. DOHERTY
Nobel Prize in Physiology or Medicine
1996

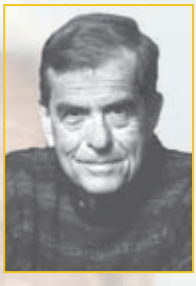
Prize motivation:
"for their discoveries concerning the specificity of the cell mediated immune defence"

John E. WALKER
Nobel Prize in Chemistry
1997

Prize motivation:
"for their elucidation of the enzymatic mechanism underlying the synthesis of adenosine triphosphate (ATP)"

Tim HUNT
Nobel Prize in Physiology or Medicine 2001

Prize motivation:
"for their discoveries of key regulators of the cell cycle"



Aaron CIECHANOVER
Nobel Prize in Chemistry 2004

Prize motivation:
"for the discovery of ubiquitin-mediated protein degradation"

Ada E. YONATH
Nobel Prize in Chemistry 2009

Prize motivation:
"for studies of the structure and function of the ribosome"

In this part of the meeting you will also hear
– presentations about **Albert Szent-Györgyi's life** and research activity
– introduction of the **University of Szeged and the Biological Research Center**
– information about the one-hundred-year-old tradition of **pharmaceutical manufacturing** in Hungary: the importance of medical chemistry

(II) Conference series

There will be six parallel conferences in Cardiology, Gastroenterology, Immunology & Inflammation, Molecular Biology & Genetics, Neuroscience, and Tuberculosis Evolution.

The programme of all conferences will feature **state-of-the-art lectures** by invited faculty members and **free oral and poster presentations** from submitted abstracts.

FOCUS ON: "Cardioprotection and sudden cardiac death"

Ischemic heart disease and sudden cardiac death are major factors contributing to mortality worldwide causing the premature death of millions of people every year in the western societies. The most common cause of sudden cardiac death is ischaemia-related cardiac arrhythmias as well as malfunction of cardiac ion channels due to genetic origin, electrical remodelling in cardiac hypertrophy or failure, as well as side effects of drug therapy. Therefore, protecting the heart against ischemia and reperfusion injury and its consequences including myocardial infarction and sudden death is a topic of intensive investigation. The present conference provides an excellent opportunity to bring together leading and young scientists to discuss recent advances in the field of cardioprotection and sudden cardiac death.

Faculty:

Name	City	Country
ANTZELEVITCH, Charles	Utica, NY	USA
BAXTER, Gary F.	Cardiff	UK
CERBAI, Elisabetta	Florence	Italy
EISNER, David	Manchester	UK
ESCHENHAGEN, Thomas	Hamburg	Germany
ÉDES, István	Debrecen	Hungary
FERDINANDY, Péter	Budapest	Hungary
FORSTER,Tamás	Szeged	Hungary
HAUSENLOY, Derek	London	UK
HEUSCH, Gerd	Essen	Germany
LATHROP, David	Bethesda, MD	USA
LEFER, David J.	Atlanta, GA	USA
MERKELY, Béla	Budapest	Hungary
MUNTEAN, Danina	Timisoara (Temesvár)	Romania
MURPHY, Elisabeth	Bethesda, MD	USA
NÁNÁSI, Péter	Debrecen	Hungary
PAPP, Zoltán	Debrecen	Hungary
PERNOW, John	Stockholm	Sweden
PIESKE, Burkert	Graz	Austria
PRZYKLENK, Karin	Detroit, MI	USA
RAVENS, Ursula	Dresden	Germany
RAVINGEROVA, Tatiana	Bratislava (Pozsony)	Slovakia
RŐTH, Erzsébet	Pécs	Hungary
SCHULZ, Rainer	Giessen	Germany
SCHULZ, Richard	Edmonton	Canada
SIPIDO, Karin	Leuven	Belgium
TÓSAKI, Árpád	Debrecen	Hungary
TÓTH, Kálmán	Pécs	Hungary
VARRÓ, András	Szeged	Hungary
VÉGH, Ágnes	Szeged	Hungary
VOLDERS, Paul	Maastricht	The Netherlands
VOS, Marc A.	Utrecht	The Netherlands
WILDE, Arthur	Amsterdam	The Netherlands

GASTROENTEROLOGY

FOCUS ON: "Epithelial ion transport in the gastrointestinal tract"

Epithelial ion transports play a fundamental role in the maintenance and integrity of the gastrointestinal tract. They provide proper environment for digestive processes, have a protective function, and are also involved in the homeostasis of body fluids. Importantly, derangement of epithelial secretory processes leads to a number of diseases, including hyperacidity, cystic fibrosis and secretory diarrhoeas. In addition, an increasing number of experimental studies has been published concerning the role of ion transports in the pathogenesis of inflammatory diseases, such as infectious diarrhoea or inflammatory bowel disease. Therefore, we believe that it is crucial to accelerate transfer of laboratory findings to clinical application in this field. This international gastrointestinal conference will bring together leading researchers and experts of the field to summarize recent advances in epithelial ion transport in the gastrointestinal tract.

Faculty:

Name	City	Country
ARATÓ, András	Budapest	Hungary
ARGENT, Barry E.	Newcastle	UK
BARRETT, Kim	San Diego, CA	USA
DANIEL, Hannelore	Munich	Germany
DOCKRAY, Graham	Liverpool	UK
DONOWITZ, Mark	Baltimore, MD	USA
GEIBEL, John P.	New Haven, CT	USA
GRAY, Mike A.	Newcastle	UK
HANSSON, Gunnar C.	Gothenburg	Sweden
HEGYI, Péter	Szeged	Hungary
IZBÉKI, Ferenc	Szeged	Hungary
KEELY, Stephen	Dublin	Ireland
LAMPRECHT, Georg	Tübingen	Germany
LÁZÁR, György	Szeged	Hungary
LERCH, Markus M.	Greiswald	Germany
MEDINA, Juan F.	Pamplona	Spain
MUALLEM, Shmuel	Bethesda, MD	USA
PETERSEN, Ole	Cardiff	UK
QUINTON, Paul	San Diego, CA	USA
RAKONCZAY Jr., Zoltán	Szeged	Hungary
SAHIN-TÓTH, Miklós	Boston, MA	USA
SEIDLER, Ursula	Hannover	Germany
TAKÁCS, Tamás	Szeged	Hungary
TULASSAY, Zsolt	Budapest	Hungary
VARGA, Gábor	Budapest	Hungary
VARRÓ, Andrea	Liverpool	UK
VERKMAN, Alan	San Francisco, CA	USA
WELLS, Jerry	Wageningen	The Netherlands
WILLIAMS, John	Ann Arbor, MI	USA
WITTMANN, Tibor	Szeged	Hungary
ZSEMBERY, Ákos	Budapest	Hungary

IMMUNOLOGY & INFLAMMATION

FOCUS ON: "Regulatory mechanisms"

Immunology and inflammation play central roles in all fields of medicine. Since the initiation of smallpox vaccination by Jenner, progress in immunology has been enormous. In nearly all human diseases, immunology and inflammation play fundamental roles. This conference provides a unique opportunity to learn about various aspects of immunology and inflammation from some of the world's leading researchers in these fields. Innate and adaptive immunity, immunoregulation, allergy, mediators of inflammation, antigen presentation, immunogenetics, tumor immunology, regenerative inflammatory processes will all be discussed. Special focus will be on the immunity of the gut and skin. Basic, as well as clinical research will be presented.

Faculty:

Name	City	Country
AKDIS, Cezmi	Davos	Switzerland
AKDIS, Mübeccel	Davos	Switzerland
BATA-CSÖRGŐ, Zsuzsanna	Szeged	Hungary
BENGMARK, Stig	London	UK
BÍRÓ, Tamás	Debrecen	Hungary
BOROS, Mihály	Szeged	Hungary
CHAUDRY, Irshad	Birmingham, AL	USA
ERDEI, Anna	Budapest	Hungary
FALUS, András	Budapest	Hungary
HOMEY, Bernhard	Düsseldorf	Germany
KEMÉNY, Lajos	Szeged	Hungary
KLEIN, Georg	Stockholm	Sweden
MÁNDI, Yvette	Szeged	Hungary
MENGER, Michael D.	Homburg (Saarland)	Germany
OKADA, Hidechika	Nagoya City	Japan
OPPENHEIM, Joost J.	Bethesda, MD	USA
PIVARCSI, Andor	Stockholm	Sweden
PRENS, Errol P.	Rotterdam	The Netherlands
RAJNAVÖLGYI, Éva	Debrecen	Hungary
REDL, Heinz	Vienna	Austria
RUZICKA, Thomas	Munich	Germany
STINGL, Georg	Vienna	Austria
TSCHACHLER, Erwin	Vienna	Austria
SZEGEDI, Andrea	Debrecen	Hungary
VOLLMAR, Brigitte	Rostock	Germany

MOLECULAR BIOLOGY & GENETICS

"Messages from DNA: fingerprints from the past and prospects for the future"

The tremendous progress in molecular biology and genetics during the recent decades made it possible to gain a DNA-based insight into the past of mankind, and has opened up opportunities for the provision of tailor-made personal medicine in the future. The knowledge we acquired through these disciplines has revolutionized our ideas on our past and revealed previously unforeseen possibilities for future exploitation. The „Conference on Molecular Biology and Genetics" will bring together prominent scientists who will present accounts on the most recent advances in the field. Sessions will be devoted to archeogenomics, gene regulation, oncogenomics, translational medicine including topics on stem cells, gene therapy, nanomedicine, monogenic and multifactorial human diseases and their clinical diagnosis, and animal models of human diseases. We are honored and delighted that leading scientists have accepted our invitation for them to share their results and visions with us. We invite all of those who are interested in molecular biology and genetics to take part in this conference, and we look forward to receiving abstracts for free oral and poster presentations.

Faculty:

Name	City	Country
ARIAS, Alfonso Martinez	Cambridge	UK
BANERJEE, Utpal	Los Angeles, CA	USA
BOROS, Imre	Szeged	Hungary
BRÜSTLE, Oliver	Bonn	Germany
CASSIMAN, Jean-Jacques	Leuven	Belgium
CAVAZZANA-CALVO, Marina	Paris	France
CORNEL, Martina C.	Amsterdam	The Netherlands
DAWSON, Kenneth A.	Dublin	Ireland
DINNYÉS, András	Gödöllő	Hungary
DOWNES, Stephen	Coleraine	Northern Ireland
ERDÉLYI, Miklós	Szeged	Hungary
FUXREITER, Mónika	Debrecen	Hungary
GÁSPÁR, Imre	Heidelberg	Germany
JONES, Peter A.	Los Angeles, CA	USA
KALLIONIEMI, Olli	Helsinki	Finland
KATONA, Róbert	Szeged	Hungary
KAUFMAN, Thomas	Bloomington, IN	USA
KOSZTOLÁNYI, György	Pécs	Hungary
MACEK, Milan	Prague	Czech Republic
MELEGH, Béla	Pécs	Hungary
NAGY, László	Debrecen	Hungary

OLÁH, Edit	Budapest	Hungary
OROSZ, László	Budapest	Hungary
PAGANI, Franco	Trieste	Italy
RASKÓ, István	Szeged	Hungary
REUTER, Gunter	Halle	Germany
SARKADI, Balázs	Budapest	Hungary
SCHMIDTKE, Jörg	Hannover	Germany
SCHÜPBACH, Gertrud	Princeton, NJ	USA
SILVA, Alcino J.	Los Angeles, CA	USA
SKUSE, David H.	London	UK
SZABAD, János	Szeged	Hungary
SZALAI, Csaba	Budapest	Hungary
SZÉLL, Márta	Szeged	Hungary
TAKEDA, Shin'ichi	Tokyo	Japan
THANOS, Dimitris	Athens	Greece
TÚRI, Sándor	Szeged	Hungary
UHER, Ferenc	Budapest	Hungary

NEUROSCIENCE

"The world is a construct of sensation, perception, memories."
(Erwin Schrödinger)

A number of neurological and psychiatric disorders lack effective therapies, in which the use of drugs to prevent or reduce disease progression is questionable. Other disorders of the nervous system inflict damage as a result of a single event in time, including stroke, head trauma and spinal cord injury; in these cases, there is a great need for developing effective therapies to restore lost functions. There are diseases, such as multiple sclerosis, in which drugs have been developed to slow disease progression, but there is a great need to improve or restore functions that continue to decline slowly over time. The transplantation of replacement cells into the adult nervous system or targeted delivery of therapeutic genes to areas of ongoing degeneration have received considerable attention over the last years. The next decade is likely to represent a golden era of molecular medicine that will change the landscape of neurological and psychiatric diagnosis and therapy. The European Brain Council (EBC) is a coordinating council formed by European organisations in neurology, neurosurgery, psychiatry, basic neuroscience, as well as patient organisations and industry. EBC has embarked on a major strategy, along with its other activities, to try and make 2014 the European Year of the Brain. It is our great honour that leading neuroscientists have accepted our invitation. We look forward to meeting basic neuroscientists, neurologists, psychiatrists and neurosurgeons in Szeged.

Faculty:

Name	City	Country
ÁDÁM-VIZI, Veronika	Budapest	Hungary
BÁNHEGYI, Gábor	Budapest	Hungary
BARI, Ferenc	Szeged	Hungary
BEAL, Flint	New York, NY	USA
BLAKEMORE, Colin	Oxford	UK
BORNSTEIN, R. Stefan	Dresden	Germany
BUSIJA, David W.	New Orleans, LA	USA
BUZSÁKI, György	Newark, NJ	USA
CONDORELLI, Daniele Filippo	Catania	Italy
CROW, Timothy	Oxford	UK
EDVINSSON, Lars	Lund	Sweden
FREUND, Tamás	Budapest	Hungary
JANCSÓ, Gábor	Szeged	Hungary
JANKA, Zoltán	Szeged	Hungary
MAGYAR, Kálmán	Budapest	Hungary
MIRNICS, Károly	Nashville, TN	USA
MORRIS, Richard	Edinburgh	UK
OLESEN, Jes	Glostrup	Denmark
PALKOVITS, Miklós	Budapest	Hungary
PAULUS, Walter	Göttingen	Germany
PENKE, Botond	Szeged	Hungary
RAKIC, Pasko	New Haven, CT	USA
RIEDERER, Peter	Würzburg	Germany
RIHMER, Zoltán	Budapest	Hungary
SOMOGYI, Péter	Oxford	UK
SZOLCSÁNYI, János	Pécs	Hungary
TAMÁS, Gábor	Szeged	Hungary
VÉCSEI, László	Szeged	Hungary
VIZI, Szilveszter E.	Budapest	Hungary
WOOD, John	London	UK

TUBERCULOSIS EVOLUTION

"ICEPT-2", Past & Present of Tuberculosis: a multidisciplinary overview on the origin and evolution of TB

The recent progresses in the knowledge of the evolutionary biology of tuberculosis necessitate a new synthesis on this topic. Several questions should be addressed, among them: what is the oldest evidence of this infection on human and animal remains? When did specific mutation(s) of the modern strains arise? What was the relative importance of the different pathogenic species of the *Mycobacterium* genus among past populations? Did the pre-contact American TB differ from the Old Word infection? How can we explore the dynamics of the host-pathogen co-evolution in the case of tubercular infection? Can we reconstruct a consensual phylogeny of the *Mycobacterium* genus? What do we know about the evolution of susceptibility/resistance pattern among

human populations? How paleopathology and paleomicrobiology can contribute in the research of the TB evolution? What are the main strategies to brake or to moderate the re-emergence of TB? How can the molecular phylogenetics contribute in these fights? Fifteen years after the first international "ICEPT" meeting on the evolution of tuberculosis (1997, Szeged, Hungary), a new international conference ("ICEPT-2") will be held in Szeged and will tackle these issues in order to elaborate a new multidisciplinary synthesis on the evolutionary pattern of this human infection. It will facilitate a better understanding of its past, present as well as its possible future. In choosing the exact date of the 2012 TB Evolution Meeting, we have decided to join the Albert Szent-Györgyi's Nobel Prize Award Anniversary Conference Series (23rd-25th March 2012) and the 2012 World TB day (24th March 2012) – the 130th anniversary of Robert Koch's discovery.

Faculty:

Name	City	Country
BLONDIAUX, Joël	Walincourt-Selvigny	France
BROSCH, Roland	Paris	France
BUIKSTRA, Jane E. (Co-President)	Tempe, AZ	USA
CHHEM, Rethy K.	Ulm	Germany
COLE, Stewart T. (Co-President)	Lausanne	Switzerland
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DONOGHUE, Helen D.	London	UK
DUTOUR, Olivier	Bordeaux	France
GICQUEL, Brigitte	Paris	France
HERSHKOVITZ, Israel	Tel Aviv	Israel
JAKAB, Zsuzsanna	Copenhagen	Denmark
KÉRI, György	Budapest	Hungary
MINNIKIN, David E.	Birmingham	UK
MOKROUSOV, Igor	St. Petersburg	Russia
NIEMANN, Stefan	Borstel	Germany
PÁLFI, György	Szeged	Hungary
PALKÓ, András	Szeged	Hungary
PAP, Ildikó	Budapest	Hungary
PERRIN, Pascale	Montpellier	France
RASTOGI, Nalin	Abymes/Guadeloupe	France
RAVIGLIONE, Mario	Geneva	Switzerland
ROBERTS, Charlotte A.	Durham	UK
SANTOS, Ana Luisa	Coimbra	Portugal
SCHMIDT-SCHULTZ, Tyede	Göttingen	Germany
SCHULTZ, Michael	Göttingen	Germany
SOLA, Christophe	Paris	France
SOMFAY, Attila	Szeged	Hungary
SPIGELMAN, Mark	London	UK
SUPPLY, Philip	Lille	France
TESCHLER-NICOLA, Maria	Vienna	Austria
VADÁSZ, Imre	Budapest	Hungary
VAN SOOLINGEN, Dick	Bilthoven	The Netherlands
ZINK, Albert	Bolzano	Italy

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UNIVERSITY OF SZEGED

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INFORMATICS

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László Vécsei	Dean of the Faculty of Medicine

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Klára Hernádi	Dean of the Faculty of Science and Informatics
Lajos Kemény	Vice Dean of the Faculty of Medicine
József Pál	Vice Rector of the University of Szeged
András Varró	Vice Rector of the University of Szeged

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Sándor Túri,
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László Vécsei,
Ágnes Végh,
Tibor Wittmann,
Albert Zink

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Members: Tamás Takács – Péter Hegyi – Zoltán Rakonczay Jr.– Ferenc Izbéki

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MOLECULAR BIOLOGY & GENETICS

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Members: János Szabad – Imre Dékány – László Dux – Sándor Túri – Zsolt Boldogkői

NEUROSCIENCE

Chairman: **László Vécsei**

Members: Botond Penke – Zoltán Janka – Ferenc Bari – Gábor Jancsó

TUBERCULOSIS EVOLUTION

Chairmen: **György Pálfi & Olivier Dutour**

Members: András Palkó – Pascale Perrin – Christophe Sola – Attila Somfay – Albert Zink

HOSTS OF THE MEETING

You will be mainly looked after by staff and students (graduate and post-graduate) of the University of Szeged. The conference organization and catering will be mainly taken care of by the teams of László Makó (CEO of Waldorf 2000 Kft.) and Gábor Mészáros (CEO of Congress & Hobby Service Kft.). Please do not hesitate to contact us with any questions/problems you may have. You can easily identify us by the red ties or scarfs.

András Varró

Gábor Szabó

József Pál

Rector's Office, University of Szeged

Lajos Kemény

László Vécsei

Péter Hegyi

Mészáros Gábor

Makó László

Dean's Office, University of Szeged, Faculty of Medicine

CENTRAL VENUE – Main Anniversary part (Nobel Conference)

University of Szeged, Attila József Study and Information Centre (TIK)
6722 Szeged, Ady tér 10.
Tel: +36 62 546 600, +36 62 546 601
http://www.u-szeged.hu/tik/

The history of the Attila József Study and Information Centre, University of Szeged, goes back to 1996, the beginning of the Hungarian Higher Educational Reform Programme. While initial ideas became plans, the experts taking part in the process of establishment took into consideration both the national and international practice, as well as the future needs of higher education. The foundation-stone was laid in 2002 and the Centre was opened in 2004. The Study and Information Centre provides facilities for both studying and recreation for teachers, students and visitors, including the Library of the University, the Computer Room, different servicing units, such as Student Union Offices, Career Office, University Café, University Book Shop, Souvenir Shop and the Congress Centre.

OTHER VENUES

There will be four different venues for the six conferences.

HOTEL FORRÁS

(Cardiology, Gastroenterology, Neuroscience)

6726 Szeged, Szent-Györgyi Albert u. 16-24.

Tel: +36 62 566-466

http://www.hotelforras.hunguesthotels.hu

HOTEL TISZA

(Tuberculosis Evolution)

6720 Szeged, Széchenyi tér 3

Tel: +36 62 478-278

http://www.tiszahotel.hu/

HOTEL NOVOTEL

(Immunology & Inflammation)

6721 Szeged, Maros utca 1

Tel: +36 62 562-200

http://www.novotel.com/gb/hotel-2996-novotel-szeged/index.shtml

IH EVENT CENTRE

(Molecular Biology & Genetics)

6721 Szeged, Felső Tisza-part 2.

Tel: +36 62 423-638

http://www.ihrendezvenykozpont.hu/

OFFICIAL LANGUAGE

The official language of the conference series is English.

REGISTRATION OPENING HOURS AT THE CENTRAL VENUE (TIK)

Thursday, 22nd March	**9:00-18:00**
Friday, 23rd March	**7:30-12:30**
Saturday, 24th March	**7:30-12:30**

REGISTRATION OPENING HOURS AT THE OTHER VENUES

Friday, 23rd March	**13:30-18:00**
Saturday, 24th March	**13:30-18:00**
Sunday, 25th March	**7:30-14:00**

ON-SITE REGISTRATION FEES

Young participants (<30)	**€250**
Participants (>30)	**€350**
Accompanying person	**€250**

The registration fee for participants covers the following:

- admission to all scientific sessions
- a conference bag (including a program leaflet and conference materials)
- coffee and snacks during the breaks, lunches and social events (latter are subject to a small registration fee)
- free transport from and to Budapest Ferenc Liszt International Airport

The registration fee for accompanying persons covers the following:

- social events (latter are subject to a small registration fee)
- free transport from and to Budapest Ferihegy Airport (available only before and after the meeting)

SOCIAL EVENTS	
Welcome Party **Folkdance Evening**	National Theatre Szeged, 22nd March 19:30
Faculty evening at the City Hall ***ONLY by invitation***	City Hall, 23rd March 19:30
Xaver Varnus Organ Concert	New Synagogue, 24th March 20:30
Gala Dinner **Csilla Szentpéteri Piano Concert**	TIK, 25th March 20:00

PUBLIC EVENTS	
Open Forum	TIK, 23rd March, 14:00
Tree of Sciences	TIK, 24th March, 14:00

OPTIONAL SOCIAL PROGRAMMES		
National Historical Memorial Park tour	23rd March	€ 50
Sightseeing tour in Szeged	25th March	€ 40

Name badges

Participants are requested to wear their name badges at all times.

Wireless internet

There will be free wireless internet connection for the participants at TIK and Hotel Forrás.

Internet points

Several computers will be available at your convenience at TIK in the internet room.

Invoice issues

Please note that we will only deal with invoice in TIK during registration hours.

Speaker Preview Centre

We will be providing a Speaker Preview Centre to everyone at TIK. All presenters are kindly asked to check and hand in their presentation at the Speakers Preview Centre at least 2 h prior to the lecture sessions. It would be much appreciated if you could have your presentation uploaded a day before your session. If you do not have your presentation uploaded at TIK, you will have to get this done before the beginning of your session at the other venues.

Poster exhibitions

Posters will be on display from Thursday to Sunday. The authors of the posters are asked to put up their poster on Thursday and leave them on the poster boards until the end of the Conference. At least one of the authors should be present during the poster rounds to answer questions on Friday and Saturday. All posters will be transported to the respective venues on Saturday evening by the organizers. Authors can remove their posters at the end of the day on Sunday.

TRANSPORT

Bus Transfer within Szeged

We will organize bus transfer service to TIK (the central Congress Venue) from the Faculty hotels (Forrás and Novotel) each morning (from Thursday to Saturday). A return service from TIK to the other venues will be available after lunch. The exact time-schedule of the buses will be posted in TIK and the other venues. Bus transfer will be also available for participants wanting to get to and from Social Events (from Thursday to Sunday).

Bus Transfer from/to Budapest airport

You will have to provide your flight details in advance to book transportation. Please let us no if there are any changes in your departure time ASAP. Please confirm your departure time and place (which hotel) for your transfer back to the airport.
PLEASE NOTE: if you do not provide us these information in advance we cannot guarantee your transfer.

Taxi

Radio Taxi is the official personal taxi company of the Conference. You can order a taxi by dialing +36-62-480-480. Conference participants will be entitled to discounted prices from 22nd-25th March 2012. You must show the driver your name badge or Radio Taxi card.

If you have any further requests regarding transport issues, please contact the Conference Registration Desk.

PARKING IN SZEGED

Please be advised that in downtown of Szeged there are three different designated parking zones (green, yellow and blue). To park in these zones (indicated on the traffic signs), you will have to obtain a pre-paid parking ticket. This has to be displayed in a well-visible place, possibly behind the windscreen (from Monday to Friday, 8:00-18:00). The price of a parking ticket is HUF 440 (valid for 1 h in the green zone, for 2 h in the yellow zone and for 4 h in the blue zone). A daily parking ticket is also available for

HUF 1490. PLEASE NOTE that your car may be towed if you do not have a valid ticket. Parking tickets can be bought at grocery stores, tobacconists and newsagents. We will also have some tickets available for you to purchase at the registration desk.
There will also be a limited number of parking places for conference participants in the parking garage of TIK. However, you must have your car registered beforehand at the registration desk.

CME INFORMATION

The European Accreditation Council for Continuing Medical Education (EACCME) accredited this event for a maximum of 21 European CME credits (ECMEC). Each medical specialist should claim only those hours of credit that he/she actually spent in the educational activity. To claim credits, please turn in your attendance and evaluation forms at the end of the meeting to the Organizers. EACCME credits are recognized by the American Medical Association towards the Physician's Recognition Award (PRA). To convert EACCME credit to AMA PRA category 1 credit, contact the AMA.

Smoking

Smoking is prohibited within all venues.

Health and safety

Emergency telephone numbers in Hungary
Ambulance: 104
Fire Department: 105
Police: 107
In case of any non-urgent health problems, we can help you out at the ER of the First Department of Medicine, University of Szeged (I. sz. Belgyógyászati Klinika, Szegedi Tudományegyetem). The address is: 6720 Szeged, Korányi fasor 8-10. Telephone number: +36-62-545-198 or +36-62-545-813.

Insurance/liability

The Organizers of the Conference do not accept any liability for damages and or losses of any kind which may be incurred by conference participants or accompanying persons. Delegates participate at all events at their own risk.

IMPORTANT MOBILE (CELL PHONE) NUMBERS

Registration	Viktória Venglovecz	+36-30-423-7930
Transport (from and to Budapest)	Petra Pallagi	+36-70-560-0458
Accommodation	Rea Fritz	+36-20-406-8026
Other issues	Péter Hegyi	+36-70-375-1031

HOW TO GET TO SZEGED?

BY AIR AND SHUTTLE TRANSFER

Probably the easiest and most convenient way to get to the conference is to fly to Budapest. The organizers offer you free shuttle transportation service between Budapest Ferenc Liszt International Airport and Szeged.

You will have to provide your flight details in advance to book transportation with us. There will be a meeting point at the airport for conference participants.

BY CAR

If you decide to come by car, take the M5 motorway from Budapest. Szeged is about 165 km south-east of the capital. You can reach the city within an hour and a half.

BY TRAIN

There is a regular train service (every hour) between Budapest and Szeged. The journey time is approximately two and a half hours. For details please visit: www.mav-start.hu

CONFERENCE PROGRAMME OVERVIEW

THURSDAY, 22nd March

TIME \ VENUE	TIK
	12:00-13:00 PRESS CONFERENCE
AFTERNOON PROGRAMME 13:30 -17.30	13:30-14:00 OPENING OF THE MEETING
	14:00-15:30 NOBEL SESSION I.
	COFFEE BREAK
	15:50-17:30 NOBEL SESSION II.

The colours in the programme have the following significance:
Main Plenary Programme - Nobel Conference
Cardiology Conference
Gastroenterology Conference
Neuroscience Conference
Immunology & Inflammation Conference
Molecular Biology & Genetics Conference
Tuberculosis Evolution Conference

FRIDAY, 23rd March

TIME \ VENUE	TIK	HOTEL FORRÁS		HOTEL NOVOTEL	IH EVENT CENTRE	HOTEL TISZA
MORNING PROGRAMME 8.30 -12.30	8:30-10:10 NOBEL SESSION III.					
	COFFEE BREAK					
	10:30-11:50 NOBEL SESSION IV.					
	11:50-12:30 POSTER VIEWING I.					
LUNCH BREAK						
AFTERNOON PROGRAMME 13.50 -18.40		13:50-15:45 Novel approaches to cardioprotection	14:05-15:35 Calcium signalling	13:40-15:45 Neuroscience 1.	13:50-15:45 Pathomechanism of inflammation 1.	14:00-15:20 DNA-encoded messages from the past	13:35-15:35 TB Evolution: Opening session
		COFFEE BREAK					
		16:00-17:55 Sudden cardiac death 1.	16:00-18:05 Regulation of GI secretion	16:00-17:55 Neuroscience 2.	16:00-17:55 Pathomechanism of inflammation 2.	15:40-17:50 Translational medicine: gene therapy, stem cells, nano-medicine 1.	16:05-18:15 TB Evolution II: A multidisciplinary approach

SATURDAY, 24th March

TIME \ VENUE	TIK	HOTEL FORRÁS			HOTEL NOVOTEL	IH EVENT CENTRE	HOTEL TISZA
MORNING PROGRAMME 8.30–12.30	8:30–10:10 NOBEL SESSION V.						
	COFFEE BREAK						
	10:30–11:50 NOBEL SESSION VI.						
	11:50–12:30 POSTER VIEWING II.						
LUNCH BREAK							
AFTERNOON PROGRAMME 13:40–18.20		13:35–15:45 Sudden cardiac death 2.	14:00–15:45 Regulation of bicarbonate secretion	13:50–15:45 Neuroscience 3.	14:00–15:45 Bridging innate and adaptive immunity	13:40–16:00 Gene regulation, epigenetics	13:35–15:25 World TB Day 1.
		COFFEE BREAK					
		16:00–17:55 The role of nitric oxide in cardioprotection	16:00–17:55 Mucin and water transport, epithelial interactions	16:00–17:55 Neuroscience 4.	16:00–18:00 Host defense mechanisms	16:20–18:05 Translational medicine: gene therapy, stem cells, nano medicine 2.	15:45–17:55 World TB Day 2.

SUNDAY, 25th March

TIME \ VENUE	HOTEL FORRÁS			HOTEL NOVOTEL	IH EVENT CENTRE		HOTEL TISZA
MORNING PROGRAMME 8.00–12.35	8:00–10:10 Sudden cardiac death 3.	8:00–10:20 Intestinal ion transport	8:30–10:15 Neuroscience 5.	8:30–10:15 Tumor immunology	8:00–9:55 Genomics, multifactorial human diseases		8:30–10:25 Paleopathology of TB and mycobacterial infections
	COFFEE BREAK						
	10:25–12:20 Heart failure	10:35–12:20 Pancreatitis	10:35–12:30 Neuroscience 6.	10:35–12:20 Hypersensitivity	10:15–12:00 Oncogenomics		10:40–12:35 Evolution of mycobacteria
LUNCH BREAK							
AFTERNOON PROGRAMME 13:25–18:00	13:50–15:45 Cardioprotection in the presence of cardiovascular risk factors	13:25–15:45 Clinical Gastroenterology I	13:45–15:45 Neuroscience 7.	13:50–15:45 Skin inflammation 1.	13:40–15:50 Messages from model organisms 1.	13:40–15:50 Rare diseases, clinical diagnostics 1.	13:30–15:40 Biology of mycobacteria & its applications in TB evolution research
	COFFEE BREAK						
	16:00–18:10 Cardioprotective signalling and oxidative stress	16:00–17:50 Clinical Gastroenterology II.	16:00–18:00 Neuroscience 8.	16:00–17:45 Skin inflammation 2.	16:10–18:05 Messages from model organisms 2.	16:10–17:55 Rare diseases, clinical diagnostics 2.	15:55–18:00 Different approaches to the study of 'paleotuberculosis'

SCIENTIFIC PROGRAMME BY DAY

AFTERNOON PROGRAMME — MAIN PLENARY EVENT

(Venue: TIK Main Lecture Hall)

9.00–17:00 **REGISTRATION**

12:00 **PRESS CONFERENCE**

13:30 **OPENING OF THE MEETING**

14:00–15:30 NOBEL SESSION I.

Chairs: Gábor Szabó *(Szeged, Hungary)*, József Pálinkás *(Budapest, Hungary)*

14:00 **DUX, László** *(Szeged, Hungary)*
Biography of Albert Szent-Györgyi awarded the Nobel Prize in Medicine or Physiology in 1937

14:30 **SIES, Helmut** *(Düsseldorf, Germany)*
Albert Szent-Györgyi's research at Woods Hole and the NFCR: A personal recollection

14:40 **Nob1: SAKMANN, Bert** *(Jupiter, FL, USA)*
Introduction: by Ole Petersen *(Cardiff, UK)*
Cortical columns: if you don't understand the function – do the anatomy and get numbers

15:30–15:50 **COFFEE BREAK**

15:50–17:30 NOBEL SESSION II.

Chairs: Gyula Papp *(Szeged, Hungary)*, András Varró *(Szeged, Hungary)*

15:50 **Nob2: DOHERTY, Peter C.** *(Melbourne, Australia)*
Introduction: by Anna Erdei *(Budapest, Hungary)*
The continuing challenge of virus infections

16:40 **Nob3: WALKER, John E.** *(Cambridge, UK)*
Introduction: by András Varró *(Szeged, Hungary)*
Biological combustion today

EVENING PROGRAMME

19:30 **Welcome party** *(Venue: Szeged National Theatre)*
Hungarian Folkdance Evening and Dinner
For all participants

MORNING PROGRAMME — MAIN PLENARY EVENT

(Venue: TIK Main Lecture Hall)

8:30–10:10 NOBEL SESSION III.

Chairs: Gyula Telegdy *(Szeged, Hungary)*, László Vécsei *(Szeged, Hungary)*

8:30 — **Nob4: CIECHANOVER, Aaron** *(Haifa, Israel)*
Introduction: by Shmuel Muallem *(Bethesda, MD, USA)*
The Ubiquitin Proteolytic System – From basic mechanisms through human diseases and on to drug development

9:20 — **Nob5: SCHALLY, Andrew V.** *(Miami, FL, USA)*
Introduction: by Gyula Telegdy *(Szeged, Hungary)*
Hypothalamic hormones: From Neuroendocrinology to therapy of cancer and other diseases

10:10–10:30 **COFFEE BREAK**

10:30–11:50 NOBEL SESSION IV.

Chairs: József Pál *(Szeged, Hungary)*, Klára Hernádi *(Szeged, Hungary)*

10:30 — **SZABÓ, Gábor/ORMOS, Pál** *(Szeged, Hungary)*
Introduction of the University of Szeged and the Biological Research Center

11:00 — **Nob6: YONATH, Ada** *(Rehovot, Israel)*
Introduction: by Mónika Fuxreiter *(Debrecen, Hungary)*
Combating resistance to antibiotics?

11:50–12:30 POSTER VIEWING I.

12:30–13:30 LUNCH BREAK

CARDIOLOGY

(Venue: Hotel Forrás)

13:50–15:45 SESSION I. – Novel approaches to cardioprotection

Chairs: Gerd Heusch *(Essen, Germany)*, Péter Ferdinándy *(Budapest, Hungary)*

13:50 — **C1: HEUSCH, Gerd** *(Essen, Germany)*
Remote ishemic preconditioning

14:15 — **C2: HAUSENLOY, Derek** *(London, UK)*
Mitochondrium as the therapeutic target for cardioprotection

14:40 — **C3: SCHULZ, Richard** *(Edmonton, Canada)*
Inhibition of matrix metalloproteinase-2 (MMP-2) to protect the heart from oxidative stress injury

15:05 — **C4: ESCHENHAGEN, Thomas** *(Hamburg, Germany)*
Making 3D heart tissues – state of the art and perspectives

15:30 — **O-C1: T. Csont, V. Fekete, P. Bencsik, E. Aypar, Z. Murlasits, M. Sárközy, Z. Varga, P. Ferdinandy** *(Szeged, Hungary)*
Loss of cardioprotection by ischemic postconditioning in vascular nitrate tolerance: role of survival kinaes

15:45–16:00 **COFFEE BREAK**

16:00–17:55 SESSION II. – Sudden cardiac death 1.

Chairs: Charles Antzelevitch *(Utica, NY, USA)*, Béla Merkely *(Budapest, Hungary)*

16:00 — **C5: ANTZELEVITCH, Charles** *(Utica, NY, USA)*
J wave syndromes as a cause of sudden cardiac death. From cell to bedside

16:25 — **C6: CERBAI, Elisabetta** *(Florence, Italy)*
Arrhythmogenic alterations in familial hypertrophic cardiomyopathy: molecular and functional evidence

16:50 — **C7: WILDE, Arthur** *(Amsterdam, The Netherlands)*
Sudden cardiac death, a matter of genes?

17:15 — **C8: MERKELY, Béla** *(Budapest, Hungary)*
Arrhythmogenic substrate of life threatening arrhythmias

15:40 — **O-C2: R. M. ter Bekke, K. H. Haugaa, A. van den Wijngaard, T. Edvardsen, P. G. Volders** *(Maastricht, The Netherlands)*
Electro-mechanical window is profoundly negative in genotyped long-QT patients: relation to arrhythmogenic risk

GASTROENTEROLOGY

(Venue: Hotel Forrás)

14:05–15:35 SESSION I. – Calcium signalling

Chairs: Péter Hegyi *(Szeged, Hungary)*, Shmuel Muallem *(Bethesda, MD, USA)*

14:05 **Opening of the GI Epithelial Conference**

14:15 **G1: PETERSEN, Ole** *(Cardiff, UK)*
Calcium signalling in the gastrointestinal tract

14:40 **G2: MUALLEM, Shmuel** *(Bethesda, MD, USA)*
Mechanism of epithelial HCO_3^- secretion

15:05 **O-G1: J. Maléth, Z. Rakonczay Jr., V. Venglovecz, C. Orosz, P. Hegyi** *(Szeged, Hungary)*
Non-oxidative ethanol metabolites induce intracellular ATP depletion and inhibit pancreatic ductal bicarbonate secretion in human pancreatic epithelial cell line

15:20 **O-G2: P. Ferdek, J. Gerasimenko, O. Petersen, O. Gerasimenko** *(Cardiff, UK)*
A novel role for Bcl-2 in regulation of cellular calcium extrusion

15:45–16:00 **COFFEE BREAK**

16:00–18:05 SESSION II. – Regulation of GI secretion

Chairs: Ole Petersen *(Cardiff, UK)*, Graham Dockray *(Liverpool, UK)*

16:00 **G3: DOCKRAY, Graham** *(Liverpool, UK)*
Regulatory peptides and gastric epithelial cell function

16:25 **G4: WILLIAMS, John** *(Ann Arbor, MI, USA)*
Regulating the supply of pancreatic enzymes for digestion

16:50 **G5: VARRÓ, Andrea** *(Liverpool, UK)*
Making sense of stroma

17:15 **G6: BARRETT, Kim** *(San Diego, CA, USA)*
Pathophysiology of intestinal epithelial barrier and transport functions: implications for infectious and inflammatory disease states

17:40 **G7: DANIEL, Hannelore** *(Münich, Germany)*
Intestinal peptide transporters – what are they good for?

IMMUNOLOGY & INFLAMMATION

(Venue: Hotel Novotel)

13:50–15:45 SESSION I. – Pathomechanism of inflammation 1.

Chairs: Hidechika Okada *(Nagoya City, Japan)*, László Vígh *(Szeged, Hungary)*

13:50 — **I1: OKADA, Hidechika** *(Nagoya City, Japan)*
Peptide therapy in sepsis and inflammation: A novel strategy to suppress inflammation

14:15 — **I2: BENGMARK, Stig** *(London, UK)*
Gut microbiota and immune development and function

14:40 — **I3: BÍRÓ, Tamás** *(Debrecen, Hungary)*
The role of endocannabinoids in the regulation of inflammation

15:05 — **I4: CHAUDRY, Irshad** *(Birmingham, AL, USA)*
Sex steroids and receptor antagonists for improving immune functions and decreasing mortality from sepsis following trauma-hemorrhage

15:30 — **O-I1: M. A. Deli,** *(Szeged, Hungary)*
Bacterial lipopolysaccharides damage defense mechanisms at the blood-brain barrier

15:45–16:00 COFFEE BREAK

16:00–17:55 SESSION II. – Pathomechanism of inflammation 2.

Chairs: Mihály Boros *(Szeged, Hungary)*, Brigitte Vollmar *(Rostock, Germany)*

16:00 — **I5: REDL, Heinz** *(Wien, Austria)*
Individualized goal-directed therapy for trauma-induced coagulopathy

16:25 — **I6: VOLLMAR, Brigitte** *(Rostock, Germany)*
Hepatic ischemia-reperfusion injury: pathomechanisms and therapeutic strategies

16:50 — **I7: MENGER, Michael D.** *(Homburg, Germany)*
Vascularization in tissue engineering: angiogenesis versus inosculation

17:15 — **I8: BOROS, Mihály** *(Szeged, Hungary)*
Hypoxia-induced non-microbial methane generation: mechanism and significance

17:40 — **O-I2: L. Entz, E. Dósa, G. Széplaki, A. Szabó, G. Füst** *(Budapest, Hungary)*
The role of inflammation factors in the development of restenosis after carotid endarterectomy

MOLECULAR BIOLOGY & GENETICS

(Venue: IH Event Center)

14:00–15:20 SESSION I. – DNA-encoded messages from the past

Chairs: Stephen Downes *(Coleraine, Ireland)*, Márta Széll *(Szeged, Hungary)*

14:00 **Welcome notes**

14:05 **BALÁZS, Anna** *(Budapest, Hungary)*
The power of personalization (sponsored lecture by Roche)

14:15 **M1: DOWNES, Stephen** *(Coleraine, Northern Ireland)*
Origins of human populations

14:40 **M2: RASKÓ, István** *(Szeged, Hungary)*
Archeogenetics; to study the Hungarian past

15:05 **O-M1: D. Nagy, G. Tömöry, B. Csányi, E. Bogácsi-Szabó, I. Raskó** *(Szeged, Hungary)*
Genetic testing of adult-type hypolactasia in present-day and archaic samples

15:20–15:40 COFFEE BREAK

15:40–17:50 SESSION II.

Translational medicine: gene therapy, stem cells, nanomedicine

Chairs: László Dux *(Szeged, Hungary)*, Gyula Hadlaczky *(Szeged, Hungary)*

15:40 **M3: DAWSON, Kenneth A.** *(Dublin, Ireland)*
Foundation principles of interactions of nanoparticles with cells and biological barriers (including the blood brain barrier)

16:05 **M4: CAVAZZANA-CALVO, Marina** *(Paris, France)*
Gene therapy for inherited diseases of the hematopoietic system: from bench to the bedside

16:30 **M5: BRÜSTLE, Oliver** *(Bonn, Germany)*
Stem cell-based models of neurodegenerative disease

16:55 **M6: PAGANI, Franco** *(Trieste, Italy)*
The pathology of pre-mRNA splicing: mechanistic aspects and development of novel therapeutic correction strategies

17:20 **O-M2. T. Juhász, É. Katona, C. Matta, C. Somogyi, R. Takács, E. Szentléleky, Á. Radvánszki, P. Kiss, G. Tóth, A. Tamás, D. Regldi, R. Zákány** *(Debrecen, Hungary)*
Effects of PACAP on chondrogenesis in high density mesenchymal cell cultures

17:35 **O-M3: Z. Simandi, B. L. Balint, L. Nagy** *(Debrecen, Hungary)*
Prmt1 and 8 control cell fate specification of differentiating embryonic stem cells via selectively tuning retinoid-induced gene expression

NEUROSCIENCE

(Venue: Hotel Forrás)

13:40–15:45 SESSION I. – Neuroscience 1.

Chairs: Kálmán Magyar *(Budapest, Hungary)*, László Vécsei *(Szeged, Hungary)*

13:40 — **N1: RIEDERER, Peter** *(Würzburg, Germany)*
The lateralized brain in Parkinson's Disease

14:05 — **N2: BEAL, Flint** *(New York, NY, USA)*
Mitochondria and the pathogenesis of neurodegenerative diseases

14:30 — **N3: ÁDÁM-VIZI, Veronika** *(Budapest, Hungary)*
Metabolic enzyme mutations and oxidative stress in mitochondrial diseases

14:55 — **N4: MAGYAR, Kálmán** *(Budapest, Hungary)*
The pharmacology of selegiline; past, present, future?

15:20 — **N5: VÉCSEI, László** *(Szeged, Hungary)*
Neurological disorders and kynurenines: future therapeutic possibilities

15:45–16:00 **COFFEE BREAK**

16:00–17:55 SESSION II. – Neuroscience 2.

Chairs: Zoltán Janka *(Szeged, Hungary)*, Botond Penke *(Szeged, Hungary)*

16:00 — **N6: MIRNICS, Károly** *(Nashville, TN, USA)*
Gaba-ergic dysfunction in schrizophrenia: from postmortem studies to animal models

16:25 — **N7: RIHMER, Zoltán** *(Budapest, Hungary)*
Recent advances in suicide prevention – The role of psychopharmacology

16:50 — **N8: JANKA, Zoltán** *(Szeged, Hungary)*
Biological psychiatry research in Szeged

17:15 — **N9: PENKE, Botond** *(Szeged, Hungary)*
The role of intrinsically disordered proteins (IDPs) in neurodegenerative diseases

17:40 — **O-N1: M. J. Molnár** *(Budapest, Hungary)*
New trends in the clinical neurosciences

TUBERCULOSIS EVOLUTION

(Venue: Hotel Tisza)

13:35–15:35 SESSION I. – TB Evolution: Opening session

Chairs: Jane E. Buikstra *(Tempe, AZ, USA)*, Dick van Soolingen *(Bilthoven, The Netherlands)*

13:35 — **Welcome addresses – (From ICEPT to ICEPT-2) – György Pálfi, Olivier Dutour**

13:45 — **T1: BUIKSTRA, Jane E.** – Co-President *(Tempe, AZ, USA)*
A tuberculosis: the intersection of ancient evidence and contemporary strain variation

14:15 — **T2: SPIGELMAN, Mark** *(London, UK)*
Evolutionary changes in the genome of *Mycobacterium tuberculosis* (Mtb) and the human genome from 9000 years BP until modern times

14:40 — **T3: VAN SOOLINGEN, Dick** *(Bilthoven, The Netherlands)*
The evolutionary development of *Mycobacterium tuberculosis* Beijing genotype strains and worldwide emergence of resistance

15:05 — **O-T1: J. Bryant, A. Schürch, H. van Deutekom, V. de Jager, S. Harris, K. Kremer, J. de Beer, S. van Hijum, R. Siezen, M. S. van der Loeff, M. Borgdorff, S. Bentley, J. Parkhill, D. van Soolingen** *(Hinxton, UK)*
Whole genome sequencing of 199 *Mycobacterium tuberculosis* isolates reveals an absence of a molecular clock over short time scales

15:20 — **O-T2: F. Maixner, N. Nicklisch, R. Ganslmeier, S. Friederich, V. Dresely, H. Meller, C. Sola, K. W. Alt, A. Zink** *(Bolzano, Italy)*
Tuberculosis at the onset of agriculture in central Germany

15:35–16:05 COFFEE BREAK

16:05–18:15 SESSION II. – TB Evolution: A multidisciplinary approach

Chairs: Albert Zink *(Bolzano, Italy)*, Philip Supply *(Lille, France)*

16:05 — **T4: ZINK, Albert** *(Bolzano, Italy)*
Evolution of tuberculosis in ancient mummies and skeletons

16:30 — **T5: PERRIN, Pascale** *(Montpellier, France)*
Tuberculosis: molecular and epidemiological insight – What can ancient human remains tell us?

16:55 — **T6: SOLA, Christophe** *(Paris, France)*
CRISPR genetic diversity studies as a mean to reconstitute the evolution of the *Mycobacterium tuberculosis* complex

17:20 — **T7: SUPPLY, Philip** *(Lille, France)*
A glimpse of the early evolution of the tubercle bacillus

17:45 — **O-T3: K. Manchester, S. Wood, C. A. Roberts** *(Durham, UK)*
Stannington sanatorium for TB children

18:00 — **O-T4: J. Eddy** *(Cambridge, UK)*
The city of Rome, its empire, and the spread of tuberculosis in Europe

EVENING PROGRAMME:

19:30 **Faculty evening – Hosted by the Mayor *(by invitation only)* (Venue: City Hall)**

MORNING PROGRAMME — MAIN PLENARY EVENT

(Venue: TIK Main Lecture Hall)

8:30–10:10 NOBEL SESSION V.

Chairs: Attila Dobozy *(Szeged, Hungary)*, Lajos Kemény *(Szeged, Hungary)*

8:30 — **Nob7: HUNT, Tim** *(South Mimms, UK)*
Introduction: by János Szabad *(Szeged, Hungary)*
Switches and latches: The control of entry into mitosis

9:20 — **Nob8: HUBER, Robert** *(Martinsried, Germany)*
Introduction: by Lajos Kemény *(Szeged, Hungary)*
Intracellular proteolysis: structures, molecular mechanisms and drug design

10:10–10:30  **COFFEE BREAK**

10:30–11:50 NOBEL SESSION VI.

Chairs: Ferenc Fülöp *(Szeged, Hungary)*, Imre Dékány *(Szeged, Hungary)*

10:30 — **BOGSCH, Erik** *(Budapest, Hungary)*
A hundred-year of pharmaceutical manufacturing in Hungary: The importance of medical chemistry

11:00 — **Nob9: WIESCHAUS, Eric** *(Princeton, NJ, USA)*
Introduction: by János Szabad *(Szeged, Hungary)*
Changing mesodermal cell shape during Drosophila gastrulation

11:50–12:30 POSTER VIEWING II.

12:30–13:30  **LUNCH BREAK**

CARDIOLOGY

(Venue: Hotel Forrás)

13:35–15:45 SESSION III. – Sudden cardiac death 2.

Chairs: Mark A. Vos *(Utrecht, The Netherlands)*, Ursula Ravens *(Dresden, Germany)*

13:35 — **C9: VOS, Mark A.** *(Utrecht, The Netherlands)*
Beat-to-beat variability of ventricular repolarization: biomarker to quantify repolarization reserve?

14:00 — **C10: VOLDERS, Paul** *(Maastricht, The Netherlands)*
Cardiac repolarization and arrhythmogenesis during sympathetic nervous stimulation

14:25 — **C11: VARRÓ, András** *(Szeged, Hungary)*
Possible mechanisms of sudden cardiac death in top athletes: a basic cardiac electrophysiological point of view

14:50 — **C12: NÁNÁSI, Péter** *(Debrecen, Hungary)*
Reverse rate-dependent character of action potential duration changes is a genuine property of the myocardium

15:15 — **O-C3: Z. Husti, I. Baczkó, V. Juhász, L. Virág, A. Kristóf, I. Koncz, T. Szél, N. Jost, J. G. Papp, A. Varró** *(Szeged, Hungary)*
The possible proarrhythmic effects of diclofenac

15:30 — **O-C4: A. Zaza** *(Milano, Italy)*
Catecholamines, rate and repolarization. Can we make a complex story simpler?

15:45–16:00 **COFFEE BREAK**

16:00–17:55 SESSION IV.

The role of nitric oxide in cardioprotection: From the molecule to therapy

Chairs: David Lefer *(Atlanta, GA, USA)*, Ágnes Végh *(Szeged, Hungary)*

16:00 — **C13: MURPHY, Elisabeth** *(Bethesda, MD, USA)*
Role of S-nitrosylation in cardioprotection

16:25 — **C14: PERNOW, John** *(Stockholm, Sweden)*
The importance of arginase as a regulator of NO availability: implications for myocardial ischemia-reperfusion injury

16:50 — **C15: VÉGH, Ágnes** *(Szeged, Hungary)*
Nitrite therapy against acute ischaemia and reperfusion-induced ventricular arrhythmias

17:15 — **C16: LEFER, David** *(Atlanta, GA, USA)*
Cardioprotective actions of hydrogen sulfide

17:40 — **O-C5: A. Görbe, J. Pálóczi, Z. Varga, M. Pirity, A. Dinnyés, T. Eschenhagen, T. Csont, P. Ferdinandy** *(Szeged, Hungary)*
The effect of cardioprotective agents (SNAP, BNP) against simulated ischemia/reoxygenation injury in mouse embryonic stem cell-derived cardiomyocytes

GASTROENTEROLOGY

(Venue: Hotel Forrás)

14:00–15:45 SESSION III. – Regulation of bicarbonate secretion

Chairs: Viktória Venglovecz *(Szeged, Hungary)*, Barry Argent *(Newcastle, UK)*

14:00 **G8: GRAY, Mike A.** *(Newcastle, UK)*
The role of CFTR in pancreatic ductal bicarbonate secretion

14:25 **G9: SEIDLER, Ursula** *(Hannover, Germany)*
Molecular regulation and physiological functions of intestinal bicarbonate transport

14:50 **G10: ZSEMBERY, Ákos** *(Budapest, Hungary)*
The role of CFTR in bicarbonate secretion by biliary epithelia

15:15 **O-G3: P. Pallagi, A. Kumar Singh, V. Venglovecz, R. Engelhardt, B. Riederer, T. Takács, T. Wittmann, U. Seidler, Z. Rakonczay Jr.** *(Szeged, Hungary)*
Na^+/H^+ exchanger regulatory factor-1 mediates pancreatic ductal fluid and bicarbonate secretion by affecting cystic fibrosis transmembrane conductance regulator localization in mice

15:30 **O-G4: É. Pallagi-Kunstár, K. Farkas, Z. Rakonczay., F. Nagy, T. Molnár, Z. Szepes, V. Venglovecz, Z. Rázga, J. Maléth, K. Orbán, K. Tóth, T. Wittmann, P. Hegyi** *(Szeged, Hungary)*
Non-conjugated bile acids induce ATP depletion, mitochondrial damage and inhibit the ion transport mechanisms in human colonic crypts

15:45–16:00 **COFFEE BREAK**

16:00–17:55 SESSION IV. – Mucin and water transport, epithelial interactions

Chairs: Mike Gray *(Newcastle, UK)*, Kim Barrett *(San Diego, CA, USA)*

16:00 **G11: QUINTON, Paul** *(San Diego, CA, USA)*
Normal mucin formation requires bicarbonate

16:25 **G12: HANSSON, Gunnar C.** *(Gothenburg, Sweden)*
The two mucus layers organized by the MUC2 mucin and their relation to colon inflammation

16:50 **G13: VERKMAN, Alan** *(San Francisco, CA, USA)*
New approaches for therapy of secretory diarrheas in developing countries

17:15 **G14: WELLS, Jerry** *(Wageningen, The Netherlands)*
Microbe-epithelial signalling in the intestinal tract

17:40 **O-G5: V. Venglovecz, L. V. Kemény, Z. Rakonczay Jr., I. Dékány, Á. Zvara, L. Puskás, A. Verkman, P. Hegyi** *(Szeged, Hungary)*
The effects of pancreatitis-inducing factors on ductal fluid secretion

IMMUNOLOGY & INFLAMMATION

(Venue: Hotel Novotel)

14:00–15:45 SESSION III. – Bridging innate and adaptive immunity

Chairs: Anna Erdei *(Budapest, Hungary)*, Éva Rajnavölgyi *(Debrecen, Hungary)*

14:00 — **I9: HUBER, Robert** *(Martinsried, Germany)*
Antibodies and antibody receptors, structures and application for therapy

14:25 — **I10: ERDEI, Anna** *(Budapest, Hungary)*
Complement – bridging innate and adaptive immunity

14:50 — **I11: RAJNAVÖLGYI, Éva** *(Debrecen, Hungary)*
The interplay of signalling cascades initiated by membrane and cytosolic pattern recognition receptors in human dendritic cells

15:15 — **O-I3: P. Gál, D. Héja, A. Kocsis, K. Szilágyi, J. Dobó, P. Závodszky, G. Pál** *(Budapest, Hungary)*
Deciphering the mechanism of the complement lectin pathway activation

15:30 — **O-I4: K. Juhász, Á. Zvara, A. Sonnleitner, Z. Balogi, E. Duda** *(Szeged, Hungary)*
Ckip-1 interferes with TNF reverse signaling

15:45–16:00 COFFEE BREAK

16:00–18:00 SESSION IV. – Host defense mechanisms

Chairs: Yvette Mándi *(Szeged, Hungary)*, Joost J. Oppenheim *(Bethesda, MD, USA)*

16:00 — **I12: OPPENHEIM, Joost J.** *(Bethesda, MD, USA)*
Proinflammatory alarmins promote host defense proteins

16:25 — **I13: KEMÉNY, Lajos** *(Szeged, Hungary)*
The role of immunological factors in the pathogenesis of acne

16:50 — **I14: MÁNDI, Yvette** *(Szeged, Hungary)*
Relevance of defensins in multifactorial diseases

17:15 — **O-I5: E. Klein** *(Stockholm, Sweden)*
Our equilibrated coexistence with the life endangering Epstein Barr virus, EBV

17:30 — **O-I6: F. Banati, T. Tereh, A. Koroknai, N. Kovacs, Z. Ruzics, F. Lemnitzer, J. Minarovits** *(Budapest, Hungary)*
Epigenetic regulation of the lamin a/c promoter in EBV-positive B cell lines

17:45 — **O-I7: D. P. Virok, K. Filkor, T. Mosolygo, A. Bogdanov, K. Burian, V. Endresz, J. Deak, I. Nagy** *(Szeged, Hungary)*
mRNA sequencing of the *Chlamydia trachomatis* infected and interferon-gamma treated human neutrophil granulocyte transriptom

MOLECULAR BIOLOGY & GENETICS

(Venue: IH Event Center)

13:40–16:00 SESSION III. – Gene regulation, epigenetics

Chairs: Ferenc Nagy *(Szeged, Hungary)*, Tibor Török *(Szeged, Hungary)*

13:40 — **M7: EMBO Lecture: THANOS, Dimitris** *(Athens, Greece)*
Mechanisms of stochastic gene expression

14:05 — **M8: NAGY, László** *(Debrecen, Hungary)*
Nuclear receptors link lipid metabolism to genome expression

14:30 — **M9: SILVA, Alcino J.** *(Los Angeles, CA, USA)*
Molecular and cellular mechanisms of memory allocation in neuronal networks

14:55 — **M10: BOROS, Imre** *(Szeged, Hungary)*
Histone acetyltransferases affect transcription through diverse mechanisms

15:20 — **M11: FUXREITER, Mónika** *(Debrecen, Hungary)*
Fuzzy complexes: ambiguity in protein interactions

15:45 — **O-M4: É. Margittai, P. Löw, I. Stiller, R. Sitia, G. Bánhegyi** *(Budapest, Hungary)*
Hydrogen peroxide, a prooxidant in oxidative protein folding

16:00–16:20 COFFEE BREAK

16:20–18:05 SESSION IV.

Translational medicine: gene therapy, stem cells, nanomedicine 2.

Chairs: András Dinnyés *(Gödöllő, Hungary)*, Lajos Haracska *(Szeged, Hungary)*

16:20 — **M12: UHER, Ferenc** *(Budapest, Hungary)*
The identity, plasticity and therapeutic potential of mesenchymal stem cells

16:45 — **M13: TAKEDA, Shin'ichi** *(Tokyo, Japan)*
Advances in molecular and cell therapy of Duchenne muscular dystrophy

17:10 — **M14: SARKADI, Balázs** *(Budapest, Hungary)*
Membrane transporters and calcium signaling in human pluripotent stem cells

17:35 — **O-M5: B. Gyurcsik, A. Czene, E. Németh, I. G. Zóka, E. Endreffy, H. E. M. Christensen, K. Nagata** *(Szeged, Hungary)*
Targeting the breakpoint in Duchenne muscular dystrophy

17:50 — **O-M6: B. Hegyi, Z. Környei, G. Kudlik, E. Madarász, F. Uher** *(Budapest, Hungary)*
Anti-inflammatory effects of mouse mesenchymal stem cells on microglia

NEUROSCIENCE

(Venue: Hotel Forrás)

13:50–15:45 SESSION III. – Neuroscience 3.

Chairs: Gábor Tamás *(Szeged, Hungary)*, György Buzsáki *(Newark, NJ, USA)*

13:50 — **N10: BUZSÁKI, György** *(Newark, NJ, USA)*
Brain rhythms and cell assembly sequences

14:15 — **N11: MORRIS, Richard** *(Edinburgh, UK)*
The making, keeping and loosing of memories

14:40 — **N12: CONDORELLI, Daniele Filippo** *(Catania, Italy)*
Distribution and function of neuronal gap junctions in the mammalian brain

15:05 — **N13: TAMÁS, Gábor** *(Szeged, Hungary)*
Unitary volume transmission by neurogliaform cells: broadening the functional scope of single neurons

15:30 — **O-N2: B. Rózsa, G. Katona, G. Szalay, P. Maák, A. Kaszás, M. Veress, D. Hillier, B. Chiovini, E. S. Vizi, B. Roska** *(Budapest, Hungary)*
Fast two-photon in vivo imaging with three-dimensional random-access scanning in large tissue volumes

15:45–16:00 **COFFEE BREAK**

16:00–17:55 SESSION IV. – Neuroscience 4.

Chairs: Gyula Telegdy *(Szeged, Hungary)*, Veronika Ádám-Vizi *(Budapest, Hungary)*

16:00 — **N14: FREUND, Tamás** *(Budapest, Hungary)*
Functional roles of endocannabinoid signaling in the cerebral cortex

16:25 — **N15: RAKIC, Pasko** *(New Haven, CT, USA)*
Making maps of the mind: molecular mechanisms of neuronal migration

16:50 — **N16: PAULUS, Walter** *(Göttingen, Germany)*
Modulating human cortical excitability by transcranial stimulation

17:15 — **O-N3: G. Szalai, J. Crossland** *(Columbia, SC, USA)*
The peromyscus audiogenic seizure model

17:30 — **N17: EDVINSSON , Lars** *(Lund, Sweden)*
Role of CGRP and CGRP receptors in migraine pathophysiology

TUBERCULOSIS EVOLUTION

(Venue: Hotel Tisza)

13:35–15:25 SESSION III. – World TB Day 1.

Chairs: Stewart T. Cole *(Lausanne, Switzerland)*, Zsuzsanna Jakab *(Copenhagen, Denmark)*

13:35 **T8: COLE, Stewart T.** – Co-President *(Lausanne, Switzerland)*
The evolution of *M. tuberculosis* research since Robert Koch's discovery

14:05 **T9: JAKAB, Zsuzsanna** *(Copenhagen, Denmark)*
Regional action plan to prevent and combat M/XDR-TB

14:30 **T10: LIENHARDT, Christian and RAVIGLIONE, Mario** *(Geneva, Switzerland)*
TB in 2012: burden, strategies and research needs

14:55 **O-T5: A. Somoskövi** *(Geneva, Switzerland)*
Novel laboratory diagnostic tests for tuberculosis and their potential role in an integrated and tiered laboratory network

15:10 **O-T6: K. Horváti, B. Bacsa, N. Szabó, K. Fodor, G. Balka, M. Rusvai, G. Mező, V. Grolmusz, B. Vértessy, F. Hudecz, S. Bősze** *(Budapest, Hungary)*
Antimycobacterial activity of pyridopyrimidine derivatives against *Mycobacterium tuberculosis* in a series of *in vitro* and *in vivo* models

15:25–15:45 **COFFEE BREAK**

15:45–17:55 SESSION IV. – World TB Day 2.

Chairs: Nalin Rastogi *(Abymes/Guadeloupe, France)*, Helen D. Donoghue *(London, UK)*

15:45 **T11: RASTOGI, Nalin** *(Abymes, Guadeloupe/France)*
Tuberculosis – a global emergency: tools and methods to monitor, understand and control the epidemic

16:10 **T12: VADÁSZ, Imre** *(Budapest, Hungary)*
TB in Hungary in the 20th and 21st centuries

16:35 **T13: KÉRI, György** *(Budapest, Hungary)*
Leads selection and characterization of antitubercular compounds using a Nested Chemical Library of kinase inhibitors

17:00 **T14: DONOGHUE, Helen D.** *(London, UK)*
Ancient DNA analysis – an established technique in charting the evolution of tuberculosis and leprosy

17:25 **O-T7: M. Masson, E. Molnár, H. D. Donoghue, D. Minnikin, O.Y. Lee, Gy. Pálfi** *(Edinburgh, Scotland)*
7000-year-old tuberculosis cases from Hungary – osteological and biomolecular evidence

17:40 **O-T8: S. Pfeiffer, O. Dutour** *(Toronto, Canada)*
Tuberculous skeletal lesions among precontact longhouse people of the North American Great Lakes

EVENING PROGRAMME:

20:30 **Organ Concert (Venue: Synagogue) – For all participants**

CARDIOLOGY

(Venue: Hotel Forrás)

8:00–10:10 SESSION V. – Sudden cardiac death 3.

Chairs: Karin Sipido *(Leuven, Belgium)*, David Eisner *(Manchester, UK)*

8:00 **C17: RAVENS, Ursula** *(Dresden, Germany)*
Heart failure and arrhythmias

8:25 **C18: EISNER, David** *(Manchester, UK)*
Calcium in the heart: in and out of control

8:50 **C19: SIPIDO, Karin** *(Leuven, Belgium)*
Activation of Na^+/Ca^+ exchange current in microdomains near calcium release sites

9:15 **C20: TÓSAKI, Árpád** *(Debrecen, Hungary)*
The role of carbon monoxide signalling in biological processes mediated by hemeoxygenase-1

9:40 **O-C6: L. Lu, M. Mende, H. Körber, C. Werner, U. Ravens** *(Dresden, Germany)*
Design and validation of a cyclic stretch bioreactor system for simulating the cardiac environment

9:55 **O-C7: A. Farkas, F. Rárosi, M. Szucs, D. Vincze, T. Forster, A. Varró, A. S. Farkas** *(Szeged, Hungary)*
An increase in the 'absolute' beat-to-beat variability and instability of the ECG intervals predicts dofetilide-induced torsades de pointes independently from the applied anaesthetic in rabbits, in vivo

10:10–10:25 **COFFEE BREAK**

10:25–12:20 SESSION VI. – Heart failure

Chairs: Zoltán Papp *(Debrecen, Hungary)*, Tamás Forster *(Szeged, Hungary)*

10:25 **C21: FORSTER, Tamás** *(Szeged, Hungary)*
Experimental and clinical studies in cardiomyopathies

10:50 **C22: ÉDES, István** *(Debrecen, Hungary)*
Levosimendan the cardioprotective inodilator

11:15 **C23: PAPP, Zoltán** *(Debrecen, Hungary)*
Oxidative myofilament protein alterations in the postischemic heart

11:40 **C24: PIESKE, Burkert** *(Graz, Austria)*
Diastolic heart failure – From pathophysiology to new therapeutic options

12:05 **O-C8: A. Pósafalvi, C. Weidijk, P. A. van der Zwaag, L. G. Boven, M. P. van den Berg, R. A. de Boer, R. M. Hofstra, J. P. van Tintelen, R. J. Sinke, J. D. Jongbloed** *(Groningen, The Netherlands)*
Mutation screening and functional characterisation of RNA-binding motif protein 20 in dilated cardiomyopathy

GASTROENTEROLOGY

(Venue: Hotel Forrás)

8:00–10:20 SESSION V. – Intestinal ion transport

Chairs: Ursula Seidler *(Hannover, Germany)*, Barry Argent *(Newcastle, UK)*

8:00 **G15: LAMPRECHT, Georg** *(Tübingen, Germany)*
The anion exchanger DRA and its interaction with PDZ adapter proteins

8:25 **G16: MEDINA, Juan F.** *(Pamplona, Spain)*
Role of AE2 in the pathogenesis of primary biliary cirrhosis

8:50 **G17: VARGA, Gábor** *(Budapest, Hungary)*
Evidence for electrolyte transport of two-dimensional salivary gland engineered from human submandibular tissue

9:15 **G18: DONOWITZ, Mark** *(Baltimore, MD, USA)*
Regulation of NHE3: a story of signalling complexes and cytoskeleton

9:40 **G19: KEELY, Stephen** *(Dublin, Ireland)*
Targeting bile acids for treatment of intestinal transport disorders

10:05 **O-G6: S. Yeruva, J. Goldstein, M. Lünnemann, M. Chen, A. Singh, A. Cinar, M. Luo, G. Chodisetti, L. Ludolph, M. Juric, O. Bachmann, B. Riederer , A. Bleich, M. Gereke, D. Bruder, M. P. Manns, U. Seidler** *(Hannover, Germany)*
Inflammatory cytokines downregulate the Na^+/H^+ exchanger 3 (NHE3) interacting pdz domain protein PDZK1 in ulcerative colitis patients, colitis mice and in Caco2BBE cells: link to inflammation-associated NHE3 dysfunction

10:20–10:35 **COFFEE BREAK**

10:35–12:20 SESSION VI. – Pancreatitis

Chairs: László Hunyady *(Budapest, Hungary)*, John Williams *(Ann Arbor, MI, USA)*

10:35 **G20: SALUJA, Ashok K.** *(Minneapolis, MN, USA)*
A new paradigm of pathogenesis of pancreatitis

11:00 **G21: RAKONCZAY Jr., Zoltán** *(Szeged, Hungary)*
Early intraacinar events in the pathophysiology of acute pancreatitis

11:25 **G22: HEGYI, Péter** *(Szeged, Hungary)*
The pathophysiology of pancreatic ductal bicarbonate secretion

11:50 **O-G7: M. Sahin-Tóth, A. Szabó** *(Boston, MA, USA)*
On the mechanism of hereditary chronic pancreatitis

12:05 **O-G8: J. V. Gerasimenko, P. E. Ferdek, O. V. Gerasimenko, O. H. Petersen** *(Cardiff, UK)*
Protective role of calmodulin in alcohol-induced trypsinogen activation

IMMUNOLOGY & INFLAMMATION

(Venue: Hotel Novotel)

8:30–10:15 SESSION V. – Tumor immunology

Chairs: Georg Stingl *(Vienna, Austria)*, Zsuzsanna Bata-Csörgő *(Szeged, Hungary)*

8:30 — **I15: KLEIN, Georg** *(Stockholm, Sweden)*
Tumor resistance

8:55 — **I16: STINGL, Georg** *(Wien, Austria)*
Langerhans cells: new facts – new functions

9:20 — **I17: HOMEY, Bernhard** *(Düsseldorf, Germany)*
Chemokines: from inflammation to metastasis

9:45 — **O-I8: E. Emri, G. Emri, K. Egervari, I. Veres, G. Mehes, E. Remenyik** *(Debrecen, Hungary)*
Prognostic role of CD68, CD163 and CD1a expression in human primary cutaneous malignant melanoma

10:00 — **O-I9: B. Kotlan, G. Liszkay, L. Gobor, L. Toth, V. Plotar, E. Toth, T. Balatoni, A. Ladanyi, O. Csuka, M. Kasler** *(Budapest, Hungary)*
Novel antibody profile analysis based on tracking B lymphocytes in melanomas and breast carcinomas is an asset for the new immunological score for cancer therapeutics

10:15–10:35 **COFFEE BREAK**

10:35–12:20 SESSION VI. – Hypersensitivity

Chairs: Cezmi Akdis *(Davos, Switzerland)*, András Falus *(Budapest, Hungary)*

10:35 — **I18: AKDIS, Cezmi** *(Davos, Switzerland)*
Role of tissues in immunoregulation and immune tolerance to allergens

11:00 — **I19: AKDIS, Mübeccel** *(Davos, Switzerland)*
T and B regulatory cells

11:25 — **I20: FALUS, András** *(Budapest, Hungary)*
The role of histamine in the regulation of anti-tumour immunity

11:50 — **O-I10: K. Pazmandi, B. V. Kumar, K. Szabo, I. Boldogh, E. Rajnavolgyi, A. Bacsi** *(Debrecen, Hungary)*
Reactive oxygen species generated by NADPH oxidases in ragweed subpollen particles activate human monocyte-derived dendritic cells

12:05 — **O-I11: L. Kohidai, O. Lang, K. B. Bai, E. Lajko, J. Lang, L. Polgar, I. Szabo, G. Mezo, F. Hudecz** *(Budapest, Hungary)*
Chemotactic drug targeting – a novel approach of target cell dependent drug delivery

MOLECULAR BIOLOGY & GENETICS

(Venue: IH Conference Centre)

8:00–9:55 SESSION V. – Genomics, multifactorial human diseases

Chairs: Margit Balázs *(Debrecen, Hungary)*, László Nagy *(Debrecen, Hungary)*

8:00 — **M15: SKUSE, David H.** *(London, UK)*
Genetic influences on social communication

8:25 — **M16: CORNEL, Martina C.** *(Amsterdam, The Netherland)*
Professional policy development in genetic health care: the challenge of discerning hopes from hypes

8:50 — **M17: KATONA, Róbert** *(Szeged, Hungary)*
The ACE-ing of gene- and cell therapy

9:15 — **M18: SZALAI, Csaba** *(Budapest, Hungary)*
Evaluation of a partial genome screening of two asthma susceptibility regions using Bayesian network based Bayesian multilevel analysis of relevance

9:40 — **O-M7: Z. Boldogkői, N. Póka, I. Takács, D. Tombácz** *(Szeged, Hungary)*
Transcriptional interference networks coordinate global gene expression

9:55–10:15 **COFFEE BREAK**

10:15–12:00 SESSION VI. – Oncogenomics

Chairs: Edit Oláh *(Budapest, Hungary)*, Zsuzsa Schaff *(Budapest, Hungary)*

10:15 — **M19: JONES, Peter** *(Los Angeles, CA, USA)*
The cancer epigenome

10:40 — **M20: KALLIONIEMI, Olli** *(Helsinki, Finland)*
Implementing personalized cancer medicine

11:05 — **M21: OLÁH, Edit** *(Budapest, Hungary)*
Human cancer syndromes: lessons learned

11:30 — **O-M8: A. V. Patai, O. Galamb, G. Valcz, A. Kalmar, A. Patai, B. Peterfia, B. Wichmann, K. Leiszter, K. Toth, A. Scholler, S. Spisak, F. Sipos, T. Krenacs, Z. Tulassay, B. Molnar** *(Budapest, Hungary)*
Colorectal cancer epigenetics: characteristic DNA methylation pattern upsets adenoma-dysplasia-carcinoma sequence at the epigenetic level

11:45 — **O-M9: C. G. Ziegler, G. Eisenhofer, A. V. Schally, L. Gebauer, K. Gondek, J. Engel, M. Ehrhart-Bornstein, S. R. Bornstein** *(Dresden, Germany)*
Anti-tumor effects of peptide analogues targeting neuropeptide hormone receptors in rodent pheochromocytoma cells

NEUROSCIENCE

(Venue: Hotel Forrás)

8:30–10:15 SESSION V. – Neuroscience 5.

Chairs: Péter Somogyi *(Oxford, UK)*, Szilveszter E. Vizi *(Budapest, Hungary)*

8:30 — **N18: VIZI, E. Szilveszter** *(Budapest, Hungary)*
Nonsynaptic interaction between neurons

8:55 — **N19: SOMOGYI, Péter** *(Oxford, UK)*
Co-operative chronocircuits in the hippocampus

9:20 — **N20: BLAKEMORE, Colin** *(Oxford, UK)*
Adaptation and plasticity in the development and evolution of the brain

9:45 — **O-N4: V. Vukicevic, K. Chung, J. Schmid, G. Eisenhofer, S. Bornstein, M. Ehrhart-Bornstein** *(Dresden, Germany)*
Stem/progenitor cells from adult adrenal medulla

10:00 — **O-N5: J. Kálmán** *(Szeged, Hungary)*
Stress screwing neuronal cytoskeleton to Alzheimer's

10:15 – 10:35 **COFFEE BREAK**

10:35–12:30 SESSION VI. – Neuroscience 6.

Chairs: Gábor Jancsó *(Szeged, Hungary)*, Miklós Palkovits *(Budapest, Hungary)*

10:35 — **N21: PALKOVITS, Miklós** *(Budapest, Hungary)*
Pain in the brain – Neuroanatomical and functional MRI analysis of cortical and subcortical areas activated by acute pain

11:00 — **N22: SZOLCSÁNYI, János** *(Pécs, Hungary)*
Peppers in drug discoveries: serendipity, concept and breakthrough

11:25 — **N23: WOOD, John** *(London, UK)*
Pain

11:50 — **N24: JANCSÓ, Gábor** *(Szeged, Hungary)*
Of spices, toxins and pain: a personal perspective

12:15 — **O-N6. K. Pajer, G. Feichtinger, G. Márton, D. Klein, H. Redl, A. Nógrádi** *(Szeged, Hungary)*
Cytokine signalling by grafted neuroectodermal stem cells rescues motoneurons otherwise destined to die

TUBERCULOSIS EVOLUTION

(Venue: Hotel Tisza)

8:30–10:25 SESSION V. – Paleopathology of TB and mycobacterial infections

Chairs: Charlotte A. Roberts *(Durham, UK)*, Ildikó Pap *(Budapest, Hungary)*

08:30 — **T15: ROBERTS, Charlotte A.** *(Durham, UK)*
Old World tuberculosis: evidence from human remains with a review of current research and future prospects

08:55 — **T16: TESCHLER-NICOLA, Maria** *(Vienna, Austria)*
The early mediaeval manor-place Gars/Thunau (Lower Austria): a terrain of endemic tuberculosis

09:20 — **T17: PAP, Ildikó** *(Budapest, Hungary)*
The impact of tuberculosis to the 18th century Vác population, Hungary

09:45 — **T18: PÁLFI, György** *(Szeged, Hungary)*
Paleopathology of mycobacterial infections in Hungary: new results

10:10 — **O-T9: G. Maász, Zs. Lutz, J. Schmidt, L. Márk** *(Pécs, Hungary)*
Mycobacterial biomarker discovery

10:25–10:40 **COFFEE BREAK**

10:40–12:35 SESSION VI. – Evolution of mycobacteria

Chairs: Roland Brosch *(Paris, France)*, Igor Mokrousov *(St. Petersburg, Russia)*

10:40 — **T19: BROSCH, Roland** *(Paris, France)*
ESX/type VII secretion systems of mycobacteria: insights into evolution, pathogenicity and protection

11:05 — **T20: NAMOUCHI, Amine and GICQUEL, Brigitte** *(Paris, France)*
Horizontal transfer in *Mycobacterium tuberculosis* complex

11:30 — **T21: NIEMANN, Stefan** *(Borstel, Germany)*
Pathobiological variability of *Mycobacterium tuberculosis* complex strains

11:55 — **T22: MOKROUSOV, Igor** *(St. Petersburg, Russia)*
A tale of two genotypes: contrasting phylogeography of *Mycobacterium tuberculosis* Beijing and Ural families

12:20 — **O-T10: C. R. McEvoy, R. Cloete, B. Müller, A. C. Schürch, P. D. van Helden, S. Gagneux, R. M. Warren, N. C. Gey van Pittius** *(Tygerberg, South Africa)*
Evolution of the *Mycobacterium tuberculosis* complex *pe* and *ppe* genes

12:35–13:30 **LUNCH BREAK *(Lunch will be served at the venues)***

CARDIOLOGY

(Venue: Hotel Forrás)

13:50–15:45 – SESSION VII.

Cardioprotection in the presence of cardiovascular risk factors

Chairs: Karin Przyklenk *(Worchester, MA, USA)*, Rainer Schulz *(Giesen, Germany)*

13:50 — **C25: FERDINANDY, Péter** *(Budapest, Hungary)*
Endogenous cardioprotection is a healthy heart phenomenon? Cardioprotective signaling in hyperlipidemia

14:15 — **C26: SCHULZ, Rainer** *(Giesen, Germany)*
Cardioprotection and aging

14:40 — **C27: PRZYKLENK, Karin** *(Detroit, MI, USA)*
Cardioprotection in diabetic and aging cohorts: getting to the 'heart' of the matter

15:05 — **C28: RAVINGEROVA, Tatiana** *(Bratislava, Slovakia)*
Lifestyle-related risk factors and cardiac response to ischemia: possibilities to restore impaired ischemic tolerance of the heart

15:30 — **O-C9: Z. Szelid, Z. Bagyura, P. Soós, O. Szenczi, Z. Andrási, Á. Lux, E. Édes, P. Maurovich-Horvat, N. Pintér, P. Józan, B. Merkely** *(Budapest, Hungary)*
Primary prevention population cohort: Budakalász epidemiology study

15:45–16:00 **COFFEE BREAK**

16:00–18:10 – SESSION VIII. Cardioprotective signalling and oxidative stress

Chairs: Gary F. Baxter *(Cardiff, UK)*, Erzsébet Rőth *(Pécs, Hungary)*

16:00 — **C29: BAXTER, Gary F.** *(Cardiff, UK)*
H_2S and cardioprotection

16:25 — **C30: RŐTH, Erzsébet** *(Pécs, Hungary)*
How the inhibition of glutathione S-transferase can modulate stress response of cardiac myocytes

16:50 — **C31: TÓTH, Kálmán** *(Pécs, Hungary)*
Myocardial and vascular protection by PARP inhibitors

17:15 — **C32: MUNTEAN, Danina** *(Timisoara, Romania)*
The emerging role of magnesium orotate in cardioprotection against acute ischemia-reperfusion injury

17:40 — **O-C10: G. Á. Fülöp, I. Rutkai, E. T. Pásztorné, I. S. Mányiné, I. Édes, Z. Papp, A. Tóth** *(Debrecen, Hungary)*
Effects of hydrogen peroxide and MPO in the rat basilar artery

17:55 — **O-C11: A. Ziegelhöffer, M. Ferko, J. Mujkošová, M. Cagalinec, I. Waczulíková, D. Kincelová, T. Goliášová, S. Pastoreková, T. Ravingerová, B. Ziegelhöffer** *(Bratislava, Slovakia)*
Regulatory mechanisms in protection of cell energetics in hypoxic and diabetic myocardium: role of calcium and the mitochondrial signaling

GASTROENTEROLOGY

(Venue: Hotel Forrás)

13:25–15:45 – SESSION VII. Clinical Gastroenterology I.

Chairs: Zsolt Tulassay *(Budapest, Hungary)*, Ferenc Izbéki *(Szeged, Hungary)*

13:25 — **G23: WITTMANN, Tibor** *(Szeged, Hungary)*
Novel pathogenetical factors of irritable bowel syndrome (IBS)

13:50 — **G24: TULASSAY, Zsolt** *(Budapest, Hungary)*
From chronic inflammation to cancer

14:15 — **O-G9: T. Molnár, K. Farkas, F. Nagy, P. L. Lakatos, Z. Szepes, P. Miheller, G. Horváth, M. Papp, K. Palatka, T. Nyári, T. Wittmann** *(Szeged, Hungary)*
High restarting rate among patients with Crohn's disease after cessation of one-year treatment period with biologicals: result of national RASH study

14:30 — **O-G10: K. Toth, J. Beck, K. Buser, Z. Tulassay, R. Stöhr, H. Golcher, V. Schellerer, B. Molnar** *(Budapest, Hungary)*
Plasma methylated SEPT9 is a screening marker in both left and right– sided colon cancer. Comparison to FOBT and CEA results

14:45 — **O-G11: A. Rosztoczy, F. Izbeki, R. Roka, I. Nemeth, K. Gecse, K. Vadaszi, J. Kadar, E. Vetro, L. Tiszlavicz, T. Wittmann** *(Szeged, Hungary)*
The evaluation of oesophageal function in patients with different types of oesophageal metaplasia

15:00 — **O-G12: A. Kalmar, S. Spisak, O. Galamb, B. Wichmann, F. Sipos, K. Toth, K. Leiszter, B. Peterfia, G. Valcz, A. V. Patai, A. Scholler, B. Molnar, Zs. Tulassay** *(Budapest, Hungary)*
Methylation-related biomarker identification by gene expression analysis of laser microdissected colonic cells

15:15 — **O-G13: T. Várkonyi, É. Börcsök, R. Takács, C. Lengyel, M. Lázár, M. Papós, L. Pávics, P. Kempler, T. Wittmann** *(Szeged, Hungary)*
Determination of gastric emptying, the current glucose levels and neuropathy in patients with type 1 diabetes mellitus

15:30 — **O-G14: F. Izbéki, D. Asuzu, H. Yurio, M. Bardsley, T. Wittmann, T. Ördög** *(Szeged, Hungary)*
Gastrointestinal neuromuscular dysfunction in klotho mouse model of ageing

15:45–16:00 COFFEE BREAK

GASTROENTEROLOGY

(Venue: Hotel Forrás)

16:00–17:50 SESSION VIII. Clinical Gastroenterology II.

Chairs: Tibor Wittmann *(Szeged, Hungary)*, Ákos Pap *(Budapest, Hungary)*

16:00 **G25: ARATÓ, András** *(Budapest, Hungary)*
Innate and adaptive immunity in the pathogenesis of coeliac disease

16:25 **G26: LÁZÁR, György** *(Szeged, Hungary)*
The effects of laparoscopic nissen fundoplication on Barrett's esophagus: long-term results

16:50 **O-G15: K. Leiszter, O. Galamb, F. Sipos, T. Krenács, S. Spisák, G. Veres, B. Wichmann, Á. V. Patai, K. Tóth, G. Valcz, A. Kalmár, B. Molnár, Z. Tulassay** *(Budapest, Hungary)*
Decreased somatostatin production in colorectal cancer with uncontrolled cell proliferation, as compared to controlled cell growth in young and adult colonic mucosa

17:05 **O-G16: Z. Szepes, K. Farkas, T. Kiss, T. Nyári, F. Nagy, T. Wittmann, T. Molnár** *(Szeged, Hungary)*
Endoscopic activity at the time of diagnosis does not predict disease course in Crohn's disease, while endoscopic finding is worsened by smoking

17:20 **O-G17: V. Terzin, I. Földesi, L. Kovács, G. Pokorny, T. Wittmann, L. Czakó** *(Szeged, Hungary)*
Association between autoimmune pancreatitis and systemic autoimmune diseases

17:35 **O-G18: A. Schnúr, P. Hegyi, M. Sahin-Tóth** *(Boston, MA, USA)*
Rare cationic trypsinogen mutations found in patients with chronic pancreatitis are harmless variants

IMMUNOLOGY & INFLAMMATION

(Venue: Hotel Novotel)

13:50–15:45 – SESSION VII. Skin inflammation 1.

Chairs: Thomas Ruzicka *(Munich, Germany)*, Lajos Kemény *(Szeged, Hungary)*

13:50 — **I21: PRENS, Errol P.** *(Rotterdam, The Netherlands)*
Linking innate and adaptive immunity in psoriasis

14:15 — **I22: PIVARCSI, Andor** *(Stockholm, Sweden)*
MicroRNAs: novel regulators in psoriasis

14:40 — **I23: TSCHACHLER, Erwin** *(Vienna, Austria)*
New aspects of the skin nervous system

15:05 — **I24: RUZICKA, Thomas** *(Munich, Germany)*
Why some get psoriasis while others don't – understanding the Koebner Phenomenon

15:30 — **O-I12: L. Lakatos, E. Szabó, M. Manczinger, A. Göblös, L. Kemény** *(Budapest, Hungary)*
Silencing suppressor activity remodelled

15:45–16:00 **COFFEE BREAK**

16:00–17:45 – SESSION VIII. Skin inflammation 2.

Chairs: Andrea Szegedi *(Debrecen, Hungary)*, Sarolta Kárpáti *(Budapest, Hungary)*

16:00 — **I25: KÁRPÁTI, Sarolta** *(Budapest, Hungary)*
Transglutaminases in skin pathology

16:25 — **I26: SZEGEDI, Andrea** *(Debrecen, Hungary)*
T cells in atopic dermatitis

16:50 — **I27: BATA-CSÖRGŐ, Zsuzsanna** *(Szeged, Hungary)*
Psoriasis susceptibility factors

17:15 — **O-I13: K. V. Vukman, P. N. Adams, M. Metz, M. Maurer, S. M. O'Neill** *(Dublin, Ireland)*
Fasciola hepatica tegumental antigens suppress TH1-promoting mast cells

17:30 — **O-I14: N. Sándor, A. Erdei, Z. Bajtay** *(Budapest, Hungary)*
Cr3 is the main phagocytic receptor for IC3B opsonized particles on dendritic cells while Cr4 plays supporting role

MOLECULAR BIOLOGY & GENETICS

(Venue: IH Conference Centre)

Please NOTE that sessions VII.a and VII.b as well as sessions VIII.a and VIII.b will run in parallel!

13:40–15:50 SESSION VII.a – Messages from model organisms 1.

Chair: Utpal Banerjee *(Los Angeles, CA, USA)*

13:40 **M22: GÁSPÁR, Imre** *(Heidelberg, Germany)*
Microtubules, motors, mRNAs: the transport of oskar RNP within the Drosophila oocyte

14:05 **M23: BANERJEE, Utpal** *(Los Angeles, CA, USA)*
Signal transduction and metabolic control of cell fate

14:30 **M24: REUTER, Gunter** *(Halle, Germany)*
Conserved epigenetic mechanisms control gene silencing in fungi, animals and plants

14:55 **M25: DINNYÉS, András** *(Gödöllő, Hungary)*
Induced pluripotent stem cells to create 3D neuronal tissue models

15:20 **O-M10: K. Bakos, Z. Csenki, B. Kovács, R. Kovács, D. Kánainé Sipos, D. Bencsik, Y. Hadzhiev, F. Müller, B.Urbányi** *(Gödöllő, Hungary)*
Establishment of a liver transgenic zebrafish line for screening estrogenic compounds

15:35 **O-M11: B. Csorgo, T. Fehér, E. Tímár, F. R. Blattner, G. Pósfai** *(Szeged, Hungary)*
Low-mutation-rate, reduced-genome Escherichia coli: an improved host for faithful maintenance of engineered genetic constructs

13:40–15:50 SESSION VII.b – Rare diseases, clinical diagnostics 1.

Chairs: György Kosztolányi *(Pécs, Hungary)*, Béla Melegh *(Pécs, Hungary)*

13:40 **M30: SCHMIDTKE, Jörg** *(Hannover, Germany)*
A role for Europe in genetic service provision

14:05 **M31: CASSIMAN, Jean-Jacques** *(Leuven, Belgium)*
EuroGentest: the way forward to quality genetic services

14:30 **M32: KOSZTOLÁNYI, György** *(Pécs, Hungary)*
Time to take timing seriously in human genetics

14:55 **M33: MACEK, Milan** *(Prague, Czech Republic)*
Organization of care for genetic diseases in a diverse Europe

15:20 **O-M12: M. Marazita, A. Czeizel, F. Deleyainnis, J. Resick, M. Ford, C. Brandon, M. Cooper** *(Pittsburgh, PA, USA)*
Velopharyngeal insufficiency (VPI) in relatives of individuals with cleft lip/palate (CL/CP)

15:35 **O-M13: N. Szabó, D.J. Morris-Rosendahl, A. Mokánszki, É. Oláh, G. Gergev, S. Túri, L. Sztriha** *(Szeged, Hungary)*
Tubulin-related cerebral dysgenesis, novel paradigms for understanding the tubulin's role in the brain development

MOLECULAR BIOLOGY & GENETICS

(Venue: IH Conference Centre)

Please NOTE that sessions VII.a and VII.b as well as sessions VIII.a and VIII.b will run in parallel!

15:50 – 16:10 COFFEE BREAK

16:10–18:05 SESSION VIII.a – Messages from model organisms 2.

Chair: Eric Wieschaus *(Princeton, NJ, USA)*

16:10 — **M26: SCHÜPBACH, Gertrude** *(Princeton, NJ, USA)*
Control of EGF receptor activity and axis establishment in Drosophila oogenesis

16:35 — **M27: ERDÉLYI, Miklós** *(Szeged, Hungary)*
Functional analysis of the Drosophila embryonic germ cell transcriptome by RNAi

17:00 — **O-M14: P. Nagy, A. Varga, K. Pircs, G. Juhasz** *(Budapest, Hungary)*
An in vivo, whole-genome RNAi screen for genes involved in autophagy in drosophila

17:15 — **M28: SZABAD, János** *(Szeged, Hungary)*
Poking microtubules bring about nuclear wriggling to position nuclei

17:40 — **M29: OROSZ, László** *(Budapest, Hungary)*
Wonder deer, antler, osteoporosis

16:10–17:55 SESSION VIII.b – Rare diseases, clinical diagnostics 2.

Chairs: János Szabó *(Szeged, Hungary)*, Sándor Túri *(Szeged, Hungary)*

16:10 — **M34: TÚRI, Sándor** *(Szeged, Hungary)*
Rare inherited diseases and newborn screening in Szeged, Hungary

16:35 — **M35: MELEGH, Béla** *(Pécs, Hungary)*
Genetics of Romani people

17:00 — **M36: SZÉLL, Márta** *(Szeged, Hungary)*
Germline and somatic mutations in melanoma pathogenesis

17:25 — **O-M15: A. Gal, V. Remenyi, A. Racz D., A. Kekesi, B. Bereznai, G. Csabi, K. Komlosi, M.J. Molnar** *(Budapest, Hungary)*
Phenotype-genotype correlation in patients with nuclear and mitochondrial intergenomial communication disturbances

17:40 — **O-M16: R. Sepp, L. Losonczi, T. Tóth, V. Nagy, A. Orosz, K. Kádár, M. Hőgye, G. Fekete, M. Csanády, T. Forster** *(Szeged, Hungary)*
Screening for sarcomeric gene mutations in hungarian patients with hypertrophic cardiomyopathy

NEUROSCIENCE

(Venue: Hotel Forrás)

13:45–15:45 SESSION VII. – Neuroscience 7.

Chairs: József Hámori *(Budapest, Hungary)*, Timothy Crow *(Oxford, UK)*

13:45 — **N25: CROW, Timothy** *(Oxford, UK)*
Dezső Miskolczy & the speciation of modern Homo sapiens

14:10 — **N26: BORNSTEIN, Stefan R.** *(Dresden, Germany)*
The neuro-adrenal stress axis: Role of Vitamin C

14:35 — **N27: BÁNHEGYI, Gábor** *(Budapest, Hungary)*
Ascorbate compartmentation

15:00 — **O-N7: F. Walter, S. Veszelka, C. Ábrahám, G. Rákhely, A. Tóth, B. Ózsvári, L. Puskás, M. A. Deli** *(Szeged, Hungary)*
The effects of tesmilifene, a chemopotentiating agent, on brain endothelial cells

15:15 — **O-N9: I. Krizbai, C. Fazakas, P. Nagyoszi, J. Haskó, J. Molnár, G. Végh, F. Ayaydin, G. Váró, I. Wilhelm** *(Szeged, Hungary)*
Mechanisms of the interaction between melanoma cells and cerebral endothelial cells

15:30 — **O-N9: A. Tóth, L. Kiss, S. Veszelka, B. Ózsvári, L. G. Puskás, A. Tóth, G Rákhely, S. Dohgu, Y. Kataoka, M. A. Deli** *(Szeged, Hungary)*
Protection against methylglyoxal-induced toxicity in human brain endothelial cells

15:45 – 16:00 COFFEE BREAK

16:00–18:00 SESSION VIII. – Neuroscience 8.

Chairs: Ferenc Bari *(Szeged, Hungary)*, Jes Olesen *(Copenhagen, Denmark)*

16:00 — **N28: OLESEN, Jes** *(Glostrup, Denmark)*
Migraine from man to molecule

16:25 — **O-N4: Z. T. Kincses, N. Szabó, I. Valálik, Z. Kopniczky, L. Dézsi, P. Klivényi, M. Jenkinson, A. Király, M. Babos, E. Vörös, P. Barzó, L. Vécsei** *(Szeged, Hungary)*
Tractography guided target identification for thalamotomy

16:40 — **N29: BUSIJA, David W.** *(New Orleans, LA, USA)*
Mitochondrial mechanisms in the cerebral vasculature in health and disease

17:05 — **N30: BARI, Ferenc** *(Szeged, Hungary)*
Neurovascular coupling in the injured barin

17:30 — **O-N11: D. L. Clark, A. Institoris, E. Farkas, F. Bari** *(Szeged, Hungary)*
The impact of aging on focal cerebral ischemia-induced periinfarct depolarization with multimodal imaging in the rat brain

17:45 — **O-N12: F. Domoki, O. Oláh, V. Tóth-Szőki, F. Bari** *(Szeged, Hungary)*
Severe subacute neurovascular dysfunction is alleviated by hydrogen in asphyxiated newborn pigs

TUBERCULOSIS EVOLUTION *(Venue: Hotel Tisza)*

13:30–15:25 SESSION VII.
Biology of mycobacteria & its applications in TB evolution research

Chairs: Mamadou Daffe *(Toulouse, France)*, David E. Minnikin *(Birmingham, UK)*

13:30 — **T23: DAFFE, Mamadou** *(Toulouse, France)*
The cell envelope of tubercle bacilli

13:55 — **T24: MINNIKIN, David E.** *(Birmingham, UK)*
Ancient mycobacterial lipids: key reference biomarkers in charting the evolution of leprosy and tuberculosis

14:20 — **T25: SCHMIDT-SCHULTZ, Tyede** *(Göttingen, Germany)*
AG 85: a major secretion protein of *Mycobacterium tuberculosis* can be identified in ancient bone

14:45 — **T26: HERSHKOVITZ, Israel** *(Tel Aviv, Israel)*
Detection and molecular characterization of 9000-year-old *Mycobacterium tuberculosis* from a Neolithic settlement in the Eastern Mediterranean

15:10 — **O-T11: O.Y. Lee** *(Birmingham, UK)*
Lipid biomarkers provide evolutionary signposts for the oldest known cases of tuberculosis

15:25 — **O-T12: P. Suffys** *(Rio de Janeiro, Brazil)*
Characterization of *Mycobacterium tuberculosis* isolates from Brazil, Mozambique and Russia

15:40–15:55 COFFEE BREAK

15:55–18:10 SESSION VIII. – Different approaches to the study of 'paleotuberculosis'

Chairs: Michael Schultz *(Göttingen, Germany)*, Joël Blondiaux *(Walincourt-Selvigny, France)*

15:55 — **T27: SANTOS, Ana Luisa** *(Coimbra, Portugal)*
Sanatoria, archives and skeletons: an interdisciplinary approach to the study of paleotuberculosis

16:20 — **T28: SCHULTZ, Michael** *(Göttingen, Germany)*
Is it possible to diagnose TB in ancient bone using microscopy?

16:45 — **T29: CHHEM, Rethy K.** *(Ulm, Germany)*
Paleopathology and paleoradiology data as sources for the history of tuberculosis: The epistemic and histographical issues?

17:10 — **T30: DUTOUR, Olivier** *(Bordeaux, France)*
Contribution of 3D reconstructions to the paleopathology of tuberculosis

17:35 — **T31: BLONDIAUX, Joël** *(Wallincourt-Selvigny, France)*
Tuberculosis and survival in past populations: a paleo-epidemiological appraisal

18:05 — **Conclusions and closing remarks – Olivier Dutour, György Pálfi**

EVENING PROGRAMME:

20:00 **Gala dinner and closing remarks**
Piano concert and dinner *(Venue: TIK)* – For all participants

POSTER VIEWING I. FRIDAY 23 MARCH 11:50–12:30

CARDIOLOGY

Chairs: Danina Muntean *(Timisoara, Romania)*, Norbert Jost *(Szeged, Hungary)*

P-C1. **A. Adameova, T. Rajtik, S. Carnicka, A. Szobi, S. Jankyova, P. Svec, P. Krenek, T. Ravingerova** *(Bratislava, Slovakia)*
A role of CaMKIIδ in cardiac injury caused by ischemia and reperfusion

P-C2. **B. Balatonyi, V. Kovács, B. Gasz, J. Lantos, G. Jancsó, N. Marczin, E. Rőth** *(Pécs, Hungary)*
The effect of GST inhibition on cell viability and MAPK pathways on cultured cardiomyocytes in the process of ischaemic postconditioning

P-C3. **Á. Balogh, S. Vandenwijngaert, P. Pokreisz, S. Janssens, Z. Papp** *(Debrecen, Hungary)*
Myocardial phosphodiesterase 5 overexpression modulates cardiomyocyte contractility

P-C4. **V. Barsukevich, M. Basalay, A. Mrochek, A. V. Gourine, A. Gourine** *(Minsk, Belarus)*
Cardioprotective effect of delayed ischaemic postconditioning is mediated by mitochondrial KATP channels in the rat heart in vivo

P-C5. **M. Basalay, V. Barsukevich, S. Mastitskaya, A. Mrochek, J. Pernow, P. Sjöquist, G. L. Ackland, A. V. Gourine, A. Gourine** *(Minsk, Belarus)*
Remote ischaemic pre- and delayed postconditioning – similar degree of cardioprotection but distinct mechanisms

P-C6. **V. Csató, Á. Koller, A. Tóth, I. Édes, Z. Papp** *(Debrecen, Hungary)*
Different vascular effects of hydrogen peroxide in rat microvessels

P-C7. **O. Duicu, N. Mirica, D. Gheorgheosu, S. Trancota, O. Fira-Mladinescu, D. Muntean** (Timisoara, Romania)
Ageing associated decrease in cardiac mitochondria functions in healthy rats

P-C8. **A. S. Farkas, S. Orosz, T. Forster, A. Varró, A. Farkas** *(Szeged, Hungary)*
Proarrhythmia predictors in a reduced repolarisation reserve isolated rabbit heart model

P-C9. **M. Gönczi, M. Kovács, G. Seprényi, Á. Végh** *(Szeged, Hungary)*
Role of gap junctions in cardiac pacing – induced delayed antiarrhythmic protection

POSTER VIEWING I. FRIDAY 23 MARCH 11:50–12:30

P-C10. **A. Horvath, Z. Kohajda, A. Kristof, C. Corici, L. Virag, F. Fulop, A. Varro, N. Jost** *(Szeged, Hungary)*
R-L3 enantiomers have adverse modulating effects on IKs in rabbit ventricular myocytes

P-C11. **L. Juhász, V. Demeter-Haludka, G. Seprényi, J. Kaszaki, J. Gardi, Á. Végh** *(Szeged, Hungary)*
Acute inhibition of monoamine oxidases does not modify the severity of ischaemia and reperfusion-evoked ventricular arrhythmias in dogs

P-C12. **J. Kalász, Á. Balogh, E. Pásztor, M. Fagyas, S. Pahlavan, A. Tóth, I. Édes, Z. Papp, A. Borbély** *(Debrecen, Hungary)*
Cardiomyocyte contractile dysfunction in the human myocardium: the role of myofilament protein oxidation

P-C13. **G. Kisvári, M. Kovács, J. Kaszaki, Á. Végh** *(Szeged, Hungary)*
The effect of acute simvastatin administration on ventricular arrhythmias in a canine model of ischaemia and reperfusion

P-C14. **Z. Kohajda, A. Kristof, T. Szel, Z. Husti, I. Baczko, A. Varro, N. Jost, L. Virag** *(Szeged, Hungary)*
Transient outward potassium current in dog atrial preparations

P-C15. **Á. Kovács, Á. Balogh, Z. Papp, N. S. Dhalla, J. Barta** (Debrecen, Hungary)
Global and cellular alterations of myocardial contractility in a rat model of calcium paradox

P-C16. **M. Kovács, M. Gönczi, G. Seprényi, Á. Végh** *(Szeged, Hungary)*
Gene expression changes in the canine heart following rapid cardiac pacing

P-C17. **V. Ledvenyiova, D. Pancza, J. Matejikova, T. Ravingerova** (Bratislava, Slovakia)
Influence of maturation on resistance to ischemia-reperfusion injury in langendorff perfused female rat hearts

P-C18. **P. Major, L. Hiripi, N. Jost, A. Varró, V. Szűts, Z. Miklós, T. Ivanics, Z. Bősze** *(Gödöllő, Hungary)*
Transgenic mouse model of LQT5 syndrome

POSTER VIEWING I. FRIDAY 23 MARCH 11:50–12:30

P-C19. **N. Morvay, I. Leprán** *(Szeged, Hungary)*
Effects of a polyunsaturated fatty acid-rich diet on the development of heart failure after myocardial infarction in rats

P-C20. **N. Nagy, A. Kormos, Á. Szebeni, K. Acsai, J. G. Papp, A. Varró, A. Tóth** *(Szeged, Hungary)*
Partial NCX inhibition exerts protective role against Na^+ induced Ca^{2+} load by restricting $[Ca^{2+}]_i$ elevation in canine ventricular myocardium

P-C21. **J. Radosinska, V. Knezl, J. Slezak, N. Tribulova** *(Bratislava, Slovakia)*
Implication of electrical coupling protein, connexin-43, in termination of ventricular fibrillation and sinus rhythm restoration demonstrated in isolated perfused rat heart.

P-C22. **A. Sarusi, A. S. Farkas, S. Orosz, T. Forster, A. Varró, A. Farkas** *(Szeged, Hungary)*
Reduced repolarization reserve in langendorff perfused rabbit hearts: a new proarrhythmia model

P-C23. **S. L. Trancota, N. Mirica, O. Duicu, A. Anechitei, O. Fira Mladinescu, D. Muntean** *(Timisoara, Romania)*
Association of diazoxide and cyclosporine A elicit deleterious effects on mitochondrial function after prolonged global ischemia

P-C24. **N. Tribulova, J. Radosinska, B. Bacova, T. Benova1, V. Knezl, J. Slezak** *(Bratislava, Slovakia)*
Implication of myocardial connexin-43 and PKC signaling in antiarrhythmic effects of omega-3 fatty acids demonstrated in sponatenously hypertensive rats.

P-C25. **E. M. Végh, B. Sax, V. Kékesi, V. Wagner, T. Bárány, V. Kutyifa, G. Szücs, B. Merkely** *(Budapest, Hungary)*
Adrenomedullin, ghrelin and leptin as potential biomarkers of chronic heart failure: an experimental study

GASTROENTEROLOGY

Chairs: Barry Argent *(Newcastle, UK)*, Petra Pallagi *(Szeged, Hungary)*

P-G1. **A. Balázs, P. Hegyi, M. Sahin-Tóth** *(Szeged, Hungary)*
Functional characterization of the p.L104P human cationic trypsinogen variant

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P-G2. **A. Geisz, A. Szabó, P. Hegyi, Z. Rakonczay Jr., M. Széll, M. Sahin-Tóth** *(Boston, MA, USA)*
Activation of human chymotrypsinogen isoforms

P-G3. **A. Scholler, S. Spisak, O. Galamb, A. V. Patai, B. Wichmann, A. Kalmar, B. Molnar, Z.Tulassay** *(Budapest, Hungary)*
Detection of methylation profile changes of cfdna fractions in patients with colorectal cancer compaired to adenoma and healthy controls

P-G4. **D. Gheorgheosu, O. Duicu, S. Trancota, N. Nicoleta Mirica, C. Dehelean, D. Muntean** *(Timisoara, Romania)*
Liver mitochondrial respiratory function is decreased in senescent rats

P-G5. **L. Judák, P. Hegyi, Z. Rakonczay Jr., M. A. Gray, V. Venglovecz** *(Szeged, Hungary)*
Ethanol and its non-oxidative metabolites inhibit CFTR activity in guinea pig pancreatic duct cells

P-G6. **L. V. Kemény, P. Hegyi, Z. Rakonczay Jr., K. Borka, A. Korompay, M. A. Gray, B. E. Argent, V. Venglovecz** *(Szeged, Hungary)*
Substance P inhibits ductal bicarbonate secretion in guinea pig pancreatic ducts via neurokinin receptors 2 and 3

P-G7. **G. Kovács, G. Biczó, S. Dósa, N. Shalbuyeva, S. Berczi, Z. Hracskó, Z. Balla, B. Kui, A. Siska, Z. Kukor, V. Venglovecz, I. S. Varga, B. Iványi, T. Wittmann, A. Gukovskaya, T. Takács, P. Hegyi, Z. Rakonczay Jr.** *(Szeged, Hungary)*
The possible role of mitochondial injury in l-lysine-induced acute pancreatitis

IMMUNOLOGY & INFLAMMATION

Chairs: Tamás Bíró *(Debrecen, Hungary)*, József Kaszaki *(Szeged, Hungary)*

P-I1. **D. Erces, J. Kaszaki, M. Nogrady, I. Laszlo, E. Nagy, H. Okada, M. Boros** *(Szeged, Hungary)*
Reduced plasma big-endothelin level after complement C5A antagonist treatment accompanied by improved small intestinal microcirculation in experimental model of cardiac tamponade

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P-I2. **G. Erős, K. Szentner, P. Hartmann, I. Németh, L. Kemény, G. Szolnoky** *(Szeged, Hungary)*
The role of lymphangiogenesis in wound healing

P-I3. **O. Lang, E. Lajko, L. Polgar, J. Lang, L. Kohidai** *(Budapest, Hungary)*
Cell physiological applications of impedimetry with a special respect to cell adhesion and migration

P-I4. **M. Kürthy, D. Kovács, Z. Miklós, E. Ranczinger, J. Lantos, V. Kovács, G. Jancsó, E. Rőth** *(Pécs, Hungary)*
Effects of hypercholesterolemia induced inflammation and insulin resistance on postconditioned rats

P-I5. **H. Polyánka, K. Szabó, G. Tax, V. Tubak, R. L. Katona, E. Kusz, Z. Újfaludi, Á. Kinyó, I. Boros, Z. Bata-Csörgö, L. Kemény, M. Széll** *(Szeged, Hungary)*
Primary characterization of a novel immortalized cell line for studying keratinocyte innate immune functions

P-I6. **A. Szabo, K. Bene, R. E. Varga, A. Lanyi, B. Rethi, P. Gogolak, E. Rajnavolgyi** *(Debrecen, Hungary)*
Human $CD1a^+$ dendritic cells mediate efficient anti-viral immune responses via RIG-I and MDA5 signaling

P-I7. **A. Szabo, R. M. Osman, I. Bacskai, E. Rajnavolgyi** *(Debrecen, Hungary)*
Consecutive treatment of human melanoma cells by ATRA and polyi:C results in distinct inflammatory cytokine and chemokine responses via TLR3 and MDA5

P-I8. **T. Tőkés, G. Varga, E. Tuboly, L. Major, M. Ghyczy, J. Kaszaki, M. Boros** *(Szeged, Hungary)*
Anti-inflammatory effects of l-alpha glycerylphosphorylcholine treatment in a rat model of mesenteric ischaemia-reperfusion injury

P-I9. **G. Toldi, A. Bajnok, D. Dobi, A. Kaposi, L. Kovács, B. Vásárhelyi, A. Balog** *(Budapest, Hungary)*
The effects of kv1.3 and IKCA1 potassium channel inhibition on calcium influx of human peripheral T lymphocytes in rheumatoid arthritis

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P-I10. **E. Tuboly, G. Varga, T. Tőkés, J. Kaszaki, M. Ghyczy, M. Boros** *(Szeged, Hungary)*
The effects of exogenous methane inhalation on macro- and microcirculatory changes during intestinal ischemia/reperfusion in rats

P-I11. **G. Varga, T. Kovács, T. Tőkes, D. Érces, J. Kaszaki, M. Ghyczy, M. Boros** *(Szeged, Hungary)*
Dietary phosphatidylcholine protects against inflammatory activation in experimental colitis in the rat

P-I12. **K. Vas, B. Guban Konczne, A. Bebes, B. Kormos, N. Belso, R. Kui, M. Szell, L. Kemeny, Z. Bata-Csorgo** *(Szeged, Hungary)*
Alpha5-integrin and its ligand, the oncofetal fibronectin (EDA+FN) are differentially expressed in psoriatic uninvolved and healthy skin

MOLECULAR BIOLOGY & GENETICS

Chairs: Judit Oláh *(Szeged, Hungary)*, Ildikó Unk *(Szeged, Hungary)*

P-M1. **M. Barath, A. Szoor, E. Rajnavolgyi, M. Geiszt, G. Vereb, A. Lanyi** *(Debrecen, Hungary)*
Modulation of RAC1-dependent cellular functions by the SH3PX-domain adaptor HOFI/TKS4/SH3PXD2B

P-M2. **A. Bebes, I. Németh, T. Nagy, Z. Bata-Csörgö, L. Kemény, M. Széll** *(Szeged, Hungary)*
Overexpression of the ABCG2 protein in non-melanoma skin cancer could affect photodynamic therapy outcome

P-M3. **B. Bontovics, J. Slamecka, P. Maraghechi, L. Hiripi, A. V. Makarevich, P. Chrenek, Z. Bösze, E. Gócza** *(Gödöllő, Hungary)*
Expression pattern of pluripotency markers in rabbit epiblast and embryonic stem cells

P-M4. **G. Boros, D. Rózsa, E. Miko, E. Emri, G. Nagy, A. Juhász, I. Juhász, G. van der Horst, H. Muramatsu, D. Weissman, K. Karikó, I. Horkay, É. Remenyik, G. Emri** *(Debrecen, Hungary)*
Functional photolyase synthesis in cultured human keratinocytes induced by a novel mRNA-based gene therapy method

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P-M5. **K. Csepregi, A. Valasek, Á. Pénzes, Z. Tóth, Í. Kiss, I. Kerepesi, J. Hunyadkürti, B. Horváth, I. Nagy, C. Fekete** *(Pécs, Hungary)*
Structural and functional characterization of polyketide synthase gene clusters found in newly sequenced bacterial genome

P-M6. **J. Deák, V. Papp, C. H. Rama , J. Eluf-Neto** *(Szeged, Hungary)*
Differentiation between Hungarian and Brazil human papillomavirus types among female anogenital HPV infections

P-M7. **Z. Erdei, G. Vofély, T. I. Orbán, A. Péntek, K. Szebényi, A. Sebe, L. Rosivall, Á. Apáti, B. Sarkadi** *(Budapest, Hungary)*
Calcium signals in pluripotent stem cells

P-M8. **K. Farkas, N. Nagy, D. Beke, Á. Kinyó, L. Kemény, M. Széll** *(Szeged, Hungary)*
A newly identified missense mutation of the HR gene is possibly associated with a novel phenotype of marie unna hereditary hypotrichosis 1

P-M9. **A. Göblös, S. Bacsa, K. Szegedi, M. Antal, I. Németh, E. Sonkoly, A. Dobozy, Z. Bata-Csörgö, L. Kemény, M. Széll** *(Szeged, Hungary)*
Prins, the psoriasis susceptibility related non-coding RNA regulates the UV-B-induced intracellular shuttling of nucleophosmin

P-M10. **O. I. Hoffmann, L. Hiripi, L. Mátés, A. Kerekes, Z. Izsvák, Z. Ivics, Z. Bösze** *(Gödöllő, Hungary)*
Rabbit transgenesis with sleeping beauty transposon system

P-M11. **T. Juhász, C. Matta, J. Fodor, Á. Bartók, Z. Varga, P. Gergely, R. Zákány** *(Debrecen, Hungary)*
NMDA-type ionotropic glutamate receptors regulate commitment of chondrogenic cells

P-M12. **O. Kapuy, G. Bánhegyi** *(Budapest, Hungary)*
mTOR pathway-dependent autophagy due to NADPH/$NADP^+$ imbalance in endoplasmic reticulum

P-M13. **L. Képíró, A. Meszes, R. Gyulai, L. Kemény, M. Széll** *(Szeged, Hungary)*
Tnfsf15 single nucleotide polymorphisms and haplotypes in psoriasis and psoriatic arthritis

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P-M14. **M. Kósa, E. Zádor** *(Szeged, Hungary)*
Distribution of transfected fibres along the regenerating soleus muscle

P-M15. **G. Kudlik, B. Hegyi, B. Sági, G. Mészáros, É. Monostori, F. Uher** *(Budapest, Hungary)*
Phenotypic and functional switch of peritoneal macrophages induced by mesenchymal stem cells

P-M16. **P. I. Kulcsár, E. Tóth, E. Welker** *(Szeged, Hungary)*
Identification and characterization the functional NLS of Shadoo protein

TUBERCULOSIS EVOLUTION

Chairs: Maria Teschler-Nicola *(Vienna, Austria)*, Mark Spigelman *(London, UK)*

P-T1. **O. Baker, B. Chamel, R. Khawam, E. Coqueugniot, D. Helmer, L. Gourichon, F. Le Mort, A. Colombo, B. Dutailly, H. Coqueugniot, O. Dutour** *(Talence, France)*
Evidence of tuberculosis in ancient Syria dating from pre and early domestication

P-T2. **Zs. Baranyai, J. Vinšová, N. Szabó, S. Bősze** *(Budapest, Hungary)*
In vitro antimycobacterial activity of substituted salicylanilides against *Mycobacterium tuberculosis* H37RV and multidrug-resistant A8 cultures

P-T3. **A. Béleczki, K. Szalontai, K. Ugocsai, A. Somfay** *(Szeged, Hungary)*
Endobronchial tuberculosis in patients with active disease

P-T4. **K. Holloway, A. Bouwman, K. Link, M. Henneberg, F. Rühli** *(Zürich, Switzerland)*
Changes in the disease profile of tuberculosis during the introduction of antibiotics – a study of 20th century swiss pathological skeletal samples

P-T5. **A. Buzhilova, N. Berezina** *(Moscow, Russia)*
Spina ventosa: two cases of osteo-articular tuberculosis of children from Königsberg, Prussia

P-T6. **A. Colombo, H. Coqueugniot, C. Saint-Pierre, S. Naji, O. Dutour** *(Talence, France)*
Possible association between Langerhans Cell Histiocytosis and Tuberculosis, in a medieval child from the archaeological site of La Granède (Millau, France)

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P-T7. **H. Coqueugniot, O. Dutour, P. Desbarats, B. Dutailly, K. Karlinger, B. Kovács, A. Palkó, E. Riedl, I. Szikossy, I. Pap, G. Pálfi** *(Talence, France)*
From virtuality to reality: 3D reconstructions of tuberculosis processes using VIRCOPAL® chain

P-T8. **S. Évinger, Zs. Bernert, T. Hajdu, A. Marcsik, K. K. Kiss, K. Köhler** *(Budapest, Hungary)*
A new spinal tuberculosis case from the Árpád period (11th–13th centuries AD) from Zalavár, western Hungary

P-T9. **M. Faerman, L. Zamstein, C. L. Greenblatt, P. Smith** *(Jerusalem, Israel)*
Growth and disease patterns in infants and children from the Ottoman Dor, northern Israel

P-T10. **W. Frigui, A. Pawlik, D. Bottai, S. Mangenot, L. Fiette, M. Orgeur, R. Siméone, V. Barbe, C. Medigue, R. Brosch** *(Paris, France)*
Variable host susceptibility to infection with *Mycobacterium tuberculosis* linked to the genotype of strain.

P-T11. **M. K. Gomgnimbou, E. Abadia, J. Zhang, G. Refrégier, S. Panaiotov, E. Bachivska, C. Sola** *(Orsay, France)*
Spoligoriftyping – A new DPO-based direct-hybridization assay for effective TB control on a multianalyte microbead-based hybridization system

P-T12. **R. Hirmondó, I. Pécsi, A. Lopata, A. Brown C., T. Parish, B. Vértessy, J. Tóth** *(Budapest, Hungary)*
The mycobacterial dUTPase: biochemistry, physiology and molecular intervention

P-T13. **K. Köhler, I. Zalai-Gaál, A. Osztás, E. Bánffy, K. Kirinó, K. K. Kiss, G. Pálfi, B. G. Mende** *(Budapest, Hungary)*
Skeletal tuberculosis in a Late Neolithic series from Hungary

P-T14. **Á. Lehőcz, K. Szalontai, K. Ugocsai, A. Hajnal, A. Somfay** *(Szeged, Hungary)*
Evaluation of interferon-gamma release assay for the detection of active *Mycobacterium Tuberculosis* infection

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P-T15. **S. Lösch, M. Kim, O. Dutour, P. Courtaud, T. Romon, C. Sola, A. Zink** *(Talence, France)*
Evidence of *Mycobacterium tuberculosis* at 18th/19th century slaves in Anse Sainte-Marguerite (Guadeloupe)

P-T16. **C. Mitterer, G. Cipollini, A. Graefen, D. Piombino-Mascali, A. Zink, F. Maixner** *(Bolzano, Italy)*
A novel polymer-based DNA purification method supports removal of co-purified PCR-inhibitors from ancient tissue extracts

P-T17. **T. Hajdu, E. Fóthi, I. Kővári, M. Merczi, A. Marcsik, L. Márk** *(Szeged, Hungary)*
Bone tuberculosis in the Roman period Pannonia (western part of Hungary) – case report

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CARDIOLOGY

Chairs: István Baczkó *(Szeged, Hungary)*, Tatiana Ravingerova *(Bratislava, Slovakia)*

P-C26. **Z. Bagyura, Z. Szelid, P. Soós, O. Szenczi, Z. Andrási, L. Kiss, P. Józan, B. Merkely** *(Budapest, Hungary)*
Role of a primary prevention cohort based screening in the adherence of the cardiovascular target-values

P-C27. **P. Bencsik, K. Kupai, V. Sasi, T. Csont, I. Ungi, P. Ferdinandy** *(Szeged, Hungary)*
Matrix metalloproteinases and nitrosative stress in patients with coronary artery disease

P-C28. **L. Gellér, S. Szilágyi, E. Zima, T. Tahin, V. Kutyifa, E. Végh, H. Vágó, I. Osztheimer, G. Széplaki, B. Merkely** *(Budapest, Hungary)*
Coronary sinus side branch stenting a new tool for left ventricular lead fixation

P-C29. **Z. Kahán, F. Rárosi, A. Cserháti, Z. Együd, Z. Varga** *(Szeged, Hungary)*
Individual positioning for maximum heart protection during breast radiotherapy: the development of a practical tool based on a complex model

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P-C30. **V. Kovács, B. Balatonyi, B. Borsiczky, B. Gasz, J. Lantos, G. Jancsó, N. Marczin, E. Rőth** *(Pécs, Hungary)*
Role of glutathione S-transferase p1 gene polymorphism in patients underwent cardiac surgery

P-C31. **P. Maurovich-Horvat, H. Alkadhi, C. Schlett, M. Kriegel, M. Nakano, F. Isuka, P. Stolzmann, H. Scheffel, M. Ferencik, R. Virmani, B. Merkely, U. Hoffmann** *(Budapest, Hungary)*
Vulnerable plaque detection with coronary CT angiography: the napkin-ring sign

P-C32. **S. N. Mirica, O. M. Duicu, A. Anechitei, O. Fira-Mladinescu, M. D. Muntean** *(Timisoara, Romania)*
Cardioprotection by magnesium orotate and orotic acid at reperfusion: comparable effects on functional recovery but not on infarct size

P-C33. **T. Radovits, G. Veres, B. Németh, R. Tóth, L. Hidi, T. Németh, I. Hartyánszky, B. Merkely, G. Szabó** *(Budapest, Hungary)*
Succesful heart transplantation after 12 hours of ischemic donor organ storage with the new organ preservation solution custodiol-n

P-C34. **G. Széplaki, Z. Bagyura, H. Vágó, A. Tóth, B. Sax, E. Édes, S. Walentin, G. Füst, Z. Prohászka, B. Merkely** *(Budapest, Hungary)*
Serum complement C3 levels are associated with ventricular volumes and mass in top athletes

P-C35. **K. Úri, M. Fagyas, A. Kertész, Z. Csanádi, M. Clemens, G. Sándorfi, I. Ményiné Siket, Z. Papp, A. Tóth, I. Édes, E. Lizanecz** *(Debrecen, Hungary)*
Changes in ACE2 enzyme activity in systolic heart failure

GASTROENTEROLOGY

Chairs: Mark Donowitz *(Baltimore, MD, USA)*, József Maléth *(Szeged, Hungary)*

P-G8. **G. Valcz, F. Sipos, T. Krenács, A. Kalmár, A. V Patai, K. Leiszter, K. Tóth, B. Wichmann, B. Molnár, Z. Tulassay** *(Budapest, Hungary)*
The increasing appearance of epithelial-to-myofibroblast transition in line with transforming growth factor beta II receptor and toll-like receptor 9 protein expression during colorectal carcinogenesis

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P-G9. **W. Xia, Y. Qin, B. Riederer, A. K. Singh, R. Engelhardt, P. Song, D. Tian, M. Soleimani, U. Seidler** *(Hannover, Germany)*
The anion transporter slc26a6 (putative anion transporter-1) regulates CO_2/HCO_3^- induced murine small intestinal fluid absorption

P-G10. **K. Farkas, Z. Rakonczay Jr., F. Nagy, T. Molnár, Z. Szepes, L. Varga, T. Takács, T. Wittmann, A. Schnúr, V. Venglovecz, Y. Sunil, J. Hubricht, B. Riederer, M. Király, Á. Zsembery, G. Varga, U. Seidler, P. Hegyi** *(Szeged, Hungary)*
The role of ion transporters in the pathogenesis of ulcerative colitis

P-G11. **L. Kiss, F. Walter, A. Bocsik, S. Veszelka, B. Ózsvári, L. Puskás, A. Szüts, P. Révész, M. Deli** *(Szeged, Hungary)*
Toxicity and absorption enhancer profile of surfactants on a human intestinal barrier model

P-G12. **L. Kotsis, M. Póczi** *(Salgótarján, Hungary)*
Original esophageal surgical procedures

P-G13. **D. Laczkó, A. Rosztóczy, P. Hegyi, Z. Rakonczay Jr., T. Wittmann, M. Katona, V. Venglovecz** *(Szeged, Hungary)*
Functional characterization of human oesophageal epithelial cells

IMMUNOLOGY & INFLAMMATION

Chairs: Rolland Gyulai *(Szeged, Hungary)*, Kornelia Szabó *(Szeged, Hungary)*

P-I13. **A. Marton, C. Vizler, E. Kusz, Z. Szathmary, K. Nagy, Z. Szegletes, G. Varo, L. Siklos, R. Katona, Z. Howard, E. Duda, J. Minarovits, K. Buzas** *(Szeged, Hungary)*
Melanoma cell-derived exosomes alter macrophage and dendritic cell functions in vitro

P-I14. **K. Szabó, E. Tóth-Molnár, K. Balogh, H. Polyánka, H. Orvos, H. Ócsai, L. Kemény, M. Széll, J. Oláh** *(Szeged, Hungary)*
Neonatal blue light phototherapy and melanocytic nevi: a twin study

P-I15. **R. Gyulai, F. Kovács-Sólyom, J. Prihoda, L. Kemény** *(Szeged, Hungary)*
Interleukin-1 receptors are differentially expressed in normal and psoriatic T lymphocytes

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P-I16. **Á. Kinyó, S. Hambalkó, A. Bebes, Z. Kiss-László, Z. Bata-Csörgő, M. Széll, F. Nagy, L. Kemény** *(Szeged, Hungary)*
Cop1, a p53 interacting protein, is strongly expressed by proliferating keratinocytes, its expression decreases as cells differentiate and after UVB irradiation

P-I17. **G. Nagy, K. Gáspár, D. Minh, Z. Bacsó, G. Mócsai, D. Törcsik, E. Gyimesi, T. Bíró, É. Remenyik, A. Szegedi** *(Debrecen, Hungary)*
The role of myeloid dendritic cells in the polarization of effector T cells in atopic dermatitis

P-I18. **I. B. Nemeth, T. Krenacs, G. Kiszner, M. Kurunczi, E. Varga, A. Kinyo, Z. Bata-Csorgo, I. Korom, J. Olah, M. Szell, F. Nagy, L. Kemeny** *(Szeged, Hungary)*
Expression of human constitutive photomorphogenic protein-1 (COP1) in melanocytic and non- melanocytic tumours. An immunohistochemical study.

P-I19. **M. Resch, L. Marsovszky, E. Medgyessi, L. Kovács, J. Németh, A. Balog** *(Budapest, Hungary)*
In vivo examination of corneal langerhans cells in systemic lupus erythematosus (SLE) with confocal microscopy

P-I20. **H. Szabados, K. Uray, P. Silló, F. Hudecz, S. Kárpáti, S. Bösze** *(Budapest, Hungary)*
Determination of T-cell epitope regions of protein desmoglein 3 using synthetic oligopeptides: design, synthesis and in vitro activity

P-I21. **K. Szabó, G. Tax, E. Urbán, L. Kemény** *(Szeged, Hungary)*
The role of keratinocyte activation in acne pathogenesis

P-I22. **P. Szeremy, I. Makai, M. Jani, L. Marton, S. Gedey, K. Jakab, P. Krajcsi, J. Marki-Zay** *(Szeged, Hungary)*
Investigation of transporter interactions of antimalarials *in vitro*

P-I23. **G. Tax, K. Szabó, E. Urbán, L. Kemény** *(Szeged, Hungary)*
Real-time monitoring of the interaction of keratinocytes and propionibacterium acnes bacterium

P-I24. **S. Dalmády, F.A. Kovács, M. Kiss, L. Kovács, R. Gyulai, L. Kemény** *(Szeged, Hungary)*
The occurence of autoantibodies targeting mutated citrullinated vimentin in patients with psoriatic arthritis and psoriasis

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MOLECULAR BIOLOGY & GENETICS

Chairs: Judit Deák *(Szeged, Hungary)*, Árpád Lányi *(Debrecen, Hungary)*

P-M17. **C. Matta, J. Fodor, R. Takács, Á. Papp, T. Oláh, L. Csernoch, P. Gergely, R. Zákány** *(Debrecen, Hungary)*
Investigation of high-frequency Ca^{2+} oscillations in chondrifying cell cultures

P-M18. **A. I. Nagy, A. E. Herlihy, R. Gatsi, R. P. Vazquez-Manrique, B. Merkely, H. A. Baylis** *(Budapest, Hungary)*
Manipulating the rnai response by suppressing IP_3 signalling in Caenorrhabditis Elegans

P-M19. **N. Nagy, K. Farkas, Á. Kinyó, A. Meszes, K. Szentner, L. Kemény, M. Széll** *(Szeged, Hungary)*
The rs3185480 polymorphism of the adenomatosis polyposis coli down-regulated 1 (APCDD1) gene is associated with androgenic alopecia

P-M20. **J. Oláh, K. Balogh, E. Nemes, G. Uhercsák, Z. Kahán, G. Lázár, G. Farkas, H. Polyánka, E. Kiss, R. Gyulai, E. Varga, I. Korom, E. Keresztné Határvölgyi, L. Kaizer, L. Haracska, L. Tiszlavicz, L. Kemény, M. Széll** *(Szeged, Hungary)*
Identification of the R24P melanomapredisposing CDKN2A mutation in a patient with multiple primary malignancies

P-M21. **A. Péntek, K. Szebényi, Z. Erdei, G. Vofély, T. I. Orbán, B. Sarkadi, Á. Apáti** *(Budapest, Hungary)*
Directed differentiation of human embryonic stem cells into cardiomyocytes

P-M22. **B. Sági, P. Maraghechi, V. S. Urbán, B. Hegyi, R. Fajka-Boja, G. Kudlik, É. Monostori, E. Gócza, F. Uher** *(Budapest, Hungary)*
Comprehensive analysis of mouse mesenchymal stromal cells derived from various tissues and organs

P-M23. **G. Somlyai, A. Kovács, I. Guller, Z. Gyöngyi, K. Krempels, I. Somlyai** *(Pécs, Hungary)*
Deuterium has a key role in tumour development – a new submolecular regulatory system

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P-M24. **J. E. Szabó, G. Merényi, B. Vértessy, J. Tóth** *(Budapest, Hungary)*
Functional adaptation and allosteric regulation of the dUTPase superfamily

P-M25. **Á. Szepesi, Z. Matula, A. Szigeti, K. Német, P. Tátrai** *(Budapest, Hungary)*
An in vitro model for the study of enhanced cyclic AMP signaling in mesenchymal stem cells and their differentiated derivatives

P-M26. **P. Tátrai, Á. Szepesi, Z. Matula, A. Szigeti, F. Uher, K. Német** *(Budapest, Hungary)*
Immortalization of human adipose tissue derived stromal cells with human telomerase reverse transcriptase, BMI-1, and SV40 large T antigen

P-M27. **S. Tóth, A. Füredi, D. Türk, A. Síkhegyi, E. Kanta, G. Szakács** *(Budapest, Hungary)*
Screening and testing compounds killing selectively multidrug resistant cancer cells

P-M28. **Z. Tóth, Á. Pénzes, J. Pongrácz, J. Hunyadkürti, A. Valasek, B. Horváth, I. Nagy, C. F. Fekete** *(Pécs, Hungary)*
Whole transcriptome profiling of mono- and co-cultured two- and three dimensional in vitro liver models

P-M29. **S. Z.Tóth, V. Nagy, R. Tengölics, G. Schansker, G. Rákhely, K. L. Kovács, G. Garab** *(Szeged, Hungary)*
Novel role of ascorbatein the photosynthetic electrontransport. Physiological significance and potential biotechnological application

P-M30. **A. Valasek, K. Csepregi, Z. Tóth, I. Kerepesi, B. Frey, Á. Pénzes, Á. Juhász, B. Horváth, I. Nagy, C. Fekete** *(Pécs, Hungary)*
In silico analysis of thiotemplate multidomain gene clusters in Saccharomonospora Azurea

P-M31. **E. Varga, M. Kiss, K. Szabó, L. Kemény** *(Szeged, Hungary)*
Merkel cell carcinoma and merkel cell polyomavirus: a hungarian experience

P-M32. **I. Vida, A. Borsy, E. Tóth, E. Welker** *(Szeged, Hungary)*
Examination of nucleic acid binding of the newest prion protein, Shadoo, using agarose gel shift assay

P-M33. **B. Vodicska, A. Nyeste, A. Borsy, E.Tóth, E. Welker** *(Budapest, Hungary)*
Examination of the effect of a downstream translation initiation site on the localization of proteins using the secretory pathway

POSTER VIEWING II. SATURDAY 24 MARCH 11:50–12:30

NEUROSCIENCE

Chairs: Péter Klivényi *(Szeged, Hungary)*, Ferenc Bari *(Szeged, Hungary)*

P-N1. **B. Barkóczi, G. Juhász, Z. Bozsó, B. Penke, V. Szegedi** *(Szeged, Hungary)*
Abeta$_{1-42}$ impairs theta coupled firing of CA1 cells *in vivo*

P-N2. **P. G. Bencsura, A. Nyeste, E. Welker** *(Budapest, Hungary)*
The consequence of PRPC or Shadoo overexpression on the cytotoxic effect of the PRPC DCR mutant phenotype

P-N3. **A. Bocsik, A. N. M. Kablan, S. Veszelka, M. Pásztói, L. Vigh, B. Csiszár, F. Walter, L. Siklós, E. Búzás, A. Falus, D. Virgintino, M. A. Deli** *(Szeged, Hungary)*
Injury-induced glial cell reactions in histamine deficient (HDC-KO) mice

P-N4. **E. Borbély, J. Horváth, Á. Kasza, Zs. Frank, S. Furdan, G. Főr, T. Szögi, K. Németh, L. Fülöp, Z. Bozsó, Z. Penke, B. Penke** *(Szeged, Hungary)*
Intracerebroventricular administration of the syntethic AB$_{1-42}$ to the rat brain. Connection of spatial memory and spine density.

P-N5. **J. Horváth, T. Szögi, G. Müller, B. Penke, V. Szegedi** *(Szeged, Hungary)*
Different coping strategy of mice having high- or- low-anxiety related behavior

P-N6. **Z. Máté, S. Takács, E. Horváth, A. Szabó, A. Papp** *(Szeged, Hungary)*
Animal experiments on the functional neurotoxicity of metal nanoparticles

P-N7. **K. Pesti, A. Szabo, A. Mike** *(Budapest, Hungary)*
A fast method for assessing the type of sodium channel inhibitors

P-N8. **J. Samardzic, R. Matunovic, D. Obradovic** *(Belgrade, Serbia)*
Memory effects of benzodiazepine-site inverse agonists: are they potential cognition enhancers?

P-N9. **E. Varga, G. Juhász , Z. Bozsó, B. Penke, V. Szegedi** *(Szeged, Hungary)*
How Abeta$_{1-42}$ disrupts synaptic plasticity: effects on ltp and spiking activity in hippocampal slices

P-N10. **S. Veszelka, Z. Datki, A. Toth, F. Walter, E. Mózes, L. Fülöp, Z. Bozsó, B. Penke, M. A. Deli** *(Szeged, Hungary)*
Docosahexaenoic acid reduces beta-amyloid induces toxicity in cells of the neurovascular unit

POSTER VIEWING II. SATURDAY 24 MARCH 11:50–12:30

TUBERCULOSIS EVOLUTION

Chairs: Israel Hershkovitz *(Tel Aviv, Israel)*, Christophe Sola *(Paris, France)*

P-T18. **V. M. Matos, A. L. Santos** *(Coimbra, Portugal)*
Living and dying with tuberculosis as revealed by the archives of the Portuguese sanatorium Carlos Vasconcelos Porto (1918–1991)

P-T19. **E. Molnár, Z. Bereczki, G. Pálfi, A. Marcsik** *(Szeged, Hungary)*
Infants with atypical skeletal tuberculosis from the 8–9th century of Hungary

P-T20. **R. Müller, T. Brown, C. A. Roberts** *(Manchester, UK)*
Tuberculosis across Europe – an ancient DNA study

P-T21. **E. Neparáczki, A. Pósa, T. Török, G. Lovász, Z. Bereczki, E. Molnár, F. Maixner, A. Zink, G. Pálfi** *(Szeged, Hungary)*
Preliminary results from the paleomicrobiological studies of a Hungarian anthropological series

P-T22. **L. Paja, H. Coqueugniot, A. Palkó, G. Farkas L., Z. Bereczki, J. Gervain, O. Dutour, G. Pálfi** *(Szeged, Hungary)*
Tuberculosis as probable etiology of two knee ankyloses from medieval Hungary. Contribution of medical imaging and 3D reconstruction

P-T23. **G. Pálfi, Zs. Bereczki, D. J. Ortner, O. Dutour** *(Szeged, Hungary)*
Juvenile cases of skeletal TB from the Terry Anatomical Collection (Smithsonian Institution, Washington DC, USA)

P-T24. **G. Pálfi, E. Molnár, A. Marcsik, Zs. Bereczki, I. Pap, E. Fóthi, Á. Kustár, B. G. Mende, D. E. Minnikin, O. Y. Lee, G. S. Besra, M. Spigelman, J. O'Grady, H. D. Donoghue** *(Szeged, Hungary)*
Mycobacterium tuberculosis – Mycobacterium leprae coinfections from Hungary: osteological and biomolecular findings

P-T25. **A. Pósa, G. Lovász, Zs. Bereczki, E. Molnár, F. Maixner, A. Zink, O. Dutour, J. Gervain, É. Hunyadi-Gulyás, H. Dürgő, G. Pálfi** *(Szeged, Hungary)*
Tuberculosis infection in a late-medieval Hungarian population

POSTER VIEWING II. SATURDAY 24 MARCH 11:50–12:30

P-T26. **J. Pató, R. Székely, L. Őrfi, S. Magnet, R. C. Hartkoorn, S. T. Cole, G. Kéri** *(Budapest, Hungary)*
Nitroquinoxalines: new potential anti-TB compounds

P-T27. **G. Refrégier, C. Borile, S. Franz, M. Labarre, C. Sola** *(Orsay, France)*
TB lineages: do we have the tools to identify pathogenic specificities?

P-T28. **M-C. Bernard and C. A. Roberts** *(Durham, UK)*
Tuberculosis: a demographic analysis and social study of admissions to a children's sanatorium (1936–1954) in Stannington, Northumberland

P-T29. **R. Siméone, L. Majlessi, F. Sayes, N. Honoré, A. Pawlik, W. Frigui, M. Nilges, C. Leclerc, R. Brosch** *(Paris, France)*
New insights into the complex formation of ESAT-6 and CFP-10 of *M. tuberculosis*

P-T30. **O. Spekker, G. Kozocsay, G. Pálfi, E. Molnár** *(Szeged, Hungary)*
Probable cases of skeletal tuberculosis from the Neolithic period of Hungary – a morphological study

P-T31. **R. Székely, G. Németh, N. Breza, C. Szántai-Kis, J. Pató, S. Magnet, R. C. Hartkoorn, A. Cascioferro, S. T. Cole, R. Brosch, L. Őrfi, G. Kéri** *(Budapest, Hungary)*
Drug development against *Mycobacterium tuberculosis* PknB, PknG and PknA kinases

P-T32. **I. Szikossy, I. Pap, Á. Kustár, G. Pálfi, E. Molnár, K. Karlinger, B. Kovács, M. Spigelman, H. D. Donoghue** *(Budapest, Hungary)*
Two positive TB cases in the late Nigrovits family, 18th century, Vác, Hungary

P-T33. **I. Szikossy, G. Pálfi, L. A. Kristóf, I. Horányi, K. Karlinger, B. Kovács, E. Riedl, M. Spigelman, H. D. Donoghue, O. Dutour, H. Coqueugniot, I. Pap** *(Budapest, Hungary)*
The nun without a heart – A TB case from the 18th century, Vác, Hungary

P-T34. **G. Terhes, B. Kele, A. Somfay, J. Deák** *(Szeged, Hungary)*
Molecular detection of *Mycobacterum tuberculosis* infection in patients treated in university hospitals of Szeged

The **Szeged Folkdance Ensemble and the Duna Art Ensemble** will perform together with the best Hungarian violin players **Ferenc Radics and István Pál** (Thursday), the internationally known Hungarian organ player **Xaver Varnus** (Saturday) and one of Hungary's most appreciated and popular piano players **Csilla Szentpéteri** (Sunday) will follow each other. We ensure everyone that the performances during the social events will be as high level as the presentations during the scientific programme.

On behalf of the organizing committee,

Péter Hegyi,
General Secretary of the Conference

1. Folkdance Evening

delivered by the Szeged Folkdance Ensemble, the Duna Art Ensemble and the Hungarian State Folk Orchestra lead by Ferenc Radics and István Pál.

National Theatre, Szeged, 22nd March, 2012

Dancing provided the primary source of recreation for Hungarian people before the times of electronic entertainment. The captivating motions accompanied by music and/or singing is a spectacular visualization of the Hungarian people's rich emotional world which they strive to preserve in spite of the unstoppable spread of modern lifestyle. Singing folk songs and dancing enlivened not only people's holidays but it also brought some cheerfulness in their workdays. Dancing was also an integral part of a wide range of social occasions. It provided an effective forum for group and couple interaction including courtship.

Nowadays, many young Hungarian people go to folk dance houses to keep folk dance traditions in Hungary. Numerous folk dance schools have been founded, folk dance lessons became part of school lectures, and the Hungarian Dance Academy launched a folk dance course. Today many civil associations, festivals and countless folk ensembles (both professional and amateur) cultivate folk dance traditions in Hungary. During the opening ceremony you can enjoy a composition of Hungarian Folk Dance with one of the top Hungarian Folkdance Ensembles The Szeged Folkdance Ensemble was founded over fifty years ago and has been giving performances all over the world. One of the highlights of the evening is that **Ferenc Radics**, **and István Pál** the best Hungarian violin players (both artists have received the **Cross of Merit of the Republic of Hungary** from the president of Hungary), will entertain you!

2. Organ Concert

delivered by the internationally highly ranked Hungarian organ player Xavér Varnus

New Synagogue, Szeged, 24th March, 2012

Xavér Varnus is an internationally renowned organist who has built up a world-wide reputation as an innovative musician and dazzling performer. He was born in 1964. After his Hungarian music education he became a student of Pierre Cochereau in Paris and Lorenz Stolzenbach in Leipzig. Since 1977 he has had more than 3000 concerts. His concerts are always performed to full house. His performances are known for their musicality, virtuosity and ability to excite and engage audiences of all ages. He is also famous for his art of improvisation. He received the **Officer's Cross Order of Merit of the Republic of Hungary**.

The concert will be held in the New Synagogue. The New Synagogue is the second biggest Synagogue of Hungary. The most beautiful part of the synagogue is the interior of the dome, which symbolizes the world. According to the teachings of Jewish religion, morality is determined by three factors: work, culture, good deeds. In biblical language this can be expressed by four wonds, which are painted in Hebrew on the gussets above the colums holding the dome. The 24 colums of the drum of the cupola represent the 24 hours of a day, above it the briar - bush flowers on a blue background symbolize faith. Above the greenish-brown ornamentation representing vegetation, the experience of infinite space manifests itself in the gradually darkening star-strewn blue glass dome. In the middle is the Star of David (Magen David),

around it the sun's rays, which can be illuminated, crown the firmament. The dome and all the lead glasses were created by Miksa Róth.

3. Piano Concert / Gala Dinner

delivered by the popular Hungarian piano player Csilla Szentpéteri

TIK, Concert Hall, 25th March, 2012

Genre: Csilla Szentpéteri

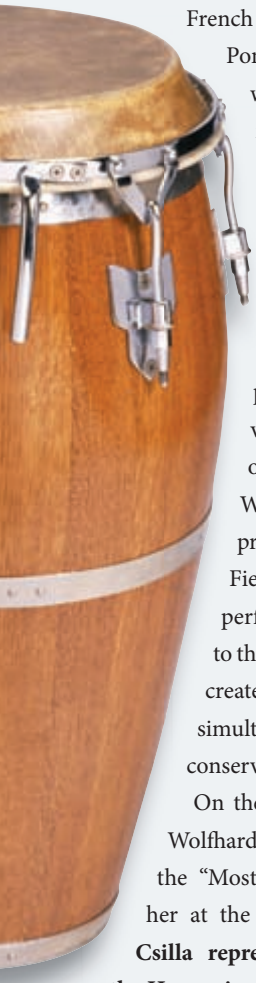
That is to say another way of playing the piano.

Concert of Csilla Szentpéteri and the Latin Trio

At the turn of the Millennium, Csilla Szentpéteri, accomplished pianist, conceived a brave idea and dash into revolutionizing classical music rules otherwise carved in stone. A creator has emerged from beneath the interpreter, who handles original classical work as a co-composer. Pending upon her feelings and mood, sometimes she continues a melody thread, sometimes cuts it, sometimes changes and sometimes amalgamates those with contemporary genres, always into something new. She bravely draws on rock, Latin, and jazz phraseology, adding stirring rhythms and passionate guitar solos to her piano playing and hence re-formulating centuries-old musical essence into a peculiar, and unique expression of world-music.

Csilla's temperament and virtuosity is best demonstrated by "Spiritus", her new record, where she follows her own spirit and freely ventures back and forth between past and present. The repertoire of Csilla Szentpéteri invites us to an exciting wandering across the world of music.

The famous Monti czardas as an Argentinean Tango is a real delicacy, not to mention Beethoven's well-known symphony presented as an alloy with assorted rock colours, the melody of Piazzolla inseminated by



French flavours, and the presentation of Ponchielli's Dance of Hours filled with spiritus. Paganini's fiery melody intoned as a deviously pulsating salsa, while the whirligig melody by Albeniz is delivered in a tempestuous flamenco rhythm.

Via her own compositions, Csilla guides us to a variety of peculiar realms. We can easily visualize a Cuban night-club or an exciting flashback of a Western, we can fully escape the present through a frisky Spanish Fiesta, and even experience the perfect state of mind while listening to the piece titled All Inclusive. Csilla creates something new, something simultaneously modern and artistic, conservative and progressive.

On the 1st of January in 1997, Baron Wolfhard von Boeselager presented with the "Most Talented Young Artist" prize her at the Academy of Music. **In 2004, Csilla represented Hungary along with the Hungarian Prime Minister in Brussels to celebrate Hungary joining the European Union.**

Contributors:

János Kormos guitars
Balázs Szendőfi bass
József Czibere percussion

www.szentpetericsilla.hu

OPEN FORUM

Venue: ***TIK, Concert Hall***
Date: ***14:00, 23rd March, 2012***
Head organizers: ***Ágnes Végh, György Pálfi, József Pál***

The presence of 9 Nobel Prize Winners in Szeged, who visit our city on the occasion of the 75th anniversary of Albert Szent-Györgyi's Nobel prize award, is a great opportunity for a meeting between prominent scientists and young people susceptible of science.

We believe that meeting the Nobel Laureates in person and the open exchanges of views can provide the future generation with not only a life-long experience but it can also strengthen their scientific interest on a long term.

Within the frames of *'Open Forum'*, which will take place on **23rd March, 2012** from **2 pm**, hundreds of secondary school students, university students and PhD students will be given the chance to meet the nine Nobel Prize winners.

The *'Open Forum'*, includes presentations on the nine Nobel Laureates performed by Hungarian secondary school students coming from 3 counties of Hungary and from Serbia; and an interactive talk show. The main participants of the talk show are secondary students, who won the multi-round contest organized by the University of Szeged.

TREE OF SCIENCES

Venue: ***TIK***
Date: ***14:00, 24th March, 2012***
Head organizers: ***Ferenc Izbéki, György Pálfi, József Pál***

The Nobel Prize Laureates visiting Szeged and the University of Szeged made a resolution that it is essential to commemorate such a special anniversary in a way that future generations will be able to experience their respect and commitment for science.

The *'Tree of Sciences'* will be planted on **24th March, 2012** at **2 pm**; in a memorial planned by István Novák – former senior architect of the City of Szeged, who has also been awarded the *Prima Primissima Prize* – located in the park of the *University of Szeged József Attila Study and Information Centre.*

The *'Tree of Sciences'* – an oak from the *Botanical Garden of the University of Szeged* – will be planted by the Nobel Laureates and the leaders of the University of Szeged together.

Mihály Fritz – sculptor and medalist, awarded with the Ligeti Prize – designed a memorial tablet, which will perpetuate the names and the main data of the Nobel Prizes of the nine laureates who visited Szeged and saluted the reminiscence of Albert Szent-Györgyi.

TUDOMÁNYOK FÁJA · TREE OF SCIENCES

SZENT-GYÖRGYI ALBERT NOBEL-DÍJÁNAK
75. ÉVFORDULÓJA ALKALMÁBÓL ÜLTETTE
A SZEGEDI TUDOMÁNYEGYETEM
ÉS 9 NOBEL-DÍJAS TUDÓS

PLANTED BY THE UNIVERSITY OF SZEGED
AND 9 NOBEL LAUREATE SCIENTISTS
ON THE OCCASION OF THE 75th ANNIVERSARY OF
ALBERT SZENT-GYÖRGYI'S NOBEL PRIZE AWARD

2012. március 24. — 24th March 2012

Laureates	Nobel Prize	Date	Affiliation
ANDREW V. SCHALLY	Physiology or Medicine	1977	Veterans Administration Hospital, New Orleans, LA, USA
ROBERT HUBER	Chemistry	1988	Max-Planck-Institut für Biochemie, Martinsried, Germany
BERT SAKMANN	Physiology or Medicine	1991	Max-Planck-Institut für Medizinische Forschung, Heidelberg, Germany
ERIC WIESCHAUS	Physiology or Medicine	1995	Princeton University, Princeton, NJ, USA
PETER C. DOHERTY	Physiology or Medicine	1996	St. Jude Children's Research Hospital, Memphis, TN, USA
JOHN E. WALKER	Chemistry	1997	MRC Laboratory of Molecular Biology, Cambridge, United Kingdom
TIM HUNT	Physiology or Medicine	2001	Imperial Cancer Research Fund, London, United Kingdom
AARON CIECHANOVER	Chemistry	2004	Technion - Israel Institute of Technology, Haifa, Israel
ADA E. YONATH	Chemistry	2009	Weizmann Institute of Science, Rehovot, Israel

Nob1:

CORTICAL COLUMN: IF YOU DON'T UNDERSTAND THE FUNCTION-DO THE ANATOMY AND GET NUMBERS

Bert Sakmann

Max Planck Florida Institute, Jupiter, FL, USA

Soma location, dendrite morphology and presynaptic innervation represent key determinants of functional responses of individual neurons, such as sensory-evoked spiking. Here, we reconstruct the three-dimensional networks formed by thalamocortical afferents from the lemniscal pathway and excitatory neurons of an anatomically defined cortical column in rat vibrissal cortex. We objectively classify nine cortical cell types and quantify the number and distribution of their somata, dendrites and thalamocortical synapses. Somata and dendrites of most cell types intermingle, while thalamocortical connectivity depends strongly upon the cell type and the three-dimensional soma location of the postsynaptic neuron. Correlating dendrite morphology and thalamocortical connectivity to functional responses revealed that the lemniscal afferents can account for cell type- and location-specific subthreshold and spiking responses after passive whisker touch. The results provide a quantitative three-dimensional anatomical description of the cell type-specific lemniscal synaptic wiring diagram and elucidate structure-function relationships of this physiologically relevant pathway at single-cell resolution.

Nob2:

THE CONTINUING CHALLENGE OF VIRUS INFECTIONS

Peter C. Doherty

Department of Microbiology and Immunology. University of Melbourne, Australia; and Department of Immunology, St Jude Children's Research Hospital, Memphis, TN, USA

Viruses are everywhere as shown, for example, by genomic analysis of the upper levels of the oceans. In the main, though, these are mainly viruses that infect bacteria or plants, with the only direct threat to us being from water-borne viruses being when we eat filter feeders, such as oysters, that have cumulated human hepatitis viruses as a consequence of exposure to sewage effluent. Perhaps the greatest pandemic risk for humans is, though, from viruses that are prominent water-borne, generally GI tract infections of aquatic birds. The influenza A viruses survive well in water to infect a broad variety of water fowl and, from time to time, marine mammals. Even if these viruses mutate to extreme virulence, the genetic diversity of the avian population ensures that some species remain relatively asymptomatic and spread the disease. That's what we saw with the high pathogenicity H5N1 influenza strains that mutated during 2005 in the Quinghai Lake region of China to kill geese and swans, but not the ducks that spread the virus westwards to Europe. The influenza A viruses "jump" occasionally into humans to become established and cause unpredicted pandemics that can, with modern air travel, spread globally with incredible speed. This is, perhaps, the greatest infectious disease threat to humanity, and will form the focus for a discussion of what immediate and longer-term steps we can take to counter the spread of a novel, virulent, pandemic virus infection.

Nob3:

BIOLOGICAL COMBUSTION TODAY

John E. Walker

Medical Research Council Mitochondrial Biology Unit, Cambridge, UK

In 1937, Albert Szent-Györgyi was awarded the Nobel Prize in Physiology or Medicine "for his discoveries in connection with biological combustion with special reference to vitamin C and the catalysis of fumaric acid". His work led to the discovery by Hans Krebs of the citric acid cycle operating in the matrix of mitochondria. The lecture will describe the development of our present understanding of the biological combustion of energy rich components in foods leading to the formation of adenosine triphosphate, ATP, which, as SzentGyörgi showed is required for the contraction of muscle fibres. It will describe briefly other important contributions from David Keilin, who discovered in the horse bot-fly the cytochromes that are required for electron transfer processes in mitochondria, and from Peter Mitchell, who established, in the face of fierce opposition, that a transmembrane proton-motive force generated by electron transfer processes in mitochondria is a key intermediate in the formation of ATP. Today, many of the main components in this process, their organisation within mitochondria and how they work together to produce ATP are understood in great detail, and these features will be described in the lecture. However, fundamental questions remain unanswered, for example, about how the proton-motive force is generated and used, but their solutions lie within our grasp. There is also a growing realisation that defective biological combustion is linked with some neuromuscular diseases, and increasingly with neurodegenerative diseases, cancer and possibly ageing.

Nob4:

THE UBIQUITIN PROTEOLYTIC SYSTEM - FROM BASIC MECHANISMS THROUGH HUMAN DISEASES AND ON TO DRUG DEVELOPMENT

Aaron Ciechanover

Cancer and Vascular Biology Research Center, Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel

Between the 50s and 80s, most studies in biomedicine focused on the central dogma - the translation of the information coded by DNA to RNA and proteins. Protein degradation was a neglected area, considered to be a non-specific, dead-end process. While it was known that proteins do turn over, the high specificity of the process - where distinct proteins are degraded only at certain time points, or when they are not needed any more, or following denaturation/misfolding when their normal and active counterparts are spared - was not appreciated. The discovery of the lysosome by Christian de Duve did not significantly change this view, as it was clear that this organelle is involved mostly in the degradation of extracellular proteins, and their proteases cannot be substrate-specific. The discovery of the complex cascade of the ubiquitin solved the enigma. It is clear now that degradation of cellular proteins is a highly complex, temporally controlled, and tightly regulated process that plays major roles in a variety of basic cellular processes such as cell cycle and differentiation, communication of the cell with the extracellular environment and maintenance of the cellular quality control. With the multitude of substrates targeted and the myriad processes involved, it is not surprising that aberrations in the pathway have been implicated in the pathogenesis of many diseases, certain malignancies and neurodegeneration among them, and that the system has become a major platform for drug targeting.

Nob5:

HYPOTHALAMIC HORMONES: FROM NEUROENDOCRINOLOGY TO THERAPY OF CANCER AND OTHER DISEASES

Andrew V. Schally

Veterans Affairs Medical Center, University of Miami Miller School of Medicine, Miami, FL, USA

Experimental and clinical studies with analogues of hypothalamic hormones will be reviewed. The synthesis of analogues of hypothalamic hormones has had a major impact on the field of medicine. The synthesis of agonists of luteinizing hormone-releasing hormone (LH-RH), also known as Gn-RH, led to new endocrine therapy for advanced prostate cancer (PC). LH-RH antagonists also have applications in PC and in IVF-ET. The presence of receptors for hypothalamic peptides on human cancers stimulated development of targeted cytotoxic analogues of LH-RH (AN-152, AEZS-108), somatostatin, and bombesin/gastrin-releasing peptide (GRP), linked to doxorubicin, which inhibit the growth of experimental human prostatic, breast, ovarian, endometrial, renal, pancreatic, colorectal, bladder, lung and gastric cancers, brain tumors, melanomas, and lymphomas. AEZS-108 is now in clinical trials on gynecologic cancers and prostate and bladder cancers. Analogues of somatostatin have clinical applications in oncology for tumor localization and treatment. We demonstrated that growth hormone-releasing hormone (GH-RH) serves as autocrine growth factor in many cancers. Antagonists of GH-RH inhibit growth of diverse experimental human tumors and show great promise for therapy of cancer and benign prostatic hyperplasia. In view of powerful inhibitory action of GHRH antagonists on cancers, we evaluated the physiopathologic effects of GHRH agonists. GHRH agonists act on cardiac myocytes and accelerate regeneration of the heart in rats after myocardial infarct, speed up wound healing in mice, and stimulate pancreatic β-islet cells. New agonists of GHRH are being evaluated for possible uses in cardiology, treatment of diabetes and wound healing. These new therapies should provide an improvement over the existing treatments.

Nob6:

COMBATING RESISTANCE TO ANTIBIOTICS?

Ada Yonath

Department of Structural Biology, Weizmann Institute, Rehovot, Israel

Ribosomes are the universal cellular apparatuses that translate the genetic code into proteins. Composed of proteins and RNA, among which the RNA moieties perform almost all functional tasks, they posses spectacular architecture accompanied by inherent mobility that facilitate their smooth and efficient performance in decoding, peptide bond formation and nascent protein elongation.

Owing to their fundamental role, ribosomes are targeted by many antibiotics, each paralyzing the ribosomes by binding to a specific functional site. Their binding modes, inhibitory action and synergism pathways have been elucidated. Similarly, the subtle differences allowing differentiation between patients and pathogens have been identified. The mechanisms leading to bacterial resistance to ribosomal antibiotics and issues concerning the ways towards combating the resistance will be discussed.

Nob7:

SWITCHES AND LATCHES: THE CONTROL OF ENTRY INTO MITOSIS

Tim Hunt

Cancer Research UK, London Research Institute, Clare Hall Laboratories, London, UK

The process of mitosis involves a comprehensive reorganization of the cell: chromosomes condense, the nuclear envelope breaks down, the mitotic spindle is assembled, cells round up and release their ties to the substrate and so on and so forth. This reorganization is triggered by the activation of the protein kinase called Cyclin-Dependent Kinase 1 (CDK1). The end of mitosis is marked by the proteolysis of the cyclin subunit of CDK1, which terminates kinase activity. At this point, the phosphate moieties that altered the properties of hundreds of proteins to bring about the cellular reorganization are removed by protein phosphatases. We recently began to pay attention to the control of these enzymes, considering it likely that they were shut off as cells enter mitosis, and reactivated when mitosis is complete, to allow the return to interphase.
At least one protein phosphatase, PP2A-B55, is shut off in mitosis. Depletion of this particular form of PP2A accelerated entry into mitosis, and blocked exit from mitosis. Control of this phosphatase is achieved by an inhibitor protein (a-endosulfine or ARPP-19) that is phosphorylated by a protein kinase called Greatwall, which is itself a substrate of CDK1. Failure to inhibit PP2A-B55 causes arrest of the cell cycle in G2 phase. I will discuss the role of this control mechanism in the control of mitosis. We still have a rather incomplete understanding of exactly how the timing of entry into mitosis is controlled.

Nob8:

INTRACELLULAR PROTEOLYSIS: STRUCTURES, MOLECULAR MECHANISMS AND DRUG DESIGN

Robert Huber

Max-Planck-Institut für Biochemie, Martinsried, Germany

Within cells or subcellular compartments misfolded and/or short-lived regulatory proteins are degraded by protease machines, cage-forming multi-subunit assemblages. Their proteolytic active sites are sequestered within the particles and located on the inner walls. Access of protein substrates is regulated by protein subcomplexes or protein domains which may assist in substrate unfolding dependent of ATP. Five protease machines will be described displaying different subunit structures, oligomeric states, enzymatic mechanisms, and regulatory properties.

Nob9:

CHANGING MESODERMAL CELL SHAPE DURING DROSOPHILA GASTRULATION

Eric F. Wieschaus[1,2], Adam C. Martin[3], Bing He[2], Matthias Kaschube[4], Oleg Polyakov[4]

[1]Howard Hughes Medical Institute, [2]Department of Molecular Biology, Princeton University, Princeton, NJ, USA, [3]Massachusetts Institute of Technology, Cambridge, MA, USA, [4]Laboratories of Physics, Princeton University, NJ, USA, [5]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, USA

The last thirty years has seen major advances in our understanding of the mechanisms that direct cells in the embryo to specific developmental pathways. In Drosophila for example, we know that cells on the ventral side of the embryo are assigned to the mesodermal cell fate maternal gradients that activated the expression the Twist and Snail transcription factors. These cell fates decisions are followed by changes in cell shape that bring the mesodermal precursors into the interior of the embryo. Significantly less is know about the mechanics of these cell shape changes and how forces are organized within the cell.

In my talk, I will describe the establishment of a novel pulsating Actin/Myosin cytoskeletal network that arises in mesodermal cells immediately before the ventral furrow. To analyze this structure, we have developed imaging approaches and analytical tools that allow tracking surface areas and volumes of all 800 mesodermal cells during gastrulation. We find that cell volume is essentially constant and that cell shape changes are pulsed in synchrony with the Actin/Myosin contractions in the apical surface. We envision that force generated apically is transmitted over large distances by the non-compressible nature of the cytoplasm.

Surprisingly, many of the morphological changes in mesodermal cells still occur in mutants in which cellularization has been blocked. We investigate the properties of the cytoplasm that transmit force in the absence of membrane by tracking fluorescent beads in living embryos and locally disrupting the cytoskeleton using laser dissections.

C1:

REMOTE ISCHEMIC PRECONDITIONING

Gerd Heusch

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Ischemic pre- and postconditioning reduce the size of myocardial infarction, but any manipulation of the culprit coronary lesion bears the risk of coronary microembolization.

Remote ischemic preconditioning (RIPC) by brief cycles of ischemia/reperfusion in an organ remote from the heart also reduces infarct size, but avoids manipulation of the culprit coronary lesion. RIPC by repeated cycles of upper or lower limb ischemia reduces troponin release in patients undergoing coronary artery bypass grafting. RIPC also reduces troponin release in patients undergoing percutaneous coronary interventions and in patients with acute myocardial infarction during ambulance transport to the hospital. The signal transduction of cardioprotection by RIPC is largely unclear; particularly unclear is the neuronal vs. humoral transfer of the signal from the ischemic/reperfused remote organ to the heart and the biochemical nature of the transferred signal.

We have recently shown reduced troponin release in patients undergoing coronary artery bypass grafting under isoflurane anesthesia and cold crystalloid cardioplegia. In myocardial biopsies, the phosphorylation of mitogen activated protein kinases, of reperfusion injury salvage kinases and of STAT (signal transducer and activator of transcription) 1 and 3 increased from baseline before ischemic cardiac arrest to early reperfusion, but not differently between patients with and without RIPC. In contrast, the phosphorylation of STAT 5 was increased during early reperfusion only in patients with RIPC.

Thus, STAT 5 activation during RIPC is the first cardioprotective signalling event identified in the human heart.

C2:

MITOCHONDRIUM AS THE THERAPEUTIC TARGET FOR CARDIOPROTECTION

Derek J. Hausenloy

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Ischemic heart disease is the leading cause of death and disability worldwide. Novel therapeutic strategies are required to protect the heart against acute ischemia-reperfusion injury (IRI) in order to preserve left ventricular function and prevent the onset of heart failure. Mitochondria, which make up a third of the volume of a single cardiomyocyte, stand at the crossroads between life and death of the cell. On the one hand, mitochondria are the ATP-producing 'powerhouse' of the cell, generating the energy required for normal cardiomyocyte function. In this regard, preventing mitochondrial dysfunction during acute IRI is a key cardioprotective strategy. In addition to this crucial role, they play a critical signaling role in ischemic preconditioning, an endogenous adaptive phenomenon which protects the heart from acute IRI. On the other hand, through the opening of the mitochondrial permeability transition pore, mitochondria are the arbitrators of cell death in the setting of acute IRI. The elucidation of these two contrasting roles of mitochondria should result in the identification of novel therapeutic strategies for protecting the heart against acute IRI and improving clinical outcomes in our patients with ischemic heart disease.

C3:

INHIBITION OF MATRIX METALLOPROTEINASE-2 (MMP-2) TO PROTECT THE HEART FROM OXIDATIVE STRESS INJURY

Richard Schulz

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Matrix metalloproteinases are best known for their biological actions to proteolyze extracellular matrix proteins to affect tissue remodeling, both physiological and pathological. My lab has discovered that MMP-2, found in almost every cell type, also localizes to specific subcellular organelles and has unique susceptible protein targets inside the cardiac myocyte. We recently found that a combination of MMP-2 signal sequence quality, as well as its splicing, dictate its distribution between the cytosol and the secretory pathway. MMP-2 is activated directly by oxidative stress to a S-glutathiolated form which is catalytically active and distinct from the secreted form. It is an integral sarcomeric protein localized to thin, thick and intermediate (titin) filaments, most prominently at the Z-line, which is also found in nuclei, mitochondria and caveolae. In oxidative stress injury to the heart MMP-2 is rapidly activated and cleaves specific sarcomeric and cytoskeletal targets including troponin I, alpha-actinin, myosin light chain-1, glycogen synthase kinase-3beta and titin. The cleavage of these proteins results in the rapid loss of contractile function. MMP inhibitor drugs prevent the cleavage of these targets and protect the heart from oxidative stress injury by preventing inefficient contractile function. Such drugs, including doxycycline, which possesses MMP inhibitory properties distinct from its antibacterial actions, may be promising drugs for the treatment of ischemic heart disease and heart failure. Post-translational modifications of MMP-2 including S-glutathiolation and its phosphorylation should allow the development of inhibitors targeting intracellular but not extracellular MMP-2.

C4:

MAKING 3D HEART TISSUES – STATE OF THE ART AND PERSPECTIVES

Thomas Eschenhagen

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Current *in vitro* models for studying cardiac function have shortcomings. They are restricted to the investigation of myocyte function, are relatively unphysiological in that myocytes are either studied as isolated cells or attached to rigid plastic surfaces and are generally restricted to short term interventions. To solve some of these issues we have developed means to generate spontaneously beating 3D force-producing engineered heart tissues (EHT) from neonatal rat heart cells, human embryonic stem cell (hESC) and induced pluripotent stem (iPS) cell-derived cardiac myocytes. The talk will give an overview on recent developments that are directed towards improving the system as an in vitro assay and for cardiac repair. (1) Miniaturization and automation for drug screening. We have set up a semi-automated 24-well-EHT assay that allows medium throughput screening of pro-arrhythmic and cardiotoxic drug effects. (2) Disease modeling. Human iPS cells have opened the exciting possibility to model human diseases in the dish. We believe that the EHT assay could serve as a robust high content readout. (3) *In vitro* hypertrophy model. EHTs subjected for 5 days to enhanced afterload develop cardiac myocyte hypertrophy with hallmarks of pathological hypertrophy. (4) Vascularization. The lack of vascularisation and directed *in vitro* perfusion limit the maximal thickness of EHTs, one of the bottlenecks of a regenerative approach. A technique to introduce simple artificial vessels will be presented.

C5:

J WAVE SYNDROMES AS A CAUSE OF SUDDEN CARDIAC DEATH. FROM CELL TO BEDSIDE

Charles Antzelevitch

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The J wave is a deflection immediately following the QRS complex in the ECG. Several lines of evidence suggest that arrhythmias associated with early repolarization (ER) pattern in the inferior or infero-lateral precordial leads or Brugada syndrome (BrS), as well arrhythmias associated with hypothermia and the acute phase of myocardial infarction, are mechanistically linked to abnormalities in the manifestation of the transient outward current (I_{to})-mediated J wave. Although BrS and ER syndrome (ERS) differ with respect to the magnitude and lead location of abnormal J wave manifestation, they are considered to represent a continuous spectrum of phenotypic expression termed J wave syndromes. ERS can be divided into three subtypes: Type 1, displaying an ER pattern predominantly in the lateral precordial leads is most prevalent among healthy male athletes and is thought to be largely benign, only rarely associated with VF; Type 2, displaying an ER pattern predominantly in the inferior or infero-lateral leads is associated with a higher level of risk; and Type 3, displaying an ER pattern globally in the inferior, lateral and right precordial leads is associated with the highest level of risk for development of malignant arrhythmias and is often associated with electrical storms. BrS has been linked to mutations in 11 different genes, whereas ERS has been associated with mutations in 6 different genes, including KCNJ8, CACNA1C, CACNB2, CACNA2D1, ABCC9 and SCN5A. In ERS and BrS, mutations in these ion channel genes create a substrate for reentrant arrhythmias in the left and right ventricles, respectively.

C6:

ARRHYTHMOGENIC ALTERATIONS IN FAMILIAL HYPERTROPHIC CARDIOMYOPATHY: MOLECULAR AND FUNCTIONAL EVIDENCE

Elisabetta Cerbai

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Introduction: Hypertrophic cardiomyopathy (HCM), the most frequent genetic cardiomyopathy, purports increased arrhythmic risk and high prevalence of diastolic dysfunction. Despite its high prevalence (1:500 worldwide) HCM is an orphan disease, in that many therapeutic decisions are not evidence based and very limited options are available.

Objectives: Using myocardial samples from HCM patients, we characterized the electrophysiological, Ca^{2+}handling and contractile abnormalities of human HCM myocardium, and assessed the effects of the late sodium current(I_{NaL}) blocker ranolazine.

Materials/patients & methods: Electrophysiological and intracellular Ca^{2+} (Ca^{2+}_i) recordings were performed in isolated myocytes from myectomy samples of obstructive HCM patients; intact trabeculae were used for mechanical measurements. Myocardial specimens from surgical patients with normal left ventricular thickness were used as controls.

Results: Compared to controls, cardiomyocytes from HCM patients exhibited: 1. prolonged action potential related to decreased repolarizing K^+ currents and increased depolarizing currents (Ca^{2+} current and I_{NaL}); 2. increased incidence of early after-depolarizations, 3. prolonged Ca^{2+}_i transients, and 4. higher diastolic $[Ca^{2+}]_i$ Inhibition of I_{NaL} with ranolazine (10μM) reversed these cellular abnormalities and accelerated the contraction-relaxation cycle of HCM trabeculae, ameliorating diastolic function. Ranolazine had negligible effects on control myocardium.

Conclusion: The favorable effects of I_{NaL} inhibition suggest that intracellular Na^+ overload leading to dysregulation of Ca^{2+}_i homeostasis contributes to the functional remodeling observed in HCM. These findings suggest a beneficial effect of I_{NaL} inhibition in the management of arrhythmias and diastolic dysfunction in HCM patients.

C7:

SUDDEN CARDIAC DEATH; A MATTER OF GENES?

Arthur A. M. Wilde

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Sudden Cardiac Death (SCD) at young age (<50y) is most often based on an inherited cardiac arrhythmia syndrome with or without a structural substrate in the heart. Genes involved in SCD in these age group include genes encoding for ionchannel and their subunits, and genes encoding for sarcomeric, cytoskeletal or desmosomal proteins.

When SCD occurs in elderly people acute myocardial ischemia or one of its sequelae is frequently involved. Population based epidemiological studies have previously indicated that sudden death of a family member is a risk factor for SCD, also at older age, suggesting a genetic component in its susceptibility. In the past years several genetic factors have been identified that seem to associate with this risk. SCD from ventricular fibrillation (VF) during myocardial infarction is the major component of this huge healthcare issue and is a

leading cause of total and cardiovascular mortality. In a Dutch cohort with first myocardial infarction with or without VF, with comparable clinical characteristics including all risk factors for coronary artery disease, a positive family history for sudden death was the most significant risk factor for VF indeed. In a genomewide association study (GWAS) for VF in this setting, the most significant association was found at chromosome 21q21 (rs2824292; odds ratio = 1.78, 95% CI 1.47–2.13, P = 3.3 × 10−10), 98 kb proximal of the CXADR gene, encoding the coxsackie and adenovirus receptor. Further research on the mechanism of this locus might ultimately provide novel insight in arrhythmia mechanisms in this condition.

C8:

ARRHYTHMOGENIC SUBSTRATE OF LIFE THREATENING ARRHYTHMIAS

Béla Merkely

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Identification of factors, which play a substantial role on the formation and maintenance of cardiac arrhythmias may contribute to the improvement of antiarrhythmic interventions. During the past two decades we aimed to have a better insight of the arrhythmia substrate.

From in situ and in vivo animal experiments, through clinical studies we step-by-step managed to reach results utilized in the daily practice.

We were the first to describe the mechanism of low-dose intracoronary endothelin-1 (ET-1) induced ventricular tachycardia. The effect of ET-1 is based on the prolongation and increased dispersion of the monophasic action potential and formation of early after depolarization with multifocal origin visualised by 3D mapping. We proved, that the direct arrhythmogenic effect is mediated by myocardial ET_A-receptors. Induction of ventricular fibrillation (VF) could be suppressed by isoproterenol infusion in experimental models. In clinical studies we were able to demonstrate, that concentrations of ET-1 and big-endothelin increased in ventricular tachycardia (VT) or VF.

Translating our experience with arrhythmias in the clinical use, we have introduced radiofrequency catheter ablation for the treatment of VT and performed more than 300 cases. Most frequently the mechanism was post myocardial infarction scar-related re-entry. However, in the past years we ablated VT in patients with non-ischemic dilated cardiomyopathy, ARVD, myocarditis or with focal idiopathic outflow tract VT, ischemic VT from the Purkinje system or Belhassen-type idiopathic VT.

Based on our experience, better understanding and accurate identification of the substrate is essential for the treatment of life threatening arrhythmias.

C9:

BEAT-TO-BEAT VARIABILITY OF VENTRICULAR REPOLARIZATION: BIOMARKER TO QUANTIFY REPOLARIZATION RESERVE?

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Individual differences in their capacity to withstand arrhythmias after multiple 'hits' on repolarization, also known as the existing repolarization reserve, makes it difficult to predict individual risk. QT-duration is still used as a primary biomarker, although the predictive value of QT prolongation has been proven questionable.

Beat to beat variability of ventricular repolarization (BVR) is determining changes in repolarization duration between subsequent beats, quantified as short-term variability (STV).

A sudden increase in $STV_{(M)APD}$ after drug administration has been associated with ventricular arrhythmias in several animal models and with early afterdepolarizations (EADs) in cardiac myocytes. Safe drugs do not change $STV_{(M)APD}$, while the QT-duration is increased to similar levels as that achieved by pro-arrhythmic drugs. Similarly, effective anti-arrhythmic interventions by drugs will reduce $STV_{(M)APD}$.

In animal studies, STV_{QT} in baseline has shown to differ between susceptible individuals and controls, indicating a possibility to identify those with a diminished repolarization reserve. This electrical biomarker was superior to QT-time. In humans, a number of descriptive studies have "confirmed" this predictive power of STV_{QT} in different patient populations (table), including heart failure. In the coming years, 2 major multicenter trials will further establish the relevance of STV_{QT}, alone or in combination, in risk stratifying patients.

In conclusion, BVR quantified as STV holds promise in identifying patients at increased risk for (drug induced) ventricular arrhythmias and sudden cardiac death

C10:

CARDIAC REPOLARIZATION AND ARRHYTHMOGENESIS DURING SYMPATHETIC NERVOUS STIMULATION

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It has long been recognized that increased sympathetic nerve activity during physiologic stress (exercise, swimming, emotion, arousal, loud noise, etc) has profound influences on the electrical and contractile functions of the heart. In the severely predisposed heart these stressors may lead to ventricular tachyarrhythmias and sudden death. Still little is known about the temporal relationship between instantaneous autonomic nerve activity and arrhythmias.

There is a large variety of autonomically-driven arrhythmias in pathological conditions to single supraventricular and ventricular extrasystolic beats in the healthy heart. The latter are considered harmless if occurring at low frequency. In the atria, mounting data indicates the presence of a sophisticated network of ganglionated plexi with major influences on cardiac function. The ablation of multiple such ganglia can suppress pulmonary-vein potentials and atrial fibrillation.

At the cellular level, recent studies have focused on the spatiotemporal details of cyclic-nucleotide signaling influencing ion-channel function during neurohumoral stimulation. We have come to understand that sarcolemmal ion channels and other electrogenic transporters are macromolecular complexes that interact with structural elements (other than the phospholipid bilayer) to promote regionalization and the targeting by regulatory proteins. Compartmentation of these regulatory proteins in subdomains of the myocyte is increasingly recognized and thought to segregate the functional (including electrogenic) responses induced by different neuromediators and hormones.

In this lecture, I will discuss arrhythmogenic mechanisms that are triggered by influences from the neurocardiac interface, covering the field from the molecular-genetic to the intact-integrated level.

C11:

POSSIBLE MECHANISMS OF SUDDEN CARDIAC DEATH IN TOP ATHLETES: A BASIC CARDIAC ELECTROPHYSIOLOGICAL POINT OF VIEW

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Introduction: Sudden death among athletes is very rare (1:50,000–1:100,000 annually) but it is still 2–4 times more frequent than in the age-matched control population and attracts significant media attention.
Objectives: A hypothetical mechanism is proposed underlying sudden cardiac death in top athletes that does not relate to myocardial ischemia but is based on possible repolarization abnormalities due to potassium channel downregulation and can also be best explained by the concurrent presence of several factors such as cardiac hypertrophy (athlete's heart), and/or hypertrophic cardiomyopathy, increased sympathetic tone, genetic defects, drugs, doping agents, food, or dietary ingredients
Patients & methods: These factors together can increase the repolarization inhomogeneity of the heart ("substrate") and an otherwise harmless extrasystole ("trigger") occurring with a very unfortunate timing may sometimes induce life-threatening arrhythmias.
Results: The effective and possible prevention of sudden cardiac death requires development of novel cost effective cardiac electrophysiological screening methods. Such a possible test could be to determine the short term QT variability based on conventional ECG recordings. We tested more than 50 professional soccer players by this method and compared with aged matched controls. These professional athletes showed no change in their QT but significantly increased QT cvariability which may reflect chronic exercise induced repolarization change and possible altered susceptibility for arrhythmias.
Conclusion: Athletes with unexpectedly high QT variability identified by this test may then be subjected to more costly examinations such as echocardiography orgenetic tests in order to uncover possible known factors for enhanced risk for arrhythmias.

C12:

REVERSE RATE-DEPENDENT CHARACTER OF ACTION POTENTIAL DURATION CHANGES IS A GENUINE PROPERTY OF THE MYOCARDIUM

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All currently used class 3 antiarrhythmics carry serious proarrhythmic risk, which is associated with lengthening of action potential duration (APD) in a reverse rate-dependent manner, i.e. lengthening of APD is greater at longer than at shorter cycle lengths. Although several theories have been developed so far to explain this reverse rate-dependency, its mechanism has not been fully clarified. Here we propose a simple mechanism to explain the reverse rate-dependency of drug effects in the mammalian heart. Rate-dependent drug-effects of various origin were studied using agents known to lengthen or shorten action potentials allowing thus to determine the drug-induced changes in APD as a function of the cycle length. Both drug-induced lengthening and shortening of APD displayed reverse rate-dependency in human, canine, and guinea pig preparations, but not in rabbit and rat myocardium. Similar results were obtained when repolarization was modified by injection of inward or outward current pulses in isolated canine cardiomyocytes. In contrast to reverse rate-dependence, drug-induced changes in APD well correlated with baseline (pre-drug) APD values in all preparations studied. Since the net membrane current, determined from the action potential waveform

was inversely proportional to APD, and consequently to cycle length, it is concluded that that reverse rate-dependency may simply reflect the inverse relationship linking net membrane current to APD. In summary, reverse rate-dependency is an intrinsic property of drug action in the hearts of species showing positive APD - cycle length relationship, including humans. This implies that development of a pure K^+ channel blocking agent without reverse rate-dependent effects has little chance to succeed.

C13:

ROLE OF S-NITROSYLATION IN CARDIOPROTECTION

Elisabeth Murphy

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Introduction: Nitric oxide (NO) plays an important role in the regulation of cardiovascular function. In addition to the classic NO activation of the cGMP-dependent pathway, NO can also regulate cell function through protein S-nitrosylation, a redox dependent, thiol-based, reversible posttranslational protein modification that involves attachment of an NO moiety to a nucleophilic protein sulfhydryl group. There are emerging data suggesting that S-nitrosylation of proteins plays an important role in regulating cardiac function.

Objectives: A better understanding of the mechanism regulating protein S-nitrosylation and its role in the heart will provide us new therapeutic opportunities and targets for interventions in cardiovascular diseases. Protein S-nitrosylation (SNO) is increased following myocardial ischemic preconditioning (IPC) and it has been proposed that SNO may provide cardioprotection, in part, by reducing cysteine oxidation during ischemia-reperfusion (IR) injury. To test this hypothesis, we developed a method to measure oxidation using resin assisted capture (RAC), similar to the SNORAC methods used in the quantification of S-nitrosylation.

Materials & methods: Langendorff perfused hearts were subjected to various perfusion protocols (control, IPC, IR, IPC-IR) and homogenized. Each sample was divided into two equal aliquots, and a modified biotin switch was performed in order to simultaneously analyze SNO and oxidation.

Results: We identified 44 different proteins which showed increased S-nitrosylation with IPC. The majority of these proteins also showed a decrease in cysteine oxidation following IR.

Conclusion: These data support the hypothesis that S-nitrosylation can protect proteins from irreversible oxidation during ischemia and reperfusion.

C14:

THE IMPORTANCE OF ARGINASE AS A REGULATOR OF NO AVAILABILITY: IMPLICATIONS FOR MYOCARDIAL ISCHEMIA-REPERFUSION INJURY

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Endothelium-derived nitric oxide (NO) protects from myocardial ischemia-reperfusion injury by its vasodilator and anti-inflammatory actions. Impairment of NO bioavailability due to reduced production and enhanced inactivation of NO during early reperfusion is a critical factor contributing to the extent of myocardial injury. Recently, arginase which hydrolyses L-arginine to ornithine and urea has emerged as an important regulator of NO production. Since both arginase and NO synthase (NOS) utilize L-arginine as substrate increased activity of arginase will reduce the availability of arginine for NO production. Ischemia-reperfusion increases myocardial arginase activity. The increase is due to an upregulation of both isoforms

of arginase (I and II). Arginase inhibition results in increased production of citrulline and NO suggesting increased activity of NOS. Intravenous or intracoronary administration of arginase inhibitor reduces infarct size in rats and pigs, respectively. The cardioprotective effect of arginase inhibition is abolished after NOS blockade or NO scavenging suggesting that it is mediated via production of NO. Furthermore, the cardioprotective effect is blocked by inhibition of mitochondrial ATP-dependent potassium channels and is associated with increased PKCe expression.

Conclusion: Increased arginase activity of arginase during myocardial ischemia-reperfusion contributes to reduced bioavailability of NO. Blockade of arginase reduces infarct size via a mechanism involving increased production of NO due to a shift in arginine utilization from arginase to NOS. Targeting arginase may be a promising therapeutic strategy for protection against IR injury in myocardial infarction.

C15:

NITRITE THERAPY AGAINST ACUTE ISCHAEMIA AND REPERFUSION-INDUCED VENTRICULAR ARRHYTHMIAS

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Introduction: It seems now little doubt that nitric oxide (NO), derived from various exogenous and endogenous sources is a critical regulator of cardiovascular function, and it may protect the myocardium against the severe consequences of ischaemia and reperfusion. We have also evidence that NO is involved in both the early and the delayed protective effects of preconditioning against the acute ischaemia and reperfusion (I/R)-induced ventricular arrhythmias, and that NO donors, such as isosorbide mononitrate, sodium nitroprusside, result in antiarrhythmic effect.

Objectives: There is also increasing evidence that inorganic nitrite and nitrate, as the most recently recognised new form of NO sources, would also be cardioprotective. The aim of the study was to examine whether nitrite exerts protection against I/R-induced arrhythmias.

Materials & methods: In anaesthetised dogs, sodium nitrite ($NaNO_2$) was administered in intravenous infusion (0.2 μmol kg^{-1} min^{-1}) either priorto and during a 25 min occlusion of the anterior branch of the left coronary artery, or just prior to reperfusion of this artery.

Results: Administration of nitrite markedly reduced the severity of ventricular arrhythmias and increased survival, compared with the untreated controls.This effect was associated with preserved NO availability during occlusion and suppressed superoxide and nitrotyrosine productions following reperfusion. Higher number of S-nitrosylated proteins with increased nitrosylation has been found in dogs when nitrite had been administered just prior to reperfusion.

Conclusion: We conclude that intravenous infusion of nitrite profoundly reduces the severity of I/R-induced arrhythmias in anaesthetised dogs. This effect may result from an NO mediated reduction in oxidative stress, perhaps through S-nitrosylation.

C16:

CARDIOPROTECTIVE ACTIONS OF HYDROGEN SULFIDE

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Introduction: Hydrogen sulfide (H_2S) has been considered a deadly environmental toxin that poses a significant health threat. Recent experimental evidence indicates that H_2S is actually synthesized by a family of enzymes in all mammals and that H_2S is a critical physiological signaling molecule that promotes cell survival.
Objectives: Our laboratory has focused on investigating the effects of H_2S therapy in the setting of acute myocardial infarction and heart failure. In addition, we have also studied the effects of either genetic deficiency or overexpression of H_2S generating enzymes (i.e., CSE, CBS, and 3-MST).
Materials & methods: Experimental studies were performed in various murine models of myocardial ischemia/reperfusion (MI/R) injury and heart failure. H_2S was administered either intravenously, intraperitoneally, or orally with a number of novel H_2S donor agents. Experiments were focused on assessing the extent of myocardial cell injury, myocardial cell death, and left ventricular function.
Results: Our studies demonstrate that the administration of physiological levels of H_2S significantly attenuates myocardial infarct size by approximately 50% following MI/R. We also observed a significant preservation of left ventricular function in mice subjected to heart failure and treated with H_2S therapies. Mice that were deficient in the H_2S generating enzyme, cystathionine gamma lyase (CSE), exhibited significantly greater pathology during MI/R and heart failure while mice with cardiac-restricted overepxression of CSE were protected.
Conclusion: The data indicate that H_2S therapy protects the murine heart during pathological states. Additional studies are required for further translation of these findings for the treatment of cardiovascular disease.

C17:
HEART FAILURE AND ARRHYTHMIAS

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Heart failure carries a poor prognosis, about half of the afflicted patients die within five years after initial diagnosis, and half of these deaths are due to severe arrhythmia. Heart failure is also associated with a high incidence of atrial fibrillation which adds to the increased mortality mainly because of stroke. The underlying proarrhythmic mechanisms in heart failure (and atrial fibrillation) consist of abnormal excitability and impulse conduction resulting in triggered activity, reentrant wave fronts and/or rotors, that are related to aberrant intracellular Ca^{2+} cycling. These concepts of arrhythmogenesis will be linked to recent reports about how altered Ca^{2+} cycling in heart failure might serve as the cellular basis for arrhythmogenesis.
Instability of the membrane potential at action potential plateau or resting level are referred to as early and delayed after depolarisations (EADs, DADs), respectively, that can serve as triggers for ectopic activity. During critical prolongation of the AP plateau phase, inactivated Na^+ and/or Ca^{2+} channels may re-open providing the extra depolarizing current for EADs. In the ventricle, EADs may lead to torsades de pointes arrhythmia or even fibrillation; in the atria EADs may provide a trigger for initiating re-entry. Conditions of abnormal cellular Ca^{2+} cycling as observed in heart failure may trigger spontaneous Ca^{2+} release from the sarcoplasmic reticulum without prior excitation. The resulting cytosolic Ca^{2+} increase activates the plasmalemmal Na^+, Ca^{2+} exchanger producing the transient inward current underlying DADs.
Detailed knowledge about cellular mechanisms of perturbed Ca^{2+} cycling during heart disease may provide a basis for novel therapeutic interventions.

C18:

CALCIUM IN THE HEART: IN AND OUT OF CONTROL

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I will begin the presentation by reviewing the mechanisms that result in Ca^{2+} cycling in the heart. Most of the Ca^{2+} that activates contraction is derived from the sarcoplasmic reticulum (SR) and is released by the process of Ca^{2+} induced Ca^{2+} release (CICR) through the Ryanodine Receptor (RyR). On this mechanism, Ca^{2+} enters the cell via the L-type Ca^{2+} current and binds to the RyR making it open thereby resulting in the release of a much larger amount of Ca^{2+} from the SR. The amount of Ca^{2+} released depends on many factors including the properties of the RyR and the Ca^{2+} content of the SR. I will discuss both the control of SR Ca^{2+} content and the relationship between SR Ca^{2+} content and the amplitude of the Ca^{2+} transient.

The rest of the talk will focus on abnormal waves of CICR. These activate the electrogenic Na^{+}-Ca^{2+} exchange and thereby produce a depolarizing, arrhythmogenic current. It appears that such waves occur when the SR Ca^{2+} content exceeds a threshold level. These waves can result either if SR Ca^{2+} content is elevated above the threshold or the threshold is decreased to a lower level. I will discuss this in the context of catecholaminergic polymorphic ventricular tachycardia (CPVT), a clinical arrhythmia syndrome associated with mutations in the RyR and other SR-associated proteins.

C19:

ACTIVATION OF NA^{+}/CA^{+} EXCHANGE CURRENT IN MICRODOMAINS NEAR CALCIUM RELEASE SITES

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A central event in the process of excitation-contraction coupling in cardiac myocytes is the release of calcium from the sarcoplasmic reticulum (SR) through ryanodine receptors (RyR). RyR are organized in clusters occurring at Z-lines. In ventricular myocytes L-type Ca^{2+} channels, LTCC in the T-tubules face RyR clusters and provide the calcium influx that triggers opening of RyR. In myocytes with lower T-tubule density several RyR clusters are not coupled to T-tubules and opening of these clusters depends on propagated calcium from neighboring clusters. The release itself induces a large surge of calcium near RyR creating a microdomain of calcium. For RyR coupled to T-tubules, Na^{+}/Ca^{2+} exchange (NCX) located in the T-tubules is directly activated and may participate in shaping this microdomain while activation of NCX by uncoupled RyR may be delayed. Conversely, from the activation of NCX we can deduce information on the location of NCX in relation to RyR clusters. Remodeling of cardiac myocytes in chronic ischemia, in particular of the organization of T-tubules and RyR clusters, may further modulate the activation of NCX.

C20:

THE ROLE OF CARBON MONOXIDE SIGNALLING IN BIOLOGICAL PROCESSES MEDIATED BY HEMEOXYGENASE-1

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Introduction: Cutting edge of biotechnology holds the potential for characterization of events which follow and contribute to pathologically altered physiological functions.

Objectives: Heme oxygenase-1 (HO-1)-related mechanisms are a major homeostatic countermeasure by which vertebrate systems manage many disease states including myocardial ischemia/reperfusion, hypertension, cardiomyopathy, organ transplantation, endotoxemia, lung diseases, and immunosuppression. In the past decades, significant progress has been made in understanding of the function and regulation of HO-1.

Materials & methods: Here a summary is presented of current understanding of the role of the HO-1 related endogenous carbon monoxide (CO) production in various diseases, focusing on myocardial ischemia/reperfusion-induced injury.

Results: Various factors are considered which influence the HO-1 system in the context of endogenous CO production. An assessment will also be made as to how this evolving understanding may contribute to pharmacological approaches to therapeutic use of HO-1 manipulation. HO-1 represents a very potent tool for maintenance of oxidative stress at levels that enable physiological processes to function efficiently and avoid tissue damage. Upregulation of HO-1 is a widely distributed adaptive response to a wide variety of influences, especially oxidative insults. Thus, strategies for use of HO-1 as definitive prophylaxis are expected to increasingly make use of pharmacological agents capable of increasing activity of the enzyme.

Conclusion: In this respect, sour cherry flavonoids are ideal, since they are capable of increasing HO-1 activity and resultant production of endogenous CO and bilirubin at levels that the author has shown to be highly effective in preventing and reversing several forms of ischemia-reperfusion injury in the myocardium.

C21:

EXPERIMENTAL AND CLINICAL STUDIES IN CARDIOMYOPATHIES

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Studies in cardiomyopathies and other myocardial diseases have been initiated in the early seventies. Hypertrophic cardiomyopathies: a family discovered with hypertrophic cardiomyopathy (HCM) and congenital deaf-mutism. This malformation has been listed by the London Dysmorphology Database as Csanady-Hogye-Forster syndrome. Further investigation showed hidden central hearing abnormality in about half of HCM patients. An increased aortic stiffness has also been demonstrated by echocardiography. Real-time 3-dimensional echocardiography gives supplementary information in the diagnosis of the disease. In HCM patients, IL-6 and sIL-6R found to be elevetad. A Gly1233ter mutation was found a causative mutation for HCM in our Hungarian population.

Dilated cardiomyopathies: preceding ACE-inhibitor era, the onset of familial DCM appeared to be 10 years earlier and survival of this patients found to be 8 years shorter. After implementation ACE-inhibitors and beta-blockers in the standard therapy of DCM, the difference in survival disappeared. TNF-alfa and IL-6 level are found to be elevated in DCM patients. An increased level of anti-HSP60 might play an important role in the pathogenesis of the disases. Genetic background in our population is also investigated.

Familial segregation of long-QT sydrome was described among the first in the early seventies of 20th century. Genetic malformations is investigated in cooperation with other European centers.

C22:

LEVOSIMENDAN THE CARDIOPROTECTIVE INODILATOR

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The background of the Ca^{2+}-sensitizing effect of the inodilator levosimendan relates to its specific interaction with the Ca^{2+}-sensor troponin C molecule in the cardiac myofilaments. It is assumed that levosimendan prolongs interaction between cardiac troponin C and troponin I, thereby promotes contractile force without an increase in the intracellular Ca^{2+} transient. The magnitude of Ca^{2+}-sensitization evoked by levosimendan is less than the maximal effect of other known Ca^{2+}-sensitizers, although similar to what expected from length-dependent Ca^{2+}-sensitization during the activation of the Frank–Starling mechanism. Diastolic function is not impaired by levosimendan because of the mild degree of Ca^{2+}-sensitization, and low diastolic Ca^{2+} levels. Over the years, significant preclinical and clinical evidence has accumulated for levosimendan and 1) exceeded the knowledge for any other positive inotropic drug in routine clinical use and 2) revealed a variety of beneficial pleiotropic effects of the drug and of its long-lived metabolite, OR-1896. The pleotropic effects of the drug are as follows. First of all, activation of ATP-sensitive sarcolemmal K^+ channels of smooth muscle cells appears as a powerful vasodilator mechanism. Additionally, activation of ATP-sensitive K^+ channels in the mitochondria potentially modulates ATP-production and implicates a mechanism for cardioprotection. Finally, levosimendan possesses an isoform-selective phosphodiesterase-inhibitory effect. These data indicate that the cardiovascular effects of levosimendan are exerted via more than an isolated drug–receptor interaction, and involve favorable energetic and neurohormonal changes that are unique in comparison to other types of inodilators.

C23:

OXIDATIVE MYOFILAMENT PROTEIN ALTERATIONS IN THE POSTISCHEMIC HEART

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Introduction: Changes in the calcium-responsiveness of cardiac myofilaments is reflected in the extents and kinetics of cardiac systoles and diastoles.

Objectives: Our group aims to study the pathophysiological significance of myofilament protein alterations of the postischemic heart.

Materials & methods: To this end, we perform direct force measurements in permeabilized isolated cardiomyocytes obtained from the hearts of humans or from various animal models of acute or chronic heart failure. Moreover, biochemical assays are employed to pinpoint structure to function relationships between protein alterations and sarcomeric dysfunctions.

Results: Phosphorylation of myofilament proteins by protein kinase A and/or protein kinase C have major impacts on the Ca^{2+}-sensitivity of force production of cardiomyocytes. Moreover, during conditions of nitro-oxidative stress (e.g. reperfusion following ischemia, postischemic remodeling, etc.) a set of biochemically different types of posttranslational protein alterations may limit physiological myofilament function. Under in vitro test conditions selective oxidation of sulfhydryl groups (SH) by dithiodipyridine, nitration of tyrosine residues by peroxynitrite, enzymatic cleavage by μ-calpain, or carbonylation by the Fenton-reaction all

decrease force production in permeabilized cardiomyocytes. However, the sarcomeric proteins affected by these insults and the mechanisms by which they evoke sarcomeric dysfunctions are divergent. Our results suggested predominant structural alterations of the sarcomere following protein cleavage or nitration, while SH-oxidation or protein carbonylation were associated with changes in the fine regulation of sarcomeric function.

Conclusion: Collectively, the end-result on force production may depend on a hierarchy of sarcomeric protein alterations and on their interactions in the diseased heart.

C24:

DIASTOLIC HEART FAILURE – FROM PATHOPHYSIOLOGY TO NEW THERAPEUTIC OPTIONS

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Diastolic heart failure (Heart failure with preserved ejection, HFPEF) is defined a signs/symptoms of HF, an ejection fraction ≥50%, and objective evidence of diastolic dysfunction. Nowadays, over 50% of HF patients suffer from HFPEF.

In contrast to systolic heart failure, the pathophysiology of HFPEF is poorly understood. Left ventricular concentric hypertrophy, left atrial dilatation, and eventually atrial fibrillation are clinical key features. Chronotropic incompetence, pulmonary hypertension, and impaired microcirculation, as well as co-morbidities, such as renal dysfunction, COPD and physical deconditioning may contribute to the clinical picture.

On the organ level, myocyte hypertrophy and remodeling, as well as interstitial fibrosis are classical findings. Novel subcellular analyses demonstrate alterations in protein kinase activities, dysfunctional protein phosphorylation, impaired sodium and calcium handling, as well as titin modifications associated with the disease.

Preventive measures include physical activity, and minute risk factor control. Therapeutically, large randomized clinically trials testing the effects of AT1-antagonists or ACE-inhibitors have failed. Novel therapeutic approaches currently under investigation include aldosterone receptor blockade (Aldo-DHF, Topcat), natriuretic peptide augmentation (Neprilysin; PARAMOUNT), PDE5-inhibition (RELAX), or late Nai current inhibition (Ranolazine). Recently, exercise training was shown to induce reverse cardiac remodeling and improve exercise capacity (Ex-DHF Trial).

C25:

ENDOGENOUS CARDIOPROTECTION IS A HEALTHY HEART PHENOMENON? CARDIOPROTECTIVE SIGNALING IN HYPERLIPIDEMIA.

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Ischemic pre- and postconditioning are well described endogenous adaptive responses of the heart to withstand ischemia/reperfusion injury. Most experimental studies on cardioprotection have been undertaken in young-adult animal models, in which ischemia/reperfusion is imposed in the absence of other disease processes. However, ischemic heart disease in humans is a complex disorder caused by or associated with known

cardiovascular risk factors including hypertension, hyperlipidemia, diabetes, insulin resistance, atherosclerosis, and heart failure; additionally, aging is an important modifying condition. In these diseases and aging, the pathological processes are associated with fundamental molecular alterations that can potentially affect the development of ischemia/reperfusion injury per se and responses to cardioprotective interventions. Among many other possible mechanisms, for example, in hyperlipidemia and diabetes, the pathological increase in reactive oxygen and nitrogen species and the use of the ATP-sensitive potassium channel inhibitor insulin secretagogue antidiabetic drugs may disrupt major cytoprotective signaling pathways thereby interfering with cardioprotective signalling. This presentation will review the evidence that comorbidities, focusing on hyperlipidemia, modify responses to cardioprotection conferred by preconditioning and postconditioning. We emphasize the critical need for preclinical studies that examine cardioprotection specifically in relation to complicating disease states. These are now essential to maximize the likelihood of successful development of rational approaches to therapeutic protection for the majority of patients with ischemic heart disease who have modifying comorbid conditions.

C26:

CARDIOPROTECTION AND AGING

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Not only the prevalence, but also the mortality due to ischaemic cardiovascular disease is higher in older than in young humans, and the demographic shift towards an ageing population will further increase the prevalence of age-related cardiovascular disease. In order to develop strategies aimed to limit reversible and irreversible myocardial damage in older patients, there is a need to better understand age-induced alterations in protein expression and cell signalling. Cardioprotective phenomena such as ischaemic and pharmacological pre- and post-conditioning attenuate ischaemia/reperfusion injury in young hearts. Whether or not pre- and post-conditioning are still effective in aged organs, animals, or patients, i.e. under conditions where such cardioprotection is most relevant, is still a matter of debate; most studies suggest a loss of protection in aged hearts. The presentation will discuss changes in protein expression and cell signalling important to ischaemia/reperfusion injury with myocardial ageing. The efficacy of cardioprotective manoeuvres, e.g. ischaemic pre- and post-conditioning in aged organs and animals will be discussed, and the development of strategies aimed to antagonize the age-induced loss of protection will be addressed.

C27:

CARDIOPROTECTION IN DIABETIC AND AGING COHORTS: GETTING TO THE 'HEART' OF THE MATTER

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Relief of myocardial ischemia in an intermittent or stuttered manner (i.e., 'postconditioning') augments myocardial salvage and profoundly limits infarct size beyond that achieved with standard, abrupt reperfusion. Postconditioning-induced cardioprotection has been documented in multiple experimental models, and there is a growing consensus that the favorable infarct-sparing effect of this phenomenon is due in part to

robust up-regulation of classic 'survival kinase' signaling (most notably, ERK 1/2) during the early minutes of reperfusion. Moreover, initial studies suggest that postconditioning may be of clinical benefit and improve outcome in patients following acute myocardial infarction. There is, however, emerging laboratory evidence that models displaying clinically relevant co-morbidities and risk factors (most notably, aging and diabetes mellitus) are refractory to infarct size reduction with postconditioning, possibly due to age- and diabetes-induced deficits in ERK signaling. These data imply that the cardioprotective effects of postconditioning may be attenuated in one or more patient sub-populations, and underscore the need for prospective clinical studies to assess the efficacy of postconditioning in aging and diabetic cohorts.

C28:

LIFESTYLE-RELATED RISK FACTORS AND CARDIAC RESPONSE TO ISCHEMIA: POSSIBILITIES TO RESTORE IMPAIRED ISCHEMIC TOLERANCE OF THE HEART

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Lifestyle-related risk factors (RF) of cardiovascular diseases including dyslipidemia, stress and hypertension lead to left ventricular hypertrophy and heart failure. The risk is increasing with age, in particular, in females. On the other hand, some stressful stimuli including reactive species, brief ischemia (hypoxia) and/or hyperglycemia, play a dual role in the pathogenesis of ischemia/reperfusion injury (IRI) and trigger adaptive processes increasing ischemic tolerance. Previously, suppression of endogenous protection by RF has not been considered. We hypothesized that RF, such as hyperlipidemia and/or hypertension, may modify cardiac response to ischemia not only by interference with pathophysiological mechanisms of IRI per se, but via suppression of intrinsic cardioprotective mechanisms known as preconditioning (PC). While hypertrophied hearts of hypertensive (SHR) rats, male counterparts of age-matched females were more sensitive to IR manifested by a larger extent of irreversible injury (infarct size), acute STZ-induced diabetes (recently termed as metabolic preconditioning) or high fat-cholesterol diet alone did not alter cardiac susceptibility to ischemia. However, combination of RF markedly exacerbated IRI. On the other hand, PC still conferred an effective protection, although its extent was lower in SHR and older animals. Research shows that protective effects of adaptation may be attenuated in heart affected by RF, although potential of intrinsic cardioprotection is still retained even in the pathologically altered myocardium that requires a higher intensity of the preconditioning stimulus. Pleiotropic effects of hypolipidemics, PPAR agonists, beyond their lipid-lowering properties indicate a promising approach to reactivate myocardial ischemic tolerance in these subjects.

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C29:

H_2S AND CARDIOPROTECTION

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Abstract is not available

C30:

HOW THE INHIBITION OF GLUTATHIONE S-TRANSFERASE CAN MODULATE STRESS RESPONSE OF CARDIAC MYOCYTES

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Introduction: Myocardial ischemia-reperfusion injury is crucially involved in the pathogenesis of cardiovascular diseases. Among numerous defense mechanisms against oxidative injury, the antioxidant enzyme glutathione S-transferase (GST) is responsible for the cellular response to stress, apoptosis and proliferation.

Aims: The main objective of this study was to identify the effect of inhibition of GST and MAP kinases (JNK, P38, ERK) on the viability and apoptosis of cardiomyocytes which were exposed to various oxidative stress stimuli (H_2O_2 and stimulated ischemia-reperfusion (I/R)).

Methods: Primary culture of neonatal rat cardiomyocytes was prepared and divided in groups according to different exposers. In the first series of experiments GST inhibitor ethacrinic acid (EA) administered to cardiomyocytes treated with H_2O_2 or exposed to I/R. In second series of experiments using similar experimental conditions JNK, p38 and ERK inhibitors were used. Viability of cardiomyocytes and the ratio of apoptosis was evaluated. The phosphorilation activity of MAP kinases were measured with flow cytometry.

Results: In the first series of our experiments it was clarified that inhibition of GST, H_2O_2 and I/R alone reduced the ratio of living cells and increased the percentage of apoptotic cells. These changes significantly enhanced following GST inhibition mainly when cells co-treated with H_2O_2 or exposed to I/R. In the second series of our investigation using MAPK inhibitors only moderate improvement can be evaluated following several stress stimuli.

Conclucions: GST activity is required for survival of cultured cardiomyocytes during oxidative stress situation suggested it's importance in clinical situation involved ischemia-reperfusion.

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C31:

MYOCARDIAL AND VASCULAR PROTECTION BY PARP INHIBITORS

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Chronic hypertension can induce cell death and fibrotic remodeling in arterial wall and in myocardium via oxidative stress and consequent poly (ADP-ribose) polymerase (PARP) activation. Thus we raised the possibility that inhibition of the nuclear PARP can improve cardiac performance and delays vascular remodeling in spontaneously hypertensive rats (SHR).

SHRs were divided into two groups. One group received no treatment (SHR-C), while the other (SHR-L) received 5 mg/kg/day L-2286 orally, for 46 weeks. Male Wistar rats belonged to the normotensive control group (WKY). By the end of the study the SHR-C group developed an eccentric hypertrophy with impaired left ventricular (LV) systolic function, while PARP-inhibitor treatment preserved systolic LV function. Moreover, intima-media thickness (IMT) and aortic stiffness index (ASI) were elevated in SHR-C compared to WKY which were

decreased significantly by L-2286 treatment ($p<0.05$). L-2286 did not affect the blood pressure of SHR rats, but moderated the level of plasma-BNP ($p<0.01$), favorably influenced the gravimetric parameters ($p<0.05$), and the extent of myocardial and vascular fibrosis ($p<0.05$). The inhibition of PARP beneficially changed the activity of intracellular signaling pathways by increasing the phosphorylation of Akt-1/GSK-3β, ERK 1/2 and PKC ε ($p<0.01$), and by decreasing the phosphorylation of JNK ($p<0.05$), p-38 MAPK, PKC pan βII and PKC ζ/λ ($p<0.01$), PKC α/βII and δ ($p<0.05$).
These data suggest that chronic inhibition of PARP prevents abnormal vascular and myocardial remodeling, preserves systolic function and delays transition of hypertensive cardiopathy to heart failure via favorable changes in the most important signaling pathways related to oxidative stress.

C32:

THE EMERGING ROLE OF MAGNESIUM OROTATE IN CARDIOPROTECTION AGAINST ACUTE ISCHEMIA-REPERFUSION INJURY

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Myocardial ischemia/reperfusion injury represents a leading cause of morbidity and mortality worldwide, being associated with acute coronary syndromes and cardiac/non-cardiac surgery. The impact of these pathological conditions on survival and life quality is mainly due to cardiomyocyte death. Therapeutic strategies aimed at limiting myocardial necrosis during the postischemic reperfusion and in the perioperative settings are nowadays extensively studied. Orotic acid is a key intermediate in the biosynthetic pathway of pyrimidines. Early investigations in the heart suggested that chronic administration of orotate and its derivatives could be of significant clinical benefit in treatment of several cardiac diseases. The presentation is concerned with the current knowledge and understanding of the major effects of these compounds in both experimental and clinical cardiology. We have recently demostrated that acute application of magnesium orotate during the postischemic reperfusion in the *ex vivo* and *in vivo* models of global and regional ischemia is associated with significant improvement of contractile function and reduction in infarct size, respectively. Evidence for a protective effect of magnesium orotate in both early and delayed administration at reperfusion is provided. The potential mechanisms and biochemical pathways responsible for cardioprotection are discussed.

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O-C1:

LOSS OF CARDIOPROTECTION BY ISCHEMIC POSTCONDITIONING IN VASCULAR NITRATE TOLERANCE: ROLE OF SURVIVAL KINASE

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Introduction: We have previously shown that the cardioprotective effect of preconditioning is attenuated by vascular nitrate tolerance.
Objectives: Here we studied whether the development of vascular nitrate tolerance affects the infarct size limiting

effect of ischemic postconditioning in the myocardium, and whether the activation of survival kinases plays a role in the molecular mechanism of postconditioning in the presence or absence of vascular nitrate tolerance.

Materials & methods: Male Wistar rats were treated with nitroglycerin or its vehicle s.c. for 3 days to induce vascular nitrate tolerance. On the fourth day, hearts were isolated, perfused according to Langendorff and subjected to 30 min coronary occlusion followed by 120 min reperfusion for infarct size determination. Postconditioning was induced by 6x10"/10" ischemia/reperfusion cycles at the onset of reperfusion.

Results: In non-tolerant hearts, postconditioning significantly decreased infarct size as compared to ischemia/reperfusion (22±2% vs. 40±4%; $p<0.05$). However, postconditioning failed to decrease infarct size in hearts of nitrate tolerant rats (29±4% vs.33±6%). Phosphorylation of ERK 1/2, Akt or eNOS showed no significant differences between groups as determined in separate experiments by Western blotting of left ventricular samples collected at the 10th min of reperfusion.

Conclusion: Vascular nitrate tolerance interferes with the infarct size limiting effect of ischemic postconditioning. Moreover, activation of survival kinases is not crucial in the molecular mechanism of postconditioning which remains unaffected in nitrate tolerance.

O-C2:

ELECTRO-MECHANICAL WINDOW IS PROFOUNDLY NEGATIVE IN GENOTYPED LONG-QT PATIENTS: RELATION TO ARRHYTHMOGENIC RISK

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Introduction: Inhomogeneous left-ventricular transmural contraction is present in symptomatic long-QT (LQT) patients with longitudinal mechanical dispersion constituting a better arrhythmic risk indicator than QTc. We focused on the "Electro-Mechanical Window" (EMW) in genotyped LQT patients. A strongly negative EMW heralds torsades de pointes in a canine model of drug-induced LQT1 syndrome.

Objectives: To examine EMW in relation to arrhythmogenic risk in genotyped LQT patients.

Patients & methods: We included 53 LQT-mutation carriers from two centres and 25 age- and gender-matched controls. EMW was measured as time from Q wave to echographic aortic-valve closure (Q-AoC) minus the QT interval, and corrected for heart rate. Ventricular tachyarrhythmia, syncope, aborted cardiac arrest, and sudden cardiac death were counted as arrhythmic events.

Results: The study group harbored 29 KCNQ1, 14 KCNH2, 2 SCN5A, 3 KCNE1, 1 KCNE2, 1 KCNJ2, and 3 compound variants (KCNQ1+KCNH2). 70% were female and 72% received beta-blockers. Thirty-one carriers experienced 1 arrhythmic event, compared with 22 event-free carriers. QT and QTc interval were significantly longer in carriers (468±61 and 490±63ms, respectively) than in controls (380±26 and 414±33ms; $P<0.0001$ for both). Mean heart rate-corrected EMW was significantly more negative in LQT carriers than in controls (-60±62 versus 5±25ms; $P<0.0001$), and even more negative in symptomatic compared with asymptomatic mutation carriers (-76±68 versus -37±36ms; $P<0.05$). EMW difference between LQT1 and LQT2 did not reach statistical significance (-55±49 versus-46±43ms; $P>0.05$).

Conclusion: LQT-mutation carriers express a profoundly negative EMW, which is most pronounced in symptomatic patients. These findings indicate that alterations in cardiac mechanics may play a role in arrhythmogenesis in LQT patients.

O-C3:

THE POSSIBLE PROARRHYTHMIC EFFECTS OF DICLOFENAC

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Introduction: Sudden cardiac death among athletes is very rare but 2-4 times more frequent than in the age-matched control population. Non-steroidal anti-inflammatory drugs (NSAIDs) like diclofenac are widely used in the treatment of sports injuries, however, their effects on the cardiac electrophysiological parameters are not properly understood. It is possible that the NSAID diclofenac might cause ventricular repolarisation abnormalities.

Objectives: The aim of our study was to characterize the cellular electrophysiological effect of diclofenac on dog right ventricular preparations.

Materials & methods: Action potential measurements were carried out by applying the standard intracellular microelectrode technique in right ventricular papillary muscle preparations isolated from mongrel dogs. Ionic currents were recorded using the whole-cell configuration of the patch-clamp technique in single ventricular myocytes isolated from dog hearts.

Results: Diclofenac slightly lengthened the action potential duration at 90% repolarisation (APD90). In the presence of 100 nM IKr blocker dofetilide, 20 μM diclofenac caused significant additional APD90 increasing. The APD lengthening effect of diclofenac was significantly augmented in preparations where the „repolarisation reserve" was attenuated by previous application of dofetilide and $BaCl_2$. In some experiments early afterdepolarisations (EADs) developed in this setting. During the experiments transmembran ionic currents were also measured. In dog ventricular myocytes the amplitude of IKr was concentration dependently decreased by diclofenac. IKs was also depressed by 30 μM diclofenac.

Conclusion: At therapeutic concentration diclofenac alone does not influence ventricular repolarisation significantly. However, in the case of impaired „repolarisation reserve" such as organic heart disease or in athlete's heart, diclofenac may enhance the arrhythmic risk and sudden cardiac death.

O-C4:

CATECHOLAMINES, RATE AND REPOLARIZATION. CAN WE MAKE A COMPLEX STORY SIMPLER?

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Repolarization course is pivotal in maintaining physiological cardiac excitation and has a major impact on contractility. Adrenergic activation targets simultaneously the sinus node and the working myocardium, conceivably to maintain the "correct" relationship between heart rate and repolarization. Membrane currents involved in repolarization are intrinsically rate-dependent, but they are, at the same time, directly modulated by adrenergic receptors. Thus, to define adrenergic modulation of repolarization we need to consider its rate-dependency. The attempt to provide a general interpretation of this issue is frustrated by an surprising inconsistency of reports, which concerns even the direction (shortening vs prolongation) of adrenergic effects on repolarization. While this is often attributed to species-specificity of repolarization

mechanisms, a thorough analysis of published data shows that apparent inconsistencies may be present even within the same species. A clue to the interpretation of this paradox may be provided by the observation that inconsistencies are eliminated if the rate-dependency of adrenergic modulation is compared between preparations with a similar action potential contour. This would imply that the dominant factor controlling rate-dependency of repolarization response to adrenergic activation is repolarization course itself, rather than the properties of individual catecholamine-responsive currents. This interpretation is supported by the notion that dynamic "expression" of currents during repolarization is strongly affected by the course of membrane potential. Many apparent discrepancies could be solved by this view which, if confirmed by direct experimental testing, would help in identifying general mechanisms underlying adrenergic modulation of repolarization.

O-C5:

THE EFFECT OF CARDIOPROTECTIVE AGENTS (SNAP, BNP) AGAINST SIMULATED ISCHEMIA/REOXYGENATION INJURY IN MOUSE EMBRYONIC STEM CELL-DERIVED CARDIOMYOCYTES

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Introduction: Embryonic stem cell (ESC)-derived cardiomyocytes are promising cell source for cardiac repair after myocardial infarction. Therefore, it is of great importance to protect graft stem cells against ischemia/reperfusion (I/R) injury.

Objectives: Here we tested whether a nitric oxide (NO)-donor and the particulate guanylate cyclase activator brain-type natriuretic peptide (BNP) can protect mouse ESC-derived cardiomyocytes against I/R injury and to investigate possible downstream pathways in this protection.

Materials & methods: Cells were subjected to 150 min simulated ischemia (SI) followed by 120 min reoxygenation (R). The following treatments were applied during SI: the NO-donor S-nitrozo-penicillamine (SNAP,10^{-5}, 10^{-6}, 10^{-7} M), B-type natriuretic peptide (BNP, 10^{-7}, 10^{-8}, 10^{-9} M), the protein kinase G (PKG) inhibitor (KT5823, $6x10^{-8}$ M), the K_{ATP} channel blocker glibenclamide (10^{-6} M), alone or in different combinations. Nonischemic control mESC-derived cardiomyocytes were subjected to 150 minutes normoxic condition followed by 120 minutes reoxygenation period. Finally, viability of cells was assayed by propidium iodine staining.

Results: SNAP decreased SI-induced cell death (5625±518 au) in a dose-dependent manner (10^{-6} M SNAP to 4234±420 au [p<0,05] and10^{-5} M SNAP to 4061±423 au, [p<0,05]) and this protection, was non-significantly attenuated by inhibition of either PKG (4284±916 au) or K_{ATP} channels (4424±1034 au). However, SI-induced cell death was not affected by BNP.

Conclusion: We conclude that SNAP protects mESC-derived cardiomyocytes against SI/R injury. Soluble guanylate-cyclase, PKG, and K_{ATP} channels may play a role in the downstream pathway of SNAP-induced protection. NO-donors are promising candidates for the enhancement of ESC survival during implantation into ischemic tissue regions.

O-C6:

DESIGN AND VALIDATION OF A CYCLIC STRETCH BIOREACTOR SYSTEM FOR SIMULATING THE CARDIAC ENVIRONMENT

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Introduction: Tissue engineering studies suggest that physical stimulation is of major importance for myocardial integrity. However, standard cell culture conditions usually do not account for the active physical environment found in the native myocardium.

Objectives: In effort to simulate the cardiac physical environment, we aim to design and validate a bioreactor which allows the application of cyclic stretch to cells cultured on polymer scaffolds.

Patients & methods: With local ethics committee's approval, human cells were isolated from patients with informed consent.

Results: Based on the motor/cam/shaft mechanism, we built a cyclic stretch bioreactor that can be conveniently fit into a standard incubator, using materials with low cytotoxicity. We first verified that precisely controlled stretch can be delivered homogeneously to the entire surface by ARAMIS surface deformation analysis. To assess cell viability under stimulation, human umbilical vein endothelial cells were stained for proliferation marker Ki-67; stretched group and unstretched control showed comparable levels of expression. Human atrial fibroblasts stimulated by 10%, 1 Hz cyclic stretch for 24 hours showed perpendicular alignment to the direction of stretch and an elevated percentage of alpha-smooth muscle actin-positive population, suggesting a transition to an activated myofibroblast phenotype.

Conclusion: We conclude that, given its capability of delivering precise cyclic stretch to cultured cells and evoking biological response without compromising cell viability, our bioreactor provides a powerful tool in studying the interaction of physical stimulation and cardiovascular (patho)physiology. Future work may incorporate more factors, such as rhythmic electrical pulses and oxygenation in to the system to better mimic the cardiac niche.

O-C7:

AN INCREASE IN THE 'ABSOLUTE' BEAT-TO-BEAT VARIABILITY AND INSTABILITY OF THE ECG INTERVALS PREDICTS DOFETILIDE-INDUCED TORSADES DE POINTES INDEPENDENTLY FROM THE APPLIED ANAESTHETIC IN RABBITS, IN VIVO

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Introduction: Recently, we showed that the 'absolute' beat-to-beat variability and instability (ABVI) parameters of the ECG intervals correlated with the occurrence of dofetilide-induced torsades de pointes tachyarrhythmia (TdP) in pentobarbital-anaesthetized rabbits.

Objectives: We tested whether ABVI parameters are able to predict dofetilide-induced TdP independently from the applied anaesthetic.

Materials & methods: Intravenous dofetilide infusion was administered to alpha$_1$-adrenoceptor stimulated rabbits anaesthetized with chloralose, propofol or pentobarbital. ECG intervals were measured retrospectively in a blinded manner at predetermined time-points and ABVI parameters were calculated.

Results: Dofetilide induced TdP in 10 out of 10 rabbits anaesthetized with chloralose, in 7 out of 10 rabbits anaesthetized with propofol and in 4 out of 10 rabbits anaesthetized with pentobarbital. ABVI parameters did not discriminate between the different anaesthesia groups. The animals were then divided into a 'TdP positive' and a 'TdP negative' group according to the presence of absence of TdP, respectively. The ABVI parameters were significantly increased in the 'TdP positive' group as compared with those in the 'TdP negative' group. An increase in the 'absolute' long-term variability (LTV) of the RR, QRS, QT and JT intervals to certain 'cut-off' levels identified 'TdP positive' and 'TdP negative' animals with a sensitivity and specificity of 95% and 78%, respectively.

Conclusion: In anaesthetized rabbits, an increase in the ABVI parameters, especially an increase in the 'absolute' LTV values predicts the occurrence of dofetilide-induced TdP with high sensitivity and specificity independently from the applied anaesthetic. Results suggest that our novel ABVI parameters may be reliable biomarkers of the proarrhythmic liability of drugs.

O-C8:

MUTATION SCREENING AND FUNCTIONAL CHARACTERISATION OF RNA-BINDING MOTIF PROTEIN 20 IN DILATED CARDIOMYOPATHY

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Introduction: Dilated cardiomyopathy (DCM) is an insidious disease of the myocardium, leading to impaired heart function. Till date, about 40 genes are known to be involved in familial DCM. One of the recently discovered DCM genes encodes the RNA-binding motif protein 20 (RBM20), which has a five aminoacid mutation hotspot in exon 9. These mutations were found to be associated with an unusually severe DCM phenotype.

Objectives: Our aim was to perform mutation screening of RBM20 and functionally evaluate the identified variants.

Patients & methods: Initially, the complete coding sequence of RBM20 was analysed by Sanger-sequencing in 63 probands with familial DCM, subsequently followed by the sequencing of exons 6, 9 and 11 encoding the functionally important domains of the protein - in an additional cohort of 380 DCM patients and 384 controls.

Results: Our mutational screening resulted in the identification of 5 novel and 4 known, mostly missense variants in 15 probands with familial DCM. 4 missense variants were located in the mutation hotspot of exon 9, which encodes the functionally important RS-rich domain. Moreover, 1 novel missense variant was detected in the RNA-recognition motif encoded by exon 6.

Conclusion: We have identified 5 novel and 4 known RBM20 variants present in ~3% of our DCM index patients, similarly to previous reports. Interestingly, 5 of these variants are located in functionally important domains of the derived protein. Hence, it is highly recommended to include exons 6 and 9 in routine diagnostic screening of DCM patients. Functional characterisation of the most promising novel variants is in progress.

O-C9:

PRIMARY PREVENTION POPULATION COHORT: BUDAKALÁSZ EPIDEMIOLOGY STUDY

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Introduction: There are no reliable morbidity data of the Hungarian population. Framingham study provides important associations between risk factors and cardiovascular disease in a US cohort.

Objectives: To start a cardiovascular screening program involving the adult population (>20y) in a Central-Hungarian town.

Patients & methods: The complete adult population (8.000) of the selected town is targeted in our voluntary programme. Protocol includes health questionnaire, non-invasive tests (cardiac- and carotid ultrasound, resting blood pressure and ankle-brachial index) and venous blood biobanking (serum, plasma and DNA), using coded samples linked to a clinical database. Low-dose cardiac CT for coronary calcium and cardiac fat determination is performed in certain age groups (>35y in males and >40y in females).

Results: Six hundred and five inhabitants were screened until mid January 2012, with a mean age of 48.9 years and a male-to-female ratio of 1:1,6. Increased body mass index (over 25 kg/m^2) was detected in 446 inhabitants (74%), in 268 (44%) enhanced systolic blood pressure, in 278 (45%) elevated HgbA1c (40 mmol/l) and pathologic LDL cholesterol level was verified in 299 inhabitants (49%). Elevated high-sensitivity CRP (2-10 mg/l) was detected in 235 people (39%) and elevated NT-proBNP level with preserved left ventricular ejection fraction in 87 inhabitants (14%). Average cardiovascular mortality risk was 20,7%.

Conclusion: First results demonstrate enhanced cardiovascular risk of this cohort in European perspectives. Completion of the protocol on the adult population in the selected town and longitudinal follow-up studies are needed to provide associations between risk factors, morbitity and mortality in this primary prevention Hungarian cohort.

O-C10:

EFFECTS OF HIDROGEN PEROXIDE AND MPO IN THE RAT BASILAR ARTERY

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Introduction: Increased expression of myeloperoxidase (MPO) plays a role in the developement of cardiovascular and cerebrovascular diseases. MPO is an important enzyme that catalyzes the formation of reactive molecules such as hypochlorous acid from hidrogen peroxide (H_2O_2).

Objectives: The aim of this study was to characterize the vasoactive effects of H_2O_2 and MPO in isolated arteries.

Materials & methods: Arteria basilaris was isolated from the rat. Arterial rings (3-5 mm in length) were fixed on an isometric contractile measurment system. H_2O_2 evoked changes in contractile force (in mN) of precontracted vascular rings were measured in the presence and absence of MPO. Expression of MPO was determined by immunhistochemistry.

Results: After precontraction of the artery with 60mM KCl we found that low dose of H2O2 (10 M 30 M) evoked contraction (9,9±0,8 mN); while higher doses (300 M - 3 mM) led to relaxation (to 3,4±0,2 mN); An

initial constriction was followed by relaxation at 100 M (from 10,4±0,9 mN to 8,1±0,9 mN); MPO shifted the H_2O_2 effects toward constriction: higher constriction was found at lower H_2O_2 concentrations (1 M –30 M) and the biphasic effect was observed not only at 100 M H_2O_2 (contraction: 12,2±1,4 mN, relaxation: to 10,5±2,6 mN) but also at 300 M H_2O_2 (contraction: 11,7±2,5 mN, relaxation: to 6,6±1,4 mN). MPO expression was found in the endothelial layer of basilar arteries.

Conclusion: MPO transforms H_2O_2 evoked relaxations to constrictions and therefore may affect circulation suggesting that MPO inhibitors may represent a new therapeutical target in the cardiovascular disease relating to systemic inflammation.

O-C11:

REGULATORY MECHANISMS IN PROTECTION OF CELL ENERGETICS IN HYPOXIC AND DIABETIC MYOCARDIUM: ROLE OF CALCIUM AND THE MITOCHONDRIAL SIGNALING

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Background: Hypoxic and diabetic heart cells experience increased calcium transients (CAT), energy dysequilibrium and disturbed mitochondrial (MIT) functions. However, enhanced Ca^{2+} and MIT perturbations associated with extrusion of succinate and radicals may also serve as signals inducing endogenous protective mechanisms.

Aims: Elucidation of functional relationships between calcium and MIT signaling, MIT membrane fluidity (MMF) and transmembrane potential (MMP), CAT and MIT energy transition pores (METP) formation in heart MIT of rats with acute (8 days) STZ-induced diabetes or in primary adult cardiomyocytes subjected to 48 h hypoxia (2% O_2).

Methods: estimation of MMF, MMP and CAT with fluorescent probes DPH, JC1 and Fluo-3; METP detection - cytochemistry and stereology; hypoxic genes (HG: HIF 1- and 2-α, VEGF, GLUT-1, iNOS and Ca IX) expression detected by RT-PCR and immunoblotting.

Results: Diabetic heart exhibited: increased CAT amplitude, 98 % increased METP formation, preserved myocardial ATP content, negative correlation between descending MMP and increasing MMF and MIT signaling with succinate and radicals. The latter inhibited the breakdown of the HIF and induced increased expression of the HG. Conclusions: In acutely diabetic or hypoxic hearts, increased CAT augments the capacity of ATP delivery to sarcoplasma via METP. In diabetic heart the formation of METP coupled with increased MMF and decreased MMP tends to preserve the total pool of ATP. Concomitantly, succinate and radicals extruded from hypoxic and diabetic heart MIT break the oxygen sensing (preserve the HIF) and augments the expression of HG. Support: VEGA grants 2/7126/27; 1/0755/09; 2/0054/11, 2/0115/10 2/0101/12 and 1/0142/09.

P-C1:

A ROLE OF CaMKIIδ IN CARDIAC INJURY CAUSED BY ISCHEMIA AND REPERFUSION

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Introduction: Although Ca^{2+}/calmodulin-dependent protein kinase II delta (CaMKIIδ) has been emerged as an important molecule in the development of cardiac diseases; the exact CaMKIIδ-dependent signalling pathways leading to contractile dysfunction, ventricular arrhythmias, and apoptosis, main phenotypes of myocardial ischemia/reperfusion injury, are not sufficiently elucidated.
Objectives: To investigate this, KN-93 (0.5 μmol/L), a CaMKII inhibitor, was given before induction of global ischemia and reperfusion in the isolated Langendorff-perfused rat hearts.
Materials & methods: Expression of CaMKIIδ and the sarcollemal Ca^{2+}-cycling proteins, known to be implicated in the reperfusion-induced disturbances of excitation-contraction coupling, was analyzed using immunoblotting. In addition, the content of proteins of intrinsic apoptosis pathway was analyzed.
Results: KN-93 reduced reperfusion-induced ectopic activity and the incidence of ventricular fibrillation. Likewise, the severity of arrhythmias was lower in KN-treated hearts. During pre-ischemia phase, neither inotropic nor chronotropic effects were elicited by KN-93, whereas post-ischemic contractile recovery was significantly improved. Ischemia/reperfusion increased the expression of CaMKIIδ and sodium-calcium exchanger (NCX1) proteins without any influence on the protein content of alpha 1c, a pore-forming subunit of L-type calcium channels (LTCCs). On the other hand, inhibition of CaMKII normalized the alterations in the expression of CaMKIIδ and NCX1. Bax/Bcl-2 ratio, cytochrome C and caspase-9 levels were increased in the ischemia/reperfused hearts, while CaMKII inhibition was capable to reduce these levels.
Conclusion: CaMKIIδ seems to regulate its own turnover and to be an important component of cascade integrating NCX1 rather than LTCCs that promotes Ca^{2+}-overload and consequently induces cardiac injury due to ischemia/reperfusion.

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P-C2:

THE EFFECT OF GST INHIBITION ON CELL VIABILITY AND MAPK PATHWAYS ON CULTURED CARDIOMYOCYTES IN THE PROCESS OF ISCHAEMIC POSTCONDITIONING

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Introduction: Ischaemic postconditioning (IPoC) is a strategy to protect heart against ischaemia and reperfusion (I/R) injury. Glutathione-S-transferase (GST) is an important antioxidant enzyme, a crucial determinants of the development of I/R injury and plays crucial role in regulating mitogen activated protein kinase (MAPK) pathways involved in cellular response to stress.
Objectives: The aim of the study was to investigate whether IPoC could be protective against the harmful effect of GST inhibition (by ethacrynic acid - EA) and assess the alteration of the activities of MAPK pathways.
Materials & methods: Experimental groups from primary culture of neonatal rat cardiomyocytes: non-treated cells (I), cells treated with EA (II), sI/R (III), sI/R and IPoC (IV), sI/R and EA (V), sI/R and IPoC together with EA (VI). Viability of cells were measured by MTT assay, the amount of apoptotic cells were assessed by flow cytometry following annexin V-FITC/propidium iodide double staining. The activation of JNK, p38, ERK/p42-p44 MAPKs and GSK-3 protein kinase were determined by flow cytometry.
Results: GST inhibition attenuate the protective effect of IPoC which resulted in increasing apoptosis and decreasing cardiomyocyte cell viability. JNK activation was elevated in GST inhibited groups while the p38

phosphorilation was diminished. The ratio of phospho-ERK/p42-44 and phospho-GSK-3 was significantly higher in IPoC groups.

Conclusion: GST activity is required for survival of cultured cardiomyocytes under stress conditions despite the application of IPoC confirming that GST plays an important role as regulator of MAP kinase pathways in I/R injury.

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P-C3:

MYOCARDIAL PHOSPHODIESTERASE 5 OVEREXPRESSION MODULATES CARDIOMYOCYTE CONTRACTILITY

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Introduction: Cardiovascular diseases (e.g. ventricular hypertrophy, pulmonary hypertension) associated with disorders in nitric oxide- and natriuretic peptide-signaling, are supposed to be accompanied by enhanced phosphodiesterase 5 (PDE5) expression, which can lead to the decrease of protein kinase G (PKG)-mediated phosphorylation processes.

Objectives: In the present study effects of PDE5 overexpression on cardiomyocyte contractility were investigated.

Materials & methods: Myocardial tissue samples derived from wild type (control) and transgenic mice with PDE5-overexpression (TG) after transverse aortic constriction. Isolated permeabilised cardiomyocytes were used to determine calcium-activated active force, passive force component and calcium-sensitivity of force production (pCa_{50}), at sarcomere lengths (SLs) of 1.9 μm and 2.3 μm. In a separate cardiomyocyte population isometric force measurements were performed before and after in vitro PKG administration.

Results: Stretching of the cardiomyocytes resulted in an increase in calcium-sensitivity of force production (pCa_{50}~0.1), indicating the preservation of the Frank-Starling mechanism. Passive force measured at SL of 2.3 μm was significantly higher in TG myocytes compared to controls, and decreased after PKG treatment in higher degree than the lower basal passive force in controls (control before PKG: 1.66±0.17 kN/m^2;TG before PKG: 3.00±0.52 kN/m^2; control after PKG: 1.09±0.13 kN/m^2;TG after PKG: 1.60±0.17 kN/m^2). PKG administration significantly reduced calcium-sensitivity in both groups at similar extent.

Conclusion: To summarize our results, genetic modification did not influence the myofibrillar length-dependent calcium-sensitisation. PDE5 overexpression significantly increased passive tension, which could be reduced by in vitro PKG administration. Molecular mechanisms responsible for the mechanical distinctions are likely alterations in phosphorylation of myofilament proteins (e.g. titin) responsible for passive cardiomyocyte characterics.

P-C4:

CARDIOPROTECTIVE EFFECT OF DELAYED ISCHAEMIC POSTCONDITIONING IS MEDIATED BY MITOCHONDRIAL KATP CHANNELS IN THE RAT HEART IN VIVO

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Introduction: Recent study in mice demonstrated that delaying the ischaemic postconditioning (IPost) intervention up to 30 min after the onset of reperfusion does not abrogate its cardioprotective effect. However, the mechanisms underlying IPost cardioprotection are still unclear.

Objectives: The first aim of this study was to determine the cardioprotective effect of IPost, when this stimulus is applied at different time points after the onset of reperfusion. The second aim was to examine the role of mitochondrial KATP-channels (mitoKATP) in mediating the cardioprotective effect of IPost.

Materials & methods: Rats were subjected to 30 min of coronary artery occlusion followed by 120 min of reperfusion. Control group (n = 8) was subjected to ischaemia/reperfusion only. IPost protocol included 6 brief (10 s each) cycles of ischemia separated by 10 s of reperfusion and were applied at various time points after the onset of reperfusion - 10 s (IPost10", n = 6), 10 min (IPost10', n = 6), 30 min (IPost30', n = 6), 45 min (IPost45', n = 6) and 60 min (IPost60', n = 6). To determine the role of mitoKATP in cardioprotection conferred by delayed IPost, the mitoKATP antagonist 5-HD (10mg/kg, i.v) was administered to the animals (n = 6) at the onset of reperfusion.

Results: IPost stimuli applied 10 s or 10, 30 and 45 min after the onset of myocardial reperfusion reduced infarct sizes by 35%, 38%, 40% and 40% respectively (all $p<0.01$). IPost60' was ineffective ($p>0.05$). 5-HD completely abolished cardioprotective effect induced by IPost10'.

Conclusion: IPost confers potent cardioprotection even if applied 45 min after the onset of myocardial reperfusion in the rat heart in vivo. This cardioprotective effect of delayed postconditioning is mediated by mitoKATP.

P-C5:

REMOTE ISCHAEMIC PRE- AND DELAYED POSTCONDITIONING – SIMILAR DEGREE OF CARDIOPROTECTION BUT DISTINCT MECHANISMS

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Introduction: Myocardial ischemia/reperfusion injury can be significantly reduced by episode(s) of ischemia/reperfusion applied prior or during myocardial ischemia (MI) to tissue located at a distance from the heart – the phenomenon called remote ischemic conditioning (RIc).

Objectives: The primary aim of this study was to determine the efficacy of RIc in protecting the heart when applied prior, during and at different time points after MI. The second aim was to determine whether neural mechanisms mediate cardioprotection induced by RIc when applied prior and after MI.

Materials & methods: Rat model of myocardial ischemia/reperfusion injury involved 30 min of left coronary artery occlusion followed by 120 min of reperfusion. RIc was induced by 15 min occlusion of femoral arteries and applied either 25 min prior to MI, 10 min or 25 min after the onset of MI, and starting 10 or 30 min after the onset of reperfusion.

Results: RIc conferred potent cardioprotection when applied prior to MI, during MI (10 and 25 min) and as late as 10 min after the reperfusion onset (reduction in infarct size by 54%, 56%, 56%, and 48% (all $P<0.001$), respectively). RIc applied 30 min into the reperfusion was ineffective. Activation of sensory nerves by capsaicin application was only effective in establishing cardioprotection when elicited prior to MI. Vagotomy or denervation of the peripheral ischemic tissue both completely abolished cardioprotection induced by RIc applied prior to MI, but had no effect on cardioprotection conferred by delayed RIc.

Conclusion: RIc establishes potent cardioprotection even if applied with a significant delay after the onset of myocardial reperfusion. Cardioprotection induced by remote ischemic preconditioning is critically dependent on afferent innervation of the remote organ and intact parasympathetic activity, while delayed remote postconditioning appears to rely on a different mechanism(s).

P-C6:

DIFFERENT VASCULAR EFFECTS OF HYDROGEN-PEROXIDE IN RAT MICROVESSELS

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Introduction: Hydrogen-peroxide (H_2O_2) has been reported to either relax or contract blood vessels depending on animal species or vessel types.

Objectives: We used various inhibitors of putative cellular signal transduction pathways to investigate the vascular effects of H_2O_2.

Materials & methods: The changes of the diameter of isolated, cannulated and pressurized (80 mmHg) arterioles were measured obtained either from skeletal muscles (diameter: 168±6 μm) or from the heart of the rat (diameter: 120±9 μm).

Results: H_2O_2 produced a biphasic response in the gracilis arteries: it evoked constrictions at lower concentrations (10 μM-100 μM; 34±3% constriction at 100 μM) and dilations at higher doses (3 mM-10 mM; 80±11% at 10 mM). In contrast, H_2O_2 caused only vasodilation (96±3%) in coronary arterioles. Thromboxane A2 receptor antagonist (SQ-29548, 1μM) abolished the H_2O_2 evoked vasoconstriction in gracilis arterioles, but did not affect dilation in coronary (96±2%) or gracilis arteries (99±3%), even if thromboxane A2 receptor stimulation (U46 0,1 nM-10 μM) evoked vasoconstriction both in skeletal muscle (69±2%) and in coronary arterioles (42±6%). H_2O_2 induced contractions (tested at 100 μM) of skeletal muscle arterioles were inhibited by preincubation with antagonists of cyclooxygenase (indomethacin 10 μM; 50±17% dilation), PLA (7,7-Dimethyl-(5Z,8Z)-eicosadienoicacid 100 μM; 9±2% constriction), PKC (chelerytrine, 10 μM; 8±13% dilation) and src kinase (Src kinase inhibitor 1 1 μM; 8±3% constriction).

Conclusion: Our data suggest that the H_2O_2 evoked vasoconstriction involves the sequential activation of src kinase, PKC,PLA, cyclooxygenase, increased synthesis of thromboxane A2 and the activationof thromboxane

A2 receptors. Moreover, coronary arterioles do not have the samefunctional pathway, suggesting differences in H_2O_2 mediated physiological responses in these arteriolar beds.

P-C7:

AGEING ASSOCIATED DECREASE IN CARDIAC MITOCHONDRIA FUNCTIONS IN HEALTHY RATS

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Introduction: Ageing is characterized by progressive decline in physiological functions, yet conflicting results are reported for the respiratory properties of isolated mitochondria describing either no changes or a decrease in respiratory rates with some but not with other substrates.

Objectives: The present study was purported to compare respiratory rates, mitochondrial membrane potential () and the sensitivity of the mitochondrial permeability transition pore (mPTP) to calcium (Ca^{2+}) in heart mitochondria isolated from old vs. adult rats.

Materials & methods: For the respirometry and y measurements an Oxygraph-2k Oroboros Ltd. equipped with an ion selective electrode filled with tetraphenylphosphonium electrode was used. The amount of total mitochondrial Ca^{2+} retained before opening of mPTP was measured spectrofluorimetrically and compared to the effect elicited by the classical pore desensitizer, cyclosporine A (CsA).

Results: Our results showed an important decline for y and the LEAK state, OXPHOS capacity, ETS and RCR (calculated as the ratio between OXPHOS and LEAK states) for both complex I and complex II supported respiration and an increased sensitivity to Ca^{2+}-induced mPTP opening in the old animals as compared to the adult ones. The protective effect of CsA on the phenomenon of permeability transition was also significantly reduced.

Conclusion: Our data suggest that a decrease in several mitochondria functions is associated with healthy ageing and these effects are independent of the respiratory substrates that are used.

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P-C8:

PROARRHYTHMIA PREDICTORS IN A REDUCED-REPOLARISATION RESERVE ISOLATED RABBIT HEART MODEL

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Introduction: The reduction of the repolarization reserve with inhibition of IKs augmented the proarrhythmic activity of dofetilide (a selective inhibitor of IKr) and led to frequent occurrence of Torsades de Pointes (TdP) and increased the sinus beat-to-beat variability of ECG intervals (BVE) in anaesthetized rabbits in vivo.

Objectives: We sought to test the effect of the reduced-repolarization reserve with IKs inhibition (HMR-1556) on proarrhythmias and on proarrhythmia predictors in an isolated, Langendorffperfused rabbit heart proarrhythmia model.

Materials & methods: 4 groups (n=8) of whole hearts from adult female NZW rabbits were compared: In the presence of cathecolamine, the hearts were perfused with: 50 nM dofetilide (DOF), 250 nM HMR + 50 nM dofetilide (HMR+DOF), Krebs (CAT CONTROL), Krebs without catecholamine (CONTROL). ECG intervals were measured; arrhythmia analysis was performed; various sinus and absolute beat-to-beat variability of ECG intervals (BVE) were assessed.

Results: HMR did not increase the incidence of arrhythmias vs. DOF group. TdP occurrence was only 13% in both groups. There was no difference neither in the onset times of arrhythmias nor in the sinus ECG values nor in the sinus BVE data between the groups. However, the absolute BVE values of HMR+DOF group were significantly higher than those of DOF group (STV QT: 7.66±1.59 vs. 2.78±0.74).

Conclusion: Only the absolute BVE biomarker was sensitive enough to reveal significant difference between the single IKr and the combined IKr+IKs pharmacologically-reduced repolarisation groups in isolated rabbit hearts. This emphasizes the importance of the validation of the absolute BVE and this *in vitro* reduced repolarisation-reserve model.

P-C9:

ROLE OF GAP JUNCTIONS IN CARDIAC PACING-INDUCED DELAYED ANTIARRHYTHMIC PROTECTION

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Introduction: We have shown previously that gap junctions (GJ) participate in the early phase of ischaemic preconditioning.

Objectives: The aim of this study was to examine whether gap junctions play a role in the delayed antiarrhythmic effect of cardiac pacing with particular reference to the alterations in connexin43 (Cx43) expression and GJ function during coronary artery occlusion and reperfusion, 24h later.

Materials & methods: Adult mongrel dogs were paced through the right ventricle (4x5 min at a rate of 240beats·min^{-1}) and 24 h later were subjected to a 25 min occlusion the left anterior descending coronary artery (LAD) followed by reperfusion.

Results: Compared to the sham-paced controls (n=20), in paced dogs (n=15) the severity of ventricular arrhythmias was markedly reduced (VPBs: 294±78 vs 63±25, VT episodes: 7.4±2.2vs 1.1±0.6) during occlusion and survival increased from 20% to 78% after reperfusion. Changes in tissue resistivity and phase angle shift, signs of gap junctional uncoupling, were also attenuated in the paced dogs. Ischaemia severity, assessed by changes in epicardial ST-segment and total activation time, was also significantly less marked in paced than in control animals. Pacing also prevented the reduction in permeability and the marked dephosphorylation of Cx43 that occurred in non-paced controls. Similarly, immunofluorescence images showed that pacing prevented the ischaemia-reperfusion induced structural impairment of intercalated discs which was apparent in the controls.

Conclusion: We conclude that cardiac pacing alters gap junction function, which may associate with the reduction of arrhythmias during a subsequent coronary artery occlusion 24h later.

This study was supported by OTKA (No. K75281).

P-C10:

R-L3 ENANTIOMERS HAVE ADVERSE MODULATING EFFECTS ON IKS IN RABBIT VENTRICULAR MYOCYTES

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Introduction: Activators of the slow delayed rectifier K^+ current (IKs) have been suggested as promising tools to suppress ventricular arrhythmias due to prolongation of repolarization.

Objectives: A recently synthesized compound, L-364,373 (R-L3), was nominated to activate IKs in ventricular cells isolated from guinea pigs, rabbits and dogs.

Materials & methods: Therefore, the aim of the present study was to analyse the effect of the R-L3 enantiomers on I_{Ks} current in isolated ventricular rabbit myocytes, by applying the whole-cell patch clamp technique at 37°C.

Results: We have synthesised two substances, ZS 1271B_R (right) and ZS 1271B_L (left) the two enantiomers of R-L3. In rabbit myocytes, ZS 1271B_R (1 μM) enhanced the I_{Ks} tail current by about 30% (at 40 mV, I_{Ks} tail current amplitude increased from 45.9±4.9 pA to 66.09±4.54 pA, after drug superfusion, n=6), while the left enantiomer ZS 1271B_L (1 μM) reduced I_{Ks} tail current by approximately 47% (at 40 mV, I_{Ks} tail current amplitude decreased from 97.1±9.7 pA to 45.8±11.6 pA after drug superfusion, n=6).

Conclusion: These results indicate that the two enantiomers of R-L3 have adverse modulating effects on I_{Ks} in the same concentration range, which may explain why the racemic drug R-L3 failed to activate I_{Ks} in previous studies. ZS 1271B_R is a potent activator of I_{Ks}, therefore, this substance is adequate to test whether I_{Ks} activators are indeed ideal tools to suppress ventricular arrhythmias originating from prolongation of action potentials.

P-C11:

ACUTE INHIBITION OF MONOAMINE OXIDASES DOES NOT MODIFY THE SEVERITY OF ISCHAEMIA AND REPERFUSION-EVOKED VENTRICULAR ARRHYTHMIAS IN DOGS

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Introduction: Enzymatic degradation of biogen amines by monoamine oxidases (MAOs) produces hydrogen-peroxide (H_2O_2) which is considered to contribute myocardial ischaemia and reperfusion (I/R)-induced oxidative injury.

Objectives: In the present work, we examined whether acute administration of pargyline, an irrreversible inhibitor of MAOs modifies ventricular arrhythmias during I/R in anaesthetized dogs.

Materials & methods: In two groups, saline (C; n=7) and pargiline (P;n=7, 10 mg/kg) were administered in intravenous bolus injection 15 min prior to a 25 min occlusion of the left anterior descending coronary artery. Severity of ventricular arrhythmias (VPBs, VT, VF), plasma adrenaline (A) and noradrenaline (NA) levels, as well as H_2O_2 and superoxide (O_2^-) productions were assessed.

Results: Compared to the controls, pargyline did not modify arrhythmia severity (VPBs: 319 ±123 vs 305 ±128; VT: 5.6 ±2.4 vs 5.0± 2.2; VT%: 86 vs 100%; VF%: 29 vs 14%; Survival: 0% vs 14%) either during occlusion or reperfusion. There were also no marked differences between the two groups in blood and tissue H_2O_2 levels or O_2^- generation. In both groups, A and NA concentrations were only increased during reperfusion, and in this respect there were no differences between the treated and untreated animals.

Conclusion: The results show that a single dose of pargyline did not influence the I/R-induced ventricular arrhythmias and the generation of H_2O_2, from which we conclude that H_2O_2, derived from the degradation of cathecolamines by MAO, does not play an essential role in the acute I/R-induced arrhythmias at least in dogs.

P-C12:

CARDIOMYOCYTE CONTRACTILE DYSFUNCTION IN THE HUMAN MYOCARDIUM: THE ROLE OF MYOFILAMENT PROTEIN OXIDATION

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Introduction: Oxidative myofilament protein alterations have been shown to contribute to myocardial contractile dysfunction.

Objectives: We aimed to characterize the effects of myeloperoxidase (MPO) on cardiomyocyte function and to identify the related myofilamentary protein modifications in left ventricular human myocardium.

Materials & methods: Ca^{2+} dependent (F_{active}) and independent ($F_{passive}$) forces were measured in permeabilized cadiomyocytes before and after applications of hydrogen peroxide (H_2O_2), MPO, MPO-inhibitor (MPO-I) and the reducing agent dithiotreitol (DTT). Ellman's and sulfhydryl (SH) group biotinylation assays were used to quantify the extent of protein SH group oxidation.

Results: Application of H_2O_2 significantly decreased cardiomyocyte F_{active} (23.1±3.7 kN/m^2 vs. 16.0±2.8 kN/m^2, P<0.01) and increased $F_{passive}$ (3.5±0.9 kN/m^2 vs. 4.0±0.9 kN/m^2,P<0.01). When H_2O_2 and MPO were applied together, a reduction in F_{active} and an additional increase in $F_{passive}$ were observed. MPO-I partially prevented the effects on F_{active} and $F_{passive}$ (16.3±3.4 kN/m^2 vs. 11.1±1.6 kN/m^2;and 1.8±0.4 kN/m^2 vs. 2.3±0.5 kN/m^2 (n=5), respectively).Combined application of H_2O_2 and MPO significantly decreased the relative SH content of myofilament proteins (87.04±1.2% vs. 100%,P<0.05), which effect was reversed by the reducing agent DTT. DTT also reversed the MPO-induced increase in $F_{passive}$ (2.4±0.3 kN/m^{2}vs. 1.4±0.2 kN/m^2). H_2O_2 and MPO significantly decreased the number of SH groups of the actin and a recently unidentified ~60kDa molecular weight protein.

Conclusion: MPO-derived oxidants may contribute to myocardial contractile dysfunction via decreasing cardiomyocyte force production and increasing $F_{passive}$ of human cardiomyocytes. These effects are mainly attributed to myofilament protein oxidation and could be partially prevented by MPO-inhibition.

P-C13:

THE EFFECT OF ACUTE SIMVASTATIN ADMINISTRATION ON VENTRICULAR ARRHYTHMIAS IN A CANINE MODEL OF ISCHAEMIA AND REPERFUSION

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Introduction: Statins are used as cholesterol-lowering drugs, but their lipid-independent pleiotropic effects which are thought to contribute to the cardioprotection, is still not fully understood.
Objectives: In this study we investigate the effect of a single bolus injection of simvastatin (0.1 mg/kg) on the ischaemia and reperfusion-induced ventricular arrhythmias in chloralose-urethaneanesthetized dogs.
Materials & methods: In one group of dogs (n=7) activated simvastatin, in the other group (n=8) the solvent of simvastatin were given just prior to a 25 min occlusion of the left anterior descending coronary artery. Severity of ischaemia (degree of inhomogenity of electrical activation, epicardial ST-segment) and of arrhythmias, as well as plasmaNOx levels were assessed during the occlusion.
Results: Compared to the control group, simvastatin decreased the total numbers of VPBs (206 ±44 vs. 139 ±50) and episodes of VT (5.8 ±1.9 vs. 0.5 ±0.3), the incidences of VT (70% vs. 38%) and VF (40% vs. 0%) during the occlusion. Survival from the combined ischaemia/reperfusion was also higher (38%) in the simvastatin treated group than in the controls (0%). Whereas in control group the plasma level of NO metabolites in the coronary sinus blood was markedly reduced, in simvastatin treated animals NOx levels were significantly increased particularly during the second phase of the occlusion.
Conclusion: We conclude that acute simvastatin treatment may provide protection against the ischaemia and reperfusion-induced severe ventricular arrhythmias perhaps through the elevation of NO availability during ischaemia.

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P-C14:

TRANSIENT OUTWARD POTASSIUM CURRENT IN DOG ATRIAL PREPARATIONS

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Introduction: The transient outward current (I_{to}) is an important repolarizing current in the atrial muscle.
Objectives: The aim of the present study was to investigate the role of Ito in atrial repolarization.
Materials & methods: I_{to} current was recorded by the whole-cell configuration of the patch clamp technique.
Results: Large I_{to} current was observed in dog atrial myocytes. Current amplitude was 15.2±1.1 pA/pF at 50 mV (n=11). The inactivation phase of the current was fitted by two exponentials (Tau1: 11.3±1.2 ms, Amp1: 10.4±0.8 pA/pF; Tau2: 116.1±8.2 ms, Amp2: 3.7±0.5 pA/pF; n=10, test potential: 50 mV). I_{to} current during the action potential plateau phase was measured as chromanol 293B sensitive current, which drug effectively suppressed not only the slow delayed rectifier potassium current but also I_{to}. An atrial action potential waveform was used for the command potential. It was found that I_{to} carries large outward current in the plateau phase of the atrial action potential. The effects of I_{to} blockade on the action potential repolarization were investigated in dog atrial muscle by the conventional microelectrode technique. The current was inhibited by application of 100 μM chromanol. The drug increased the plateau potential but failed to lengthen the action potential in atrial preparations. However, the amplitude and kinetics of the slow phase of I_{to} inactivation implied a substantial I_{to} current during the plateau phase of the atrial action potential, blockade of the current did not lengthen the action potential in dog atrial muscle.
Conclusion: Thus, understanding the role of transient outward potassium current in atrial repolarization requires further investigations.

P-C15:

GLOBAL AND CELLULAR ALTERATIONS OF MYOCARDIAL CONTRACTILITY IN A RAT MODEL OF CALCIUM PARADOX

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Introduction: Impaired myocardial contractile function is one of the main features of hearts affected by Ca^{2+} paradox that presumably develops as part of ischemia-reperfusion injury.

Objectives: We aimed to explore the potential mechanisms underlying the decreased global contractility in Ca^{2+} paradox at the level of cardiomyocytes and myofibrils.

Materials & methods: Isolatedrat hearts were perfused with physiologic Krebs-Henseleit solution by using the Langendorff method. Following stabilization, hearts were exposed to constant solution (Cont), Ca^{2+}-depletion (CaFree) and Ca^{2+}-repletion after Ca^{2+}-depletion (CaPD), while global contractile parameters were registered. The contractility of isolated cardiomyocytes from perfused right ventricles was measured, just as the activity of Ca^{2+}-stimulated and Mg^{2+} ATPase of myofibrils.

Results: The systolic left ventricular pressure developed by the Cont animals (127.4±6.1Hgmm) dramatically decreased during Ca^{2+}-depletion (9.8±1.3Hgmm) and Ca^{2+}-repletion (12.9±1.3Hgmm) with similar changes of +dP/dt and –dP/dt. In contrast, end-diastolic pressure was increased in both CaFree and CaPD groups. Although the maximal force of isolated cardiomyocytes did not considerably differ between these groups, the passive force was higher in the CaPD samples. Ca^{2+}-sensitivity of the CaPD cells was comparable to the Cont cells, while it was somewhat increased in the CaFree samples. Myofibrillar Ca^{2+}-stimulated ATPase activity was decreased in the CaFree group (Cont:12.08±0.57; CaFree:8.13±0.19 μmol Pi/mg protein/h), beside unvarying Mg^{2+} ATPase activity. CaPD samples also showed lower Ca^{2+}-stimulated ATPase (8.4±0.22 μmol Pi/mg protein/h) but higher Mg^{2+} ATPase activity (Cont:3.20±0.25; CaPD:7.21±0.36 μmol Pi/mgprotein/h).

Conclusion: Both, increased passive force of cardiomyocytes and decreased Ca^{2+}-stimulated ATPase activity may play a role in the development of impaired contractile function of CaPD hearts.

P-C16:

GENE EXPRESSION CHANGES IN THE CANINE HEART FOLLOWING RAPID CARDIAC PACING

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Introduction: In our previous study, numerous genes were found with altered expression in the delayed phase of preconditioning (PC) induced by rapid cardiac pacing. These genes encoding anti-apoptotic (Bcl2) or apoptotic proteins (casp3, casp9, BAX), antioxidants (MnSOD), NO producing enzymes (eNOS, iNOS) or members of signaling pathways are already known to be important in the delayed cardioprotection.

Objectives: The aim of this study was to examine the time course expression of these genes after rapid cardiac pacing in dogs.

Materials & methods: Under light pentobarbital anaesthesia, a pacing electrode was introduced into the right ventricle, through which the heart was paced (P group, n=12) four times for 5 min at a rate of 240 beats/min. Another group of dogs, instrumented but not paced, served as sham paced controls (SP, n=12). Three-three dogs form each group were euthanized at various time points; i.e. immediately (P-0h/SP-0h), six (P-6h/SP-

6h), twelve (P-12h/SP-12h) or twenty-four (P24h/SP-24h) hours after the pacing stimulus, and tissue samples were taken for RT-PCR, westernblot and immunofluorescent analyses.

Results: In the paced groups, genes encoding MnSOD, iNOS, eNOS, Bcl2 were up-regulated, whereas casp3, casp9, Bax were down-regulated. Cardiac pacing resulted in marked reduction of Cx43 protein content at 12hrs preceded by down-regulation of Cx43 mRNA at 6hrs.

Conclusion: We conclude that cardiac pacing induces time-dependent changes in expression of numerous genes and alters the protein content. For example, changes in gene and protein expression of Cx43 may alter gap junctions and thus influence arrhythmia generation following a subsequent ischaemia/reperfusion insult.

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P-C17:

INFLUENCE OF MATURATION ON RESISTANCE TO ISCHEMIA-REPERFUSION INJURY IN LANGENDORFF- PERFUSED FEMALE RAT HEARTS

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Introduction: Maturation represents an important risk factor for development of cardiovascular diseases. Those older than 65 years account for more than 80% of patients with ischemic heart disease. Susceptibility to arrhythmias demonstrated in humans and in animal studies is increased with age. PI3K and its downstream effectors, the system playing a crucial role in protection against ischemia/reperfusion (I/R) injury (ischemic preconditioning, IPC) has been found to have an increased importance in female hearts. Previously, we demonstrated greater resistance to I/R in female compared to male hearts.

Objectives: The present study was designed to investigate the role of maturation in response of female hearts to I/R injury, in protection by IPC and to explore a potential role of PI3K/Akt in the hearts of older female rats.

Materials & methods: IsolatedLangendorff-perfused non-preconditioned (C) and preconditioned (I/R, 5 mineach) hearts of 12- and 18-week-old female Wistar rats were subjected toregional ischemia with or without prior 15-min perfusion with PI3K inhibitorwortmannin (W).

Results: Maturationdid not modify the size of infarction (IS) in C, whereas infarct size(IS)-limiting effect of IPC in young adults was diminished in 18-week-old group.In preconditioned 12-week-old group, W increased IS, while inhibition ofPI3K/Akt did not blunt antiinfarct effect of IPC in 18-weekold group. Maturationpartially reduces the potential for activation of endogenous protection inheart.

Conclusion: Activationof PI3K/Akt during IPC plays a critical role in protection against lethal I/Rinjury conferred by IPC in 12-week-old females, while in older female hearts itappears to be PI3K/Akt-independent.

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P-C18:

TRANSGENIC MOUSE MODEL OF LQT5 SYNDROME

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Introduction: The aim of our experiments was to create a transgenic mouse model with a transgene construct carrying a missense mutation G52R-KCNE1, first identified in a long QT syndrome family (Ma et al., 2003). The missense mutation G to A at position 154 in the KCNE1 gene leads to an amino acid substitution of arginine for glycine at position 52. The mutant G52RKCNE1 has a dominant negative effect on IKs current, targeting the beta IKs channel subunit.
Objectives: The ultimate aim of the experiments is to create transgenic rabbits with the same G52R-KCNE1 mutation. Those transgenic rabbits could than utilised in pharmacological tests of candidate drugs with the risk of long QT syndrome and torsade de pointes.
Materials & methods: Transgenic mice were created with pronucleus microinjection and lentiviral transgenesis (Kvell et al 2010). The DNA construct harbours the 4.6 kb rabbit myosin heavy chain heart specific promoter and the mutant human KCNE1 cDNA. In anesthetised mice ECG was recorded using the conventional limb leads to verify the development of long QT syndrome. Left ventricular dimensions and pump function were also assessed by echocardiography.
Results: The two transgenic lines express tissue specifically the mutant human KCNE1 mRNA in the heart. In vivo measurements are in progress to obtain data on the electrophysiology and mechanical performance of transgenic compared to control mouse hearts.
Conclusion: The transgenic mouse lines expressing the human G52R-KCNE1 mutant protein serve to examine the tissue specificity and functionality of the transgene construct and to perform basic physiological tests.

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P-C19:

EFFECTS OF A POLYUNSATURATED FATTY ACID-RICH DIET ON THE DEVELOPMENT OF HEART FAILURE AFTER MYOCARDIAL INFARCTION IN RATS

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Introduction: Polyunsaturated fatty acid-rich diets decrease the risk of myocardial infarction and sudden cardiac death.
Objectives: The aim of our investigations was to study the effect of long-term polyunsaturated fatty acid-rich diet, started after the induction of myocardial infarction, on the development of heart failure in rats.
Materials & methods: Myocardial infarction was induced in male Sprague-Dawley rats by ligation of the left main coronary artery during ether anesthesia. Two weeks later in the animals surviving the acute phase of myocardial infarction, animals were randomly allocated to receive a diet enriched with 10% pork fat (containing saturated fatty acids; SF) or 10% sunflower-seed oil (containing linoleic acid, as PUFA) for 10 weeks. At the end of the feeding period during pentobarbitone anesthesia a transthoracic echocardiography was performed (GE Vivid S5, 12 MHz probe).
Results: Ejection fraction, fractional shortening and the cardiac output all decreased in the saturated fat treated animals after 10 weeks myocardial infarction, compared to the sham operated animals (48±7.1 vs.

66±7.0 %; 22±4.1 vs. 34±5.5 %; 15,2±1.2 vs. 20.3±2.8 ml/min/100g, respectively). There was no significant difference in these parameters after feeding PUFA diet, as compared to the SF diet.

Conclusion: The present investigations may suggest that –in spite of the well known effects of a polyunsaturated fatty acid-rich diet in preventing the development of acute myocardial infarction –a similar diet started 2 weeks after the induction of myocardial infarction in rats does not offer significant protection against the progression of heart failure.

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P-C20:

PARTIAL NCX INHIBITION EXERTS PROTECTIVE ROLE AGAINST NA^+ INDUCED CA^{2+} LOAD BY RESTRICTING $[CA^{2+}]I$ ELEVATION IN CANINE VENTRICULAR MYOCARDIUM

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Introduction: The late sodium current (INaL) has important role in maintaining the plateau phase of the action potential (AP). Excessive increase of the INaL leads to serious arrhythmias via Na^+- induced Ca^{2+}-load and by prolonging the action potential duration (APD). The Na^+-Ca^{2+} exchanger (NCX) has been suggested to play important role in development of these arrhythmias. In this study we hypothesize that partial inhibition of NCX may exert protective effect against arrhythmias.

Objectives: Our aim was to investigate the partial inhibition of NCX during Na^+-induced Ca^{2+}- load.

Materials & methods: The currents were measured by patch clamp technique while the Ca^{2+}-transients were monitored by fluorescent optical method in canine single ventricular cells. The APs were measured by conventional microelectrode technique in right ventricular myocardium.

Results: 2 nM ATX-II increased the I_{NaL}, causing APD prolongation, and marked increase in Ca^{2+}-transient, simultaneously with the cell shortening. During normal condition the SEA0400, despite the significant inhibition of NCX current, failed to influence neither the APD nor the Ca^{2+} transients. In field stimulated cells, pretreatment of 1M SEA0400 prevents the I_{NaL}-induced Ca^{2+} -load, and conversely, elevated Ca^{2+}-transients by application of ATX were also reduced by subsequently used SEA0400. The ATX-induced APD lengthening was not modified by SEA0400 administration. However SEA0400 pretreatment moderates the APD prolongation caused by ATX--II.

Conclusion: It appears that during activation of I_{NaL}, the impact of the NCX current on Ca^{2+}-homeostasis is increased, without marked effect on APD. We conclude that the NCX inhibition may provide effective therapeutic option against Na^+-induced Ca^{2+}-load primarily by controlling the Ca^{2+} - homeostasis.

P-C21:

IMPLICATION OF ELECTRICAL COUPLING PROTEIN, CONNEXIN-43, IN TERMINATION OF VENTRICULAR FIBRILLATION AND SINUS RHYTHM RESTORATION DEMONSTRATED IN ISOLATED PERFUSED RAT HEART.

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Introduction: Ventricular fibrillation (VF) is life-threatening arrhythmia, which occurrence precedes alterations in electrical coupling protein, connexin43.

Objectives: The purpose of this study was to examine whether myocardial expression and phosphorylated status of Cx43 is altered due to VF itself and during sinus rhythm restoration.

Materials & methods: Experiments were performed using 10-mth-old, male and female Wistar rats. Isolated spontaneously-beating heart perfused with oxygenated Krebs-Henseleit solution was subjected to the following experimental protocols: (1) ten minutes stabilization, (2) electrically-induced VF lasting two minutes, (3) electrically-induced VF lasting ten minutes, 4) two min lasting VF and its termination by stop perfusion followed by spontaneous sinus rhythm restoration. The hearts were snap frozen in liquid N_2 at each stage and ventricular tissues were taken to detect expression of functional phosphorylated (P) of Cx43 as well as its non-phosphorylated form (nonP).

Results: P-Cx43 as well as ratio of P-Cx43 to total Cx43 decreased due to 10-min VF in both genders and upon 2-min VF in males only. In parallel, there was a significant increase of nonP forms of Cx43 due to 10-min VF and upon 2-min VF in males only. Surprisingly, an enhancement of nonP-Cx43 was detected at the moment of stop perfusion-induced termination of VF followed by sinus rhythm restoration in all rat hearts. It was associated with suppression of PCx43.

Conclusion: VF itself down-regulates myocardial Cx43 that deteriorates over time and inhibits cardioversion. While sudden global myocardial cell-to-cell uncoupling due to stop flow-induced increase of nonPCx43 might be involved in termination of VF followed by restoration of sinus rhythm.

P-C22:

REDUCED REPOLARIZATION RESERVE IN LANGENDORFF PERFUSED RABBIT HEARTS: A NEW PROARRHYTHMIA MODEL

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Introduction: Proarrhythmia screening tests are not sensitive and specific enough. Recently, we developed a new in vitro proarrhythmia model based on reduction of the repolarization reserve. We also developed new biomarkers of electric instability, called 'absolute' beat-to-beat variability and instability parameters of the ECG intervals (ABVI), which predicted drug-induced TdP in anaesthetized rabbits.

Objectives: The aims of the present study were to validate the sensitivity of our model with six reference proarrhythmic drugs and to examine whether ABVI parameters predict TdP in the model.

Materials & methods: Isolated, Langendorff perfused rabbit hearts were used. After 20 minutes of drug perfusion, the I_{Ks} inhibitor HMR1556 was added to the perfusate for 40 min in order to reduce the repolarization reserve. Test drugs were dofetilide, terfenadine, cisapride, sertindole, moxifloxacin and clofilium. We determined the incidence of torsades de pointes (TdP), non-TdPtype ventricular tachycardia (VT). We also determined the ABVI parameters by measuring ECG intervals irrespective of the rhythm in 40 consecutive QRST complexes.

Results: Apart from terfenadine, all drugs significantly increased the incidence of TdP and when repolarization reserve was reduced by HMR 1556. ABVI parameters increased significantly before TdP occurrence.

Conclusion: Reduction of repolarization reserve markedly increased the sensitivity of rabbit hearts to the proarrhythmic activity of drugs and allowed TdP to be regarded as a primary end point, which validated

the model. Increased ABVI parameters predicted TdP occurrence, which suggest that ABVI parameters may be used as biomarkers of TdP. Further examinations are needed to work out why terfenadine did not evoke arrhythmias in the model.

P-C23:

ASSOCIATION OF DIAZOXIDE AND CYCLOSPORINE A ELICIT DELETERIOUS EFFECTS ON MITOCHONDRIAL FUNCTION AFTER PROLONGED GLOBAL ISCHEMIA

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Introduction: In the settings of ischemia/reperfusion injury, mitochondria have recently emerged as the major targets of cardioprotective strategies.

Objectives: We sought to investigate the effects of diazoxide (Dx), an opener of the mitochondrial ATP-dependent potassium channels, cyclosporine A (CsA), a desensitizer of the mitochondrial permeability transition pore and their association at reperfusion on mitochondria isolated from isolated rat hearts subjected to 40 min global ischemia and 15 min of reperfusion, respectively.

Materials & methods: At the end of the reperfusion mitochondria were isolated by the differential centrifugation technique. Calcium retention capacity was evaluated by luminescence spectroscopy using a calcium-sensitive probe. Oxygen consumption was measured by polarographic oxymetry in the presence of complex I (glutamate + malate) and complex II (succinate + amytalto inhibit complex I) substrates. State 2 and ADP-stimulated (state 3) respiratory rates were recorded (as nanoatoms oxygen/min/mgprotein) and the respiratory control index (RCI) was calculated.

Results: In the presence of CsA (but not of Dx) at reperfusion post-40 min of ischemia, mitochondriarespiring on CII (but not ofCI) dependent substrates were particularly resistant to calcium overload($p< 0.001$ vs. the non-treated control). Surprisingly, their association at the very onset ofreperfusion reversed this cardioprotective effect ($p< 0.001$ in CsA &Dx group vs. CsA alone) in thepresence of bothCI and CII supported respiration. In mitochondria respiring on CII substrates, Dx+CsA elicited a significant decrease of RCI.

Conclusion: Our data suggest a deleterious effect of their association on mitochondria isolatedafter a prolonged ischemic period.

Research supported by the NASR grant 42-122 and the HU-RO project 0802/011_AF.

P-C24:

IMPLICATION OF MYOCARDIAL CONNEXIN-43 AND PKC SIGNALING IN ANTIARRHYTHMIC EFFECTS OF OMEGA-3 FATTY ACIDS DEMONSTRATED IN SPONATENOUSLY HYPERTENSIVE RATS

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Introduction: Omega-3 FA exert antiarrhythmic effects in both experimental and clinical setting but mechanisms are not elucidated yet.

Objectives: We tested our hypothesis that omega-3 FA intake can protect of hypertensive rats from malignant arrhythmias via protection of intercellular communication ensured by connexin-43 (Cx43) channels.
Materials & methods: Experiments were conducted on male SHR at early and late stage of diseases as well as age-matched normotensive Wistar rats. Untreated rats were compared with animals supplemented by omega-3 (30 mg/day) for two month. Left ventricular tissues were taken for examination of Cx43 distribution and expression as well as for protein kinases C (PKC), which phosphorylates Cx43. Langendorff-isolated heart was used to test inducible VF
Results: Young SHR rat hearts exhibited LVH associated with enhanced distribution of Cx43 on lateral surfaces of the cardiomyocytes, while old SHR in addition exhibited disordered distribution of Cx43 at the area of fibrosis. Total Cx43 expression was increased in young while decreased in old SHR hearts. However, there was an increase of non-phosphorylated (non functional) form of Cx43 in young and suppression of phosphorylated (functional) forms Cx43 in old SHR hearts, which also exhibited reduction of total Cx43. Young and much more old SHR were prone to inducible VF comparing to normortensive rats. Omega-3 intake resulted in suppression of VF incidence in young and old SHR. Moreover, omega-3 diminished arrhythmogenic Cx43 distribution, attenuated down-regulation of Cx43 and enhanced PKCe expression.
Conclusion: Results indicate that SHR benefit from omega-3 intake due to alleviation of myocardial abnormalities in Cx43 that was associated with suppression of malignant arrhythmias.

P-C25:

ADRENOMEDULLIN, GHRELIN AND LEPTIN AS POTENTIAL BIOMARKERS OF CHRONIC HEART FAILURE: AN EXPERIMENTAL STUDY

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Introduction: Pathophysiology and biomarkers of heart failure are under extensive research. Recently, several regulatory peptides, primarily of non-cardiac origin, including adrenomedullin (ADM), ghrelin (GHR) and leptin (LT) were suggested to be potential biomarkers of heart failure.
Objectives: We aimed to investigate plasma concentrations of the above peptides in comparison with elevation of the marker endothelin-1 (ET-1), during development of experimental heart failure.
Materials & methods: Pacemaker was implanted in dogs (n=9) for rapid right ventricular pacing (240/min). Echocardiographic measurements were performed weekly, functional staging was assessed, and plasma concentrations of ADM, GHR, LT and ET-1 (ELISA) were measured simultaneously.
Results: NYHA IV stage heart failure developed after 22±4 days of pacing. Echocardiography revealed seriously impaired left ventricular function (EF, $p<0.01$), dilation of left (LVEDD, $p<0.01$) and right (RVEDD, $p<0.01$) ventricle, and increasing mitral and tricuspidal insufficiency. Plasma levels of peptides significantly increased during the development of heart failure (ADM: 440±53 vs 292±48 pg/ml, $p<0.01$; GHR: 1655±286 vs 952±139 pg/ml, $p<0.01$; LT: 750±241 vs 434±177 pg/ml; and ET-1: 5.27±0.94 vs 3.68±0.56 pg/ml, $p<0.05$). Positive correlation was found between GHR level and left ventricular end-diastolic and end-systolic diameters (LVEDD: $r=0.44$, $p<0.05$; LVEDS: $r=0.45$, $p<0.05$), as well as between ET-1 concentration and end-systolic diameter (LVEDS: $r=0.48$, $p<0.02$).
Conclusion: Right ventricular tachypacing induced chronic heart failure is suitable for examination of biomarker agents. Significant elevation of plasma adrenomedullin, ghrelin and leptin concentrations during the development of heart failure suggest the possible use of these peptides as novel and sensitive biomarkers of the disease.

P-C26:

ROLE OF A PRIMARY PREVENTION COHORT BASED SCREENING IN THE ADHERENCE OF THE CARDIOVASCULAR TARGET-VALUES

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Introduction: The Budakalász Epidemiology Study is aimed to perform a comprehensive cardiovascular screening programme, including health questionaire and non-invasive tests targeting the adult population (>20y) in a Central-Hungarian town.

Objectives: Estimate the patients' knowledge about their own health and the effectivity of certain therapies.

Patients & methods: In this voluntary programme non-invasive tests (cardiac- and carotid ultrasound, resting blood pressure and ankle-brachial pressure index), venous blood biobanking and laboratory analysis is beeing performed.

Results: Six hundred and five inhabitants have been screened until mid January 2012. Medical history included hypertension in 245 patiens (40,4%), among them 229 are on anti-hypertensive regime, the measured blood pressure, however was in the normal range only in 80 persons (35%). Hyperlipidaemia was previously known in 194 persons (32%), among them 106 persons (54%) takes statin daily. At the time of the screening 79 person, 75% of the treated group had the LDL cholesterol in the normal range. Diabetes was known in 55 persons (9,1%), among them 11 treated with diet, 28 persons receives oral anti-diabetics and 16 of them insulin. Carotid artery disease (intimal thickening, plaque, or stenosis) was formerly known in 29 persons but was found in 321 persons (53,6%). Significant stenosis was detected in none of the screened individuals. Pathological ankle-brachial index, an indicator of potential periferial artery disease (PAD, normal range: 0.9-1.2) was mesured in 130 persons (21,5%), but only 8.1% had PAD in the medical history.

Conclusion: Our screening programme is effective to detect the relevant cardiovascular risk factors and this could ameloriate the population's health-consciousness.

P-C27:

MATRIX METALLOPROTEINASES AND NITROSATIVE STRESS IN PATIENTS WITH CORONARY ARTERY DISEASE

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Introduction: Peroxynitrite-matrix metalloproteinse (MMPs) signalling has been shown to contribute to ischemic heart disease in preclinical models.

Objectives: We have investigated the correlation between hyperlipidemia and nitrosative stress as well as the activation of MMPs in patients with significant coronary artery disease (CAD).

Patients & methods: Venous blood samples were collected from 36 patients with CAD five min before coronaro-angioplasty. Serum total-, LDL-, HDL cholesterol and triglyceride (TG) levels were measured by conventional colorimetric assays. MMP-2 and -9 activities were determined from sera by zymography. Serum samples were deproteinized to measure nitrotyrosine (NTyr) concentration, a marker of peroxynitrite

generation, by ELISA. Left ventricular ejection fraction (LVEF) was measured by echocardiography. To show significant correlations, Pearson's correlation coefficients were determined.

Results: A positive correlation was found between serum MMP-2 activity and total ($r=0.55$; $p<0.05$) as well as LDL cholesterol ($r=0.45$; $p<0.05$) levels. However, neither HDL cholesterol, nor TG levels correlated with MMP2 activity. MMP-9 activity did not correlate with lipid levels. MMP-2 and -9 activities were not changed by statin therapy. Serum NTyr correlated significantly with TG ($r=0.47$; $p<0.05$) as well as total- ($r=0.57$; $p<0.01$) and LDL cholesterol ($r=0.65$; $p<0.01$) levels, however, negative correlation was found with HDL cholesterol ($r=-0.46$; $p<0.05$). Statin treatment reduced serum nitrotyrosine level significantly as compared to statin naive patients (from 24.1±5.1 to 12.2±1.1 ng/ml). LVEF correlated negatively with serum nitrotyrosine level ($r=-0.54$; $p<0.05$) and MMP-9 activity ($r=-0.75$; $p<0.01$), however, not with MMP-2 activity.

Conclusion: We conclude that increased peroxynitrite formation and MMP activation may play a role in the development of hyperlipidemia-related CAD.

P-C28:

CORONARY SINUS SIDE BRANCH STENTING A NEW TOOL FOR LEFT VENTRICULAR LEAD FIXATION

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Introduction: Despite of the technical development in coronary sinus (CS) lead technology dislocation rate remained high.

Objectives: The aim of our study was to investigate the effectiveness and safety of stent implantation in a CS side branch to stabilize the left ventricular lead.

Patients & methods: 335 CRT patients were treated with CS stenting. The procedure was performed because of postoperative dislocation in 22 patients, while intraoperative complications were the indications in the remaining cases. The electrode was positioned into the desired position, and a bare metal coronary stent was introduced via the same CS sheath. The stent was then deployed.

Results: During follow-up 58 patients died. Follow-up is longer than 6 months in 298 cases, longer than two years in 160 patients. Clinically important pacing threshold increase was not detected in the other cases (1.24±0.9 V vs. 1.28±1.1 V). Impedance measurements did not suggest lead insulation failure (685±197 Ohm vs. 566±147 Ohm). PNS affected by patient position was found in 15 cases. Reprogramming of the device was effective in 13 patients, while minimally invasive lead repositioning was performed with ablation catheter via the femoral vein in two cases. The stented CS leads were extracted transvenously without any complications due to pocket infection in four cases, due to endocarditis in one patient. In another four cases the leads were extracted during heart transplantation 7, 7, 26 and 27 months after the procedures.

Conclusion: Stent implantation to anchor the CS lead position seems to be an effective and safe procedure for the prevention and treatment of CS lead dislocation.

P-C29:

INDIVIDUAL POSITIONING FOR MAXIMUM HEART PROTECTION DURING BREAST RADIOTHERAPY: THE DEVELOPMENT OF A PRACTICAL TOOL BASED ON A COMPLEX MODEL

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Introduction: Radiation exposure of the heart during breast radiotherapy impairs the micro- and macrovasculature of the heart.
Objectives: The patient's prone positioning has been found feasible, and appropriate for the reduction of the radiation exposure of the lung, but its effect on heart dose remained controversial.
Patients & methods: In 83 left-sided breast cancer cases, conformal radiotherapy plans were generated in both the prone and supine positions; the benefit of one set-up over the other was analyzed according to the patient characteristics.
Results: The radiation dose to the heart in the two positions differed individually. Image fusion of CT scans revealed that prone positioning was detrimental if the heart was situated distant from the chest wall in the supine position, but moved to the chest wall in the prone position. For the characterization of the anatomical situation, a standardized method was developed by measuring the distance between the left anterior descending artery and the chest wall, and the heart area included in an imaginary field on a single CT scan, acquired at the middle of the heart in the supine position. The body mass index of the patient, the distance and the area were found the most reliable indicators for the preferred position. Based on these three parameters, a statistical model with high sensitivity and specificity was developed; its independent and prospective validation is ongoing.
Conclusion: Our work contributes to the clarification of the controversy regarding the choice of optimal positioning; a calculator has been generated that serves as a practical tool for individualized heart protection

P-C30:

ROLE OF GLUTATHIONE S-TRANSFERASE P1 GENE POLYMORPHISM IN PATIENTS UNDERWENT CARDIAC SURGERY

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Introduction: The glutathione S-transferases (GST) are a family of the most important antioxidant enzymes that play an important role in detoxification reducing the oxidative stress. Deleted polymorphisms in the GST genes may also influence the susceptibility to coronary artery disease by modulating the detoxification of genotoxic atherogens. There are three polymorphic GSTP1 alleles (GSTP1*A, GSTP1*B, GSTP1*C).
Objectives: This study was examined the genetic polymorphism of GSTP1 gene in patients who underwent cardiac surgery.
Patients & methods: 111 patients enrolled to the trial. Patients were divided the following groups: I.(n=78) without acute myocardial infarction, II.(n=33) with acute myocardial infarction in 24 hours following surgery. The ejection fraction, total bleeding, CK-MB level were measured. The presence of ST elevation, aorta cross clamp time and pump time, hospitalizing time, the surgeon, concomitant diseases were observed. Genotyping was performed by RT-PCR method.
Results: Gender, age, surgery and laboratory conditions were same in the two groups. There was no difference in the allele frequency of AA and the AB and BC alleles between the groups, while it was three times elevated

AC allele combination observed in the patient with myocardial infarction. It was 10% allele frequency of BB allele combination measured in the first group meanwhile in the second group was not found at all.

Conclusion: According to our results it seems that GSTP1 gene polymorphism play important role in complication development in patients underwent cardiac surgery. Determination of the enzyme polymorphism before surgical intervention may be an important step to prevent the serious complication.

P-C31:

VULNERABLE PLAQUE DETECTION WITH CORONARY CT ANGIOGRAPHY: THE NAPKIN-RING SIGN

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Introduction: A ring like attenuation pattern of coronary atherosclerotic plaque termed as napkin-ring sign (NRS) was described in patients who suffered acute coronary syndrome.

Objectives: Our aim was to determine the diagnostic performance of a novel plaque classification scheme based on attenuation patterns to identify high-risk plaques in coronary CT angiography.

Materials & methods: We studies 611 histological sections from21 coronaries. The histological sections were matched with the CT images in 1.5 mm increments. The images were read for the presence of non-calcified (NP), mixed (MP), calcified plaque (CP). The NCP-s and the MP-s were further classified as homogenous (HP) or heterogeneous. Plaques that appeared heterogeneous were further stratified into those with and without NRS.

Results: No plaque was detected in 134 (21.9%), NCPin 254 (41.6%), MP in 191 (31.3%), and CP in 32 (5.2%) CT cross-sections. Among NCP and MP we identified HP in 207 (46.5%), non-NRS plaque in 200 (44.9%) and NRS plaque in 38 (8.6%) cross sections. The specificities of NCP and MP to identify advanced lesions were moderate (58.1%, 95% CI:53.4-62.7% and 71.9%, 95% CI:67.4-76.0%), similarly to the homogenous and heterogeneous plaques (42.8%, 95% CI:37.4-48.2% and 57.2%, 95% CI:51.8-62.6%, respectively). In contrast, the specificity of NRS plaque was excellent (98.9%, 95% CI:97.4-99.6%). The diagnostic performance of the attenuation pattern to identify advanced lesions was better than that of the conventional scheme (AUC 0.761 vs. 0.678; p=0.0012).

Conclusion: The NRS is highly specific for advanced plaques with large necrotic core, which are often referred as vulnerable plaques. The plaque attenuation pattern has a higher accuracy to identify advanced plaques than the conventional CT plaque scheme.

P-C32:

CARDIOPROTECTION BY MAGNESIUM OROTATE AND OROTIC ACID AT REPERFUSION: COMPARABLE EFFECTS ON FUNCTIONAL RECOVERY BUT NOT ON INFARCT SIZE

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Introduction: Chronic administration of orotic acid and its derivates markedly improved the function of recently infarcted hearts. Magnesium has also been reported to elicit infarct size limiting effects in experimental models of ischemia/reperfusion.

Objectives: The present study was purported to compare the effects of 1 mM magnesium orotate (Mg-Or), orotic acid (OA) and magnesium chloride ($MgCl_2$), respectively when given throughout the postischemic reperfusion on cardiac functional parameters and infarct size.

Materials & methods: Langendorff perfused rat hearts were subjected to 30 minutes of global ischemia followed by 120 min reperfusion. Recovery of post-ischemic ventricular function was assessed by the left ventricular developed pressure (LVDP) and bythe indices of contractility and relaxation (dP/dtmax and dP/dtmin), respectively. Infarct size was quantified by the triphenyltetrazolium chloride staining.

Results: Mg-Or induced a substantial improvement in functional recovery comparable to the one elicited by OA for all functional parameters: LVDP (Mg-Or67.8±3%, OA 67±2%, vs. control 39.2±3%, $p< 0.001$), dP/dtmax (61.5±3% vs. control 38.8±4.63%, $p< 0.01$; dP/dtmin (OA 75±7%, Mg-Or 72±4% vs. control 47.6±5%, $p< 0.01$). When considering infarct size, the most important anti-infarct effect was obtained for Mg-Or (32.07±2%) as compared to OA (46.84 ±5%, $p <0.01$) suggesting a greater reduction in infarct size by the combination of orotic acid and magnesium. Magnesium chloride did not show any protective effect.

Conclusion: In conclusion, magnesium orotate and orotic acid administrated throughout the reperfusion elicited a comparable protection on cardiac function, yet a superior anti-necrotic effect was foundin the presence of magnesium orotate.

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P-C33:

SUCCESFUL HEART TRANSPLANTATION AFTER 12 HOURS OF ISCHEMIC DONOR ORGAN STORAGE WITH THE NEW ORGAN PRESERVATION SOLUTION CUSTODIOL-N

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Introduction: In heart transplantation surgery, the currently used organ preservation solution, Custodiol provides max. 4h of safe ischemic conservation time of donor organs, which severely limits the number of successful transplantations. The newly developed solution, Custodiol-N offers a better cardioprotection during cold ischemic storage and reperfusion by complex mechanisms of action (decreasing oxidative stress and histidine-toxicity).

Objectives: We examined the possibility of prolonged ischemic conservation using Custodiol-N in a canine heart transplantation model.

Materials & methods: According to the applied conservation times and solutions, our experimental groups were as follows: Custodiol 8/12h, Custodiol-N 8/12h. Hemodynamic measurements were performed in donors and after transplantation+reperfusion in recipients. Arterial pressure and coronary blood flow were registered and left ventricular pressure-volume analysis was performed using a pressure-conductance catheter. Myocardial tissue samples were collected for histological processing and for determining ATP-levels.

Results: Hearts stored in the old Custodiol solution for 8/12h showed no contractile function after implantation and histological signs of severe tissue damage could be detected. In the groups of Custodiol-N we observed spontaneous return of cardiac contractions and the animals could be weaned from extracorporal circulation after 2h reperfusion. The pressure-volume loop derived cardiac contractility parameters did not significantly differ from donor baseline values (end-systolic pressure-volume relationship (mmHg/ml) before vs. after transplantation: 4.34±1.04 vs. 3.75±1.02 8h; 3.19±0.61 vs. 2.97±0.63 12h). We found higher myocardial ATP-levels in the Custodiol-N groups compared to Custodiol.

Conclusion: Our results indicate that the currently achievable 4h of safe ischemic storage in heart transplantation could be increased up to 12h with the novel Custodiol-N solution.

P-C34:

SERUM COMPLEMENT C3 LEVELS ARE ASSOCIATED WITH WITH VENTRICULAR VOLUMES AND MASS IN TOP ATHLETES

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Introduction: The complement system is an important effector the innate immune system. The role of complement in the response of the heart to physical stress is not yet known.
Objectives: Our aim was to determine the association of total and activated complement C3 with the characteristics of the athlete's heart.
Patients & methods: We included 94 top athletes and 39 age and sex matched healthy non-athlete controls in our cross-sectional study. Total C3 and C3a concentrations were determined by immunoturbidimetry and ELISA methods. Allsubjects underwent cardiac MRi: left and right ventricular end systolic volumeindices (LVESVi, REVSVi), end diastolic volume indices (LVEDVi, RVEDVi), stroke volume indices (LVSVi, RVSVi) and mass indices (LVMi, RVMi) were determined.
Results: Total C3 levels ($p=0.03$) as well as C3a concentrations ($p=0.03$) were significantly lower in top athletes compared with non-athlete controls. Total C3 levels inversely correlated with LVESVi ($r=-0.218$, $p=0.03$), LVEDVi ($r=-0.268$, $p<0.01$), LVSVi ($r=-0.269$, $p<0.01$), as well as with RVEDVi ($r=-0.232$, $p=0.02$) and RVSVi ($r=-0.275$, $p=0.01$), but not with RVESVi ($p=0.08$), LVMi ($p=0.21$) or RVMi ($p=0.11$). C3a levels inversely correlated with LVESVi ($r=-0.304$, $p<0.01$), LVEDVi ($r=-0.353$, $p<0.01$), LVSVi($r=-0.316$, $p<0.01$), as well as with RVESVi ($r=-0.283$, $p=0.01$), RVEDVi ($r=-0.309$,$p<0.01$) and RVSVi ($r=-0.308$, $p<0.01$). We found negative correlation between C3a with LVMi ($r=-0.345$ $p<0.01$) and RVMi ($r=-0.478$, $p<0.01$).
Conclusion: Based on our results the complement system might play role in the development of athelete's heart. According to our results low C3a levels are associated with increased ventricular systolic, diastolic and stroke volumes and mass.
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P-C35:

CHANGES IN ACE2 ENZYME ACTIVITY IN SYSTOLIC HEART FAILURE

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Introduction: The angiotensin converting enzyme 2 (ACE2) is a recently identified member of the renin-angiotensin-aldosterone system (RAAS). During systolic heart failure (SHF), rising levels of serum ACE2 were observed simultaneously to worsening of left ventricular function.

Objectives: We aimed to analyze the activation of RAAS through the ACE and ACE2 levels in patients with SHF.
Patients & methods: 44 patients suffering from moderate or serious HF were involved. Left ventricular function was characterized by ejection fraction (EF). The serum ACE activity was measured spectrophotometrically while ACE2 activity of the serum was determined by fluorescence intensity measurements.
Results: A remarkable elevation of ACE2 activity was present in SHF patients compared to subjects with preserved left ventricular EF (33.64±3.237 U; 21.46±1.841 U). Parallel to worsening of EF linear rising of ACE2 activity was observed (r^2=0.2991). The same nearly linear correlation was measured between ACE2 levels and proBNP levels (r^2=0.2287). Among individuals with good ventricular function the ACE2 activity was significantly higher in men than in women (27.49±3.132 U; 15.72±0.9970 U), among heart failure patients definite, but not significant difference could be seen between genders (r^2=0.0329). No significant difference was found between ACE2 activity of diabetic and non-diabetic patients (37.28 ±12.08 U; 36.26 ±3,947 U). Activity of the ACE2 is independent from degree of the decrease in the ACE activity achieved by ACE-inhibition therapy both in case of preserved (r^2=0.0084) and damaged left ventricular systolic function (r^2=0.0279).
Conclusion: Our results suggest that increased activity of ACE2 may playinitial role in the development and progression of HF.

G1:

CALCIUM SIGNALLING IN THE GASTROINTESTINAL TRACT

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Physiological Ca^{2+} signals play crucial roles in the regulation of secretory and contractile cells in the gastrointestinal tract, whereas abnormal (excessive) Ca^{2+} signals can initiate disease processes and cause cell death. In both contractile and secretory cells the interplay between Ca^{2+} and ATP is crucial for function. The roles of these agents in muscle contraction (a subject to which Albert Szent-Györgyi made outstanding contributions) and neurotransmitter secretion were already reasonably well understood in the 1950s – 1960s, whereas it was only in the 1970s that their importance began to be recognized for regulation of exocrine secretion. The concept that local repetitive cytosolic Ca^{2+} signals regulate physiological processes, whereas global sustained elevations of the cytosolic [Ca^{2+}] initiate pathological processes (Thorn et al *Cell* 74, 661-668, 1993) has proven useful and has been worked out in detail in pancreatic acinar cells (Petersen & Tepikin *Annu Rev Physiol* 70, 273-299, 2008). These cells are classical objects for cell biological studies and have revealed many generally important aspects of intracellular Ca^{2+} handling as well as the mechanisms by which secretion of fluid and enzymes is switched on and off. More recently, studies of the mechanisms by which pathological agents - for example alcohol - initiate destruction of acinar cells have demonstrated the crucial role played by excessive release of Ca^{2+} from intracellular acid stores (Gerasimenko et al *PNAS* 106, 10758-10763, 2009), but have also revealed the intrinsic protective effect of the small intracellular Ca^{2+}-binding protein calmodulin (Gerasimenko et al *PNAS* 108, 5873-5878, 2011).

G2:

MECHANISM OF EPITHELIAL HCO_3^- SECRETION

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HCO_3^- secretion is a key function of secretory epithelia and involves HCO_3^- entry at the basolateral membrane and exit across the luminal membrane. In most epithelia the bulk of HCO_3^- entry is mediated by the Na^+-HCO_3^- co-transporter NBCe1-B and HCO_3^- exit is mediated by the combined and regulated action of CFTR and members of the SLC26 transporters. How HCO_3^- entry and exit are coordinated to ensure the fidelity of the secretory process is not know. Our recent work show that the IP_3 Receptor-binding Protein Released with IP_3 (IRBIT) plays a prominent role in regulating and coordinating the stimulated state, while the WNK and SPAK kinases stabilize and coordinate the resting state. This talk will discuss how the IRBIT/PP1 pathway and the WNK/SPAK kinases pathway alternately regulate and coordinate HCO_3^- secretion by secretory glands ducts.

G3:

REGULATORY PEPTIDES AND GASTRIC EPITHELIAL CELL FUNCTION

Graham J. Dockray

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The evolution of gastric acid secretion provides a mechanism to control ingested micro-organisms while food is temporarily retained in the stomach prior to delivery to the small intestine for breakdown and absorption. The master regulator of acid secretion is the antral hormone gastrin which is released in response to both ingested protein and bacteria including *H.pylori.* In the last few years, it has become clear that gastrin also activates many other mechanisms that are collectively associated with protection from the consequences of repeated and regular exposure of the gastric epithelium to the potentially damaging contents of the lumen. One such mechanism involves matrix metalloproteinase (MMP)-7 which is increased in epithelial cells response to gastrin and *H.pylori*; MMP-7 stimulates epithelial cell migration and can act as a gastric growth factor by liberating IGF through cleavage of an IGF-binding protein (IGFBP-5). A second system involves urokinase plasminogen activator (uPA), its receptor (uPAR) and inhibitors (PAI-1, PAI-2). These too are increased in response to gastrin and *H.pylori* in gastric epithelial cells. Mice in which PAI-1 has been deleted, or over-expressed in gastric parietal cells, reveal a role for PAI-1 in protection against tissue damage by inhibition of thrombolysis. Importantly, however, mice over-expressing PAI-1 in parietal cells are moderately obese and hyperphagic, at least partly due to inhibition of satiety signalling by the intestinal hormone cholecystokinin. In addition to established roles in control of acid secretion, the gastric regulatory system therefore embraces previously unsuspected mechanisms involving epithelial protection and maintenance of energy intake.

G4:

REGULATING THE SUPPLY OF PANCREATIC ENZYMES FOR DIGESTION

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The pancreas is the major source for digestive enzymes that break down all the major classes of nutrients in the intestinal lumen. To carry out digestion efficiently, the right amount and mix of digestive enzymes needs to be present. There are four main sites of regulation: 1) Transcription of digestive enzyme mRNA, 2) Translation of mRNA on membrane bound ribosomes to synthesize digestive enzyme protein, 3) Acute secretion of digestive enzymes by exocytosis of zymogen granules, and 4) Growth or atrophy of the pancreatic acinar cell mass. All of these occur in response to ingestion of nutrients. The major known mediators are acetylcholine,

CCK and possibly secretin, insulin and amino acids. Interestingly different pathways mediate the four sites of regulation. The transcription of different digestive enzyme mRNA is mediated by transcription factors such as the regulation of amylase mRNA by insulin in response to a high carbohydrate diet and occurs over weeks. The synthesis of digestive enzyme protein is regulated by the mTOR pathway, is activated by acetylcholine, CCK, insulin and branched chain amino acids and occurs in response to each meal. Acute secretion of digestive enzymes is mediated by an increase in intracellular free calcium ions in response to stimulation by CCK and acetylcholine. Finally, an increase in pancreatic acinar cell mass requires the calcineurin-NFAT pathway and mTOR pathways and is primarily regulated by CCK while acinar cell hypertrophy or atrophy is mediated primarily by the amount of amino acids from dietary protein. Examples will be presented and discussed.

G5:

MAKING SENSE OF STROMA

Andrea Varró

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Stromal cells help maintain normal epithelial function and in cancer they drive tumour growth. The mechanisms are unclear. Myofibroblasts are an important stromal cell type and we have defined functional changes in these cells in gastric cancer. Normal gastric myofibroblasts were shown to exhibit Ca^{2+}-dependent protein secretion in response to IGF-II but this property was lost in cells from those cancer patients with increased lymph node involvement and shorter survival. The capacity for regulated exocytosis was associated with the presence of dense-cored secretory vesicles localised in the vicinity of the Golgi complex. Using SILAC labelling and LC-MS/MS we identified approximately 60 proteins that were secreted in response to stimulation and 15 that exhibited no change. Affymetrix microarrays comparing the transcriptomes of myofibroblasts with and without regulated secretion revealed 9 differentially expressed transcripts encoding secretory proteins. The greatest difference in expression was exhibited by the neuroendocrine cell protein, secretogranin-II (Sg-II). Western blot revealed higher abundance of SgII in cells with regulated secretion than without; in the former SgII was released in response to IGF-II. Over-expression of Sg-II restored regulated secretion in cells that did not have a secretory response to IGF-II; in contrast in cells exhibiting regulated secretion, Sg-II knockdown inhibited the response. Thus stromal cells appear to have a neuroendocrine-like phenotype. Regulated secretion provides a mechanism for rapid, growth factor-dependent, changes in the tissue microenvironment that is lost with cancer progression; expression of SgII is a potential biomarker of this phenotype and a new indicator of stromal cell function in cancer.

G6:

PATHOPHYSIOLOGY OF INTESTINAL EPITHELIAL BARRIER AND TRANSPORT FUNCTIONS: IMPLICATIONS FOR INFECTIOUS AND INFLAMMATORY DISEASE STATES

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The intestinal epithelium permits the selective uptake of nutrients, electrolytes and water, while excluding toxins, pathogens, and commensal bacteria. It also secretes water and electrolytes to control luminal fluidity. Thus, the regulation of transport and barrier properties is central to appropriate gastrointestinal function. We have focused on dysregulation of epithelial function in inflammatory and infectious diarrheal diseases. Studies in cell culture

suggested that infection with *Salmonella* diminished barrier properties and upregulated the capacity for chloride secretion, secondary to enhanced expression of the chloride channel, CFTR, and the sodium/potassium/chloride cotransporter, NKCC1. However, in a murine model of *Salmonella* colitis, chloride secretion was paradoxically decreased during the onset of diarrheal symptoms. Instead, diarrhea is likely attributable to reduced expression and/or apical localization of the ENaC sodium channel and the DRA chloride/bicarbonate exchanger. A lack of crypt chloride secretion may permit bacterial invasion, whereas diminished electrolyte absorption reverses normal dehydration of the stool, resulting in diarrhea. We have also studied whether probiotic bacteria can reverse adverse effects of pathogens or inflammation. Two probiotics, *S. thermophilus* and *L. acidophilus*, reduced epithelial binding and internalization of pathogens, reversed adverse effects of pathogens and cytokines on transport and barrier function *in vitro*, and improved barrier properties in a model of spontaneous colitis. We conclude that both pathogenic and probiotic bacteria can exert profound effects on the function of the intestinal epithelium. An understanding of these actions may permit new insights into infectious and other diarrheal diseases, as well as their treatment.

G7:

INTESTINAL PEPTIDE TRANSPORTERS – WHAT ARE THEY GOOD FOR?

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While various transporters mediate the uptake of free amino acids across the brush border membrane, di- and tripeptides are selectively transported into enterocytes via the SLC15A1 peptide transporter PEPT1; a member of the proton-coupled oligopeptide transporter family found in all living organisms. Peptide transporters are electrogenicsymportersenergized by the electrochemical proton gradient. Despite extensive analysis of peptide transporter structure and function in various expression systems, it is not yet defined what the protein contributes to gastrointestinal physiology and protein nutrition. Studies employing *Pept1*$^{-/-}$ mice demonstrated a lack of intestinal transport of model dipeptides but animals otherwise do not show any obvious phenotypic alterations. To better understand the biological role of PEPT1, we analyzed the responses of transporter-deficient animals to diets either high in carbohydrates, protein or fat. Most interestingly each diet produced a different phenotype. On a high protein diet but not on a high carbohydrate control diet or a low protein diet, *Pept1*$^{-/-}$ mice showed impairments in development. Animals displayed a reduced food intake and major weight loss during the first days on this diet. Although food intake returned to normal levels after 5 days, *Pept1*$^{-/-}$ mice did not regain weight and remained significantly leaner than WT mice. Even more strikingly, *Pept1*$^{-/-}$ mice when fed with a diet comprising 48% of energy from fat show significant changes in energy balance, gut morphology and physiology and in clinical parameters. These findings suggest that PEPT1 may have other roles in the gastrointestinal tract than just transporting peptides.

G8:

THE ROLE OF CFTR IN PANCREATIC DUCTAL BICARBONATE SECRETION

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Pancreatic ducts are unique in that they can secrete a juice containing near isotonic sodium bicarbonate. This alkaline secretion helps: (i) solubilise digestive enzymes secreted from acinar cells; (ii) transport the

enzymes down the ductal tree into the duodenum, and (iii) neutralise acid chyme that enters the duodenum from the stomach, which is essential for effective digestion. The ductal secretion also has a protective role such that defects in secretion lead to pancreatic pathophysiology. This is illustrated by the inherited disease cystic fibrosis in which dysfunctional CFTR results in reduced bicarbonate and fluid secretion and ultimately to the destruction of the gland (1). Moreover, our recent studies suggest that defects in CFTR-dependent bicarbonate secretion may predispose the gland to pancreatitis induced by noxious agents such as bile acids and trypsin (2-6). Surprisingly, despite the importance of CFTR to ductal secretion its exact role is still somewhat controversial, despite more than 20 years of investigation. In this talk I will discuss the multiple roles that CFTR plays in bicarbonate secretion and describe our recent studies that have identified a novel role for CFTR as an external chloride sensor, and implications of this finding for pancreatic secretion.

G9:

MOLECULAR REGULATION AND PHYSIOLOGICAL FUNCTIONS OF INTESTINAL BICARBONATE TRANSPORT

Ursula Seidler

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The lining of the gastrointestinal tract fulfils the unique task to secrete and absorb large amounts of electrolytes, nutrients, antigens and fluids while preventing noxious and infectious agents to gain access to the circulation. It has been recognized early that the active transport of HCO_3^- ions to the lumen is a feature of all gastrointestinal epithelia from the oral cavity to the distal colon and is essential for maintaining mucosal health. In addition, large quantities of HCO_3^- are absorbed in the small intestine. This talk will review current knowledge about the nature of the ion transport proteins involved in esophagus, stomach, duodenum, jejunum and ileum, and the colon, their physiological functions, some molecular aspects of its regulation, and dysfunctions of HCO_3^- transporters that lead to disease in animal models and humans.

G10:

THE ROLE OF CFTR IN BICARBONATE SECRETION BY BILIARY EPITHELIA

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Both hepatocytes and cholangiocytes participate in the production of bile. Primary canalicular bile is produced by concerted action of ion carriers localized at the basolateral and apical membrane of hepatocytes. Cholangiocytes secrete a bicarbonate-rich watery fluid that modifies the volume and the composition of canalicular bile. The transepithelial transport of HCO_3^- starts with the accumulation of HCO_3^- in cholangiocytes. In rats, this process is carried out by electrogenic Na^+-HCO_3^- cotransport activity and carbonic anhydrase/CO_2 pathway, while in humans, bicarbonate influx is mainly due to electroneutral Na^+-dependent Cl^-/HCO_3^- anion exchange activity. The apical membrane of cholangiocytes is endowed by anion channel proteins (CFTR and Ca^{2+}-activated Cl^- channels) and AE2/SLC4A2 anion exchanger. We have demonstrated that in isolated human cholangiocytes both cAMP and protein kinase A catalytic subunit activated CFTR Cl^- channels that exhibited a non-rectifying conductance of 8 pS and appeared in clusters. Activation of Cl^- current by cAMP was associated with an increase in the rate of HCO_3^- secretion through AE2/SLC4A2 anion exchanger. Although bicarbonate permeability through activated CFTR has been shown in several cell systems, its main contribution to biliary HCO_3^- secretion appears to occur

through a coordinated action with AE2/SLC4A2. In cystic fibrosis (CF) Ca^{2+}-activated Cl^- channels can substitute for CFTR in stimulation of ductal bicarbonate secretion. Since resultant Ca^{2+}-stimulated Cl^- efflux might be up to 2-fold greater than that mediated by CFTR, this pathway could be exploited to bypass secretory defect in CF.

G11:

NORMAL MUCIN FORMATION REQUIRES BICARBONATE

Paul M. Quinton

Nancy Olmsted Professor in Pediatric Pulmonology Department of Pediatrics, UC San Diego School of Medicine, Rady Children's Hospital and Professor of Biomedical Sciences, Riverside, CA, USA

Mucins, the core components of mucus, are the largest biological molecules known. HCO_3^- is the major pH buffer in extracellular fluids (ECF). It is not intuitive that they should interact, but the hereditary, generally fatal, disease of Cystic Fibrosis indicates that they are closely associated in the process of forming the essential macromolecular barriers that protect epithelial surfaces. After synthesis, the protective gel-forming mucins are highly negatively charged polymers that must be stored and remain extremely compacted before stimulated release. Cystic Fibrosis (CF), better known for its basic impermeability to Cl^-, is also defective in HCO_3^- transport. Pathogenesis in CF has long been attributed to abnormally thick and viscid mucus. Evidence now shows that HCO_3^- secretion is required for normal mucin release and that, without this small anion, mucus properties are dramatically altered. HCO_3^- appears to act by competing Ca^{2+} and H^+ from anionic sites on mucins involved in stabilizing the condensed form of stored mucins so that in its absence, mucins fail to unravel and expand properly. The transport of poorly expanded mucins seems greatly impeded, presenting a new view of the origins of this disease as well as newly appreciated role for HCO_3^- in a crucial physiological event.

G12:

THE TWO MUCUS LAYERS ORGANIZED BY THE MUC2 MUCIN AND THEIR RELATION TO COLON INFLAMMATION

Gunnar C. Hansson

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Colon is covered with a single layer of active cells that have to have to be protect from the enormous amount of commensal bacteria. This has now been shown to be accomplished by an inner mucus layer that is impermeable to bacteria. This inner layer is converted to an outer less dense mucus that is the habitat of the microbiota. These two mucus layers are built around the MUC2 mucin, a large glycoprotein that is assembled into enormous net-like polymers that form a gel. These net-like polymers are secreted by the goblet cells together with a number of other components likely to be important for building a stable inner mucus layer.
The commensal bacteria use the MUC2 glycans as a food source, typically removing one monosaccharide at a time. In mice with truncated mucin *O*-glycans, the mucins is less efficient in protecting the epithelium. If the MUC2 mucin is less glycosylated or absent, the inner mucus layer is defective or totally absent. In this and other cases, the bacteria come in direct contact with the epithelium, penetrate into the crypts and into the cells. This triggers inflammation, bloody diarrhea and later on colon cancer. In humans, the MUC2 mucin is thicker and efficiently separates the microbiota from the epithelial cells. However, in patients with active ulcerative colitis, the mucus is thinner and penetrated by bacteria. The concept and implications of the mucus layers for the protection of colon and the bacterial habitat will be discussed.

G13:

NEW APPROACHES FOR SECRETORY DIARRHEAS IN DEVELOPING COUNTRIES

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Intestinal fluid secretion, as occurs in cholera, is driven by Cl^- secretion across the intestinal epithelium from capillaries to the gut lumen. Fluid secretion involves Cl^- entry into enterocytes via the basal membrane $Na^+/K^+/2Cl^-$ cotransporter and exit via luminal membrane Cl^- channels. Cl^- secretion creates the electrochemical and osmotic forces driving Na^+ and water secretion. The major route for luminal Cl^- secretion in cholera and other enterotoxin-mediated secretory diarrheas is the cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP-regulated Cl^- channel. Ca^{2+}-activated chloride channels (CaCCs) provide an alternative route for luminal Cl^- secretion, and perhaps the primary route for Cl^- secretion in some viral and drug-induced diarrheas. In addition there is extensive cross-talk between cAMP and Ca^{2+} signaling, whereby cAMP elevation causes CaCC activation and Ca^{2+} elevation causes CFTR activation. We have used high-throughput screening to identify and optimize small-molecule inhibitors of CFTR and CaCCs. In addition to target-based and phenotype-based high-throughput screening, we have investigated whether certain natural-product antidiarrheal 'reme dies' from around the world contain Cl^- channel inhibitors, and, if so, identify active compounds by fractionation and determine their chemical structure. A recent exciting finding is the identification of a natural herbal remedy showing strong inhibition of CFTR and CaCCs in human intestinal epithelial cells, and isolation of purified active compounds. Cl^- channel inhibition provides a rational approach to reduce morbidity and mortality from secretory diarrheas in developing countries. The goal is to develop a therapy that is inexpensive, chemically stable and effective in severe secretory diarrhea.

G14:

MICROBE-EPITHELIAL SIGNALING IN THE INTESTINAL TRACT

Jerry Wells

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Abstract is not available

G15:

THE ANION EXCHANGER DRA AND INTERACTION WITH PDZ ADAPTER PROTEINS

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DRA (SLC26A3) and NHE3 (SLC9A3) together facilitate electroneutral NaCl absorption in the distal ileum and proximal colon. Both transporters interact with PDZ adapter proteins of the so called NHERF family (NHERF, E3KARP, PDZK1 and IKEPP), which mediate regulation and intracellular trafficking of a number of transport proteins (e.g. CFTR, URAT) as well as receptors (e.g. beta-2- receptor, LPA receptor).
DRA can be expressed heterologously in HEK293 cells, which have little endogenous Cl/HCO_3 exchange activity, as well as in an inducible system in Caco2/BBE cells. Furthermore the PDZ interaction occurs via the four C-terminal amino acids; thus a deletion mutant (DRA-ETKFminus), that lacks the PDZ interaction, can be expressed. Using these cell systems we have characterized some of the transport properties of DRA.

More importantly we have found that PDZK1 is required for calcium-induced inhibition of DRA. This seems to be a two-step process, where DRA is first inhibited in and later removed from the plasma membrane into intracellular compartments. The insertion of DRA into the plasma membrane requires intact lipid rafts, PI3 kinase activity and an intact PDZ interaction motif. Furthermore the PDZ interaction of DRA influences its recycling back to the plasma membrane after endocytosis. While DRA is found in early and recycling endosomes, DRA-ETKFminus is found in early and late endosomes which are destined to the lysosomes. Correspondingly the half life of DRA is longer than that of DRA-ETKFminus. Currently it is unknown, which PDZ adapter proteins interact with DRA along the endocytosis-recycling pathway.

G16:

ROLE OF AE2 IN THE PATHOGENESIS OF PRIMARY BILIARY CIRRHOSIS

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The canalicular bile is fluidized and alkalinized along the bile ducts through hydroionic fluxes at the biliary epithelium. Secretin stimulates this process, which involves biliary secretion of bicarbonate via the Cl^-/HCO_3^- anion exchanger AE2. This membrane proteinis also relevant for the regulation of the pH_i in several cell types. We have reported a diminished expression of AE2 mRNA in liver biopsies and peripheral blood mononuclear cells from patients with primary biliary cirrhosis (PBC). Also the expression of the AE2 protein is decreased in PBC livers. Interestingly, cholangiocytes isolated from PBC patients and cultured for a few passages, exhibited defective AE2 activity. And positron emission tomography (PET) studies showed that PBC patients, even at the early stages of the disease, failed to secrete bicarbonate to bile in response to secretin, a defect that could be partially reversed after several months of treatment with UDCA. Altogether, these findings sustained our hypothesis that dysfunctions related to AE2 might have a role for the pathogenesis of PBC. Inadequate AE2 function in lymphocytes would disturb pH_i regulation in these cells and alter immune homeostasis leading to autoimmunity. On the other hand, reduced AE2 in cholangiocytes could cause cholestasis and oxidative stress of bile duct cells. Cholangiocyte changes, together with altered immune homeostasis, could then favor the development of serum antimitochondrial antibodies and nonsuppurative destructive cholangitis. Our recent findings that $Ae2_{a,b}$-deficient mice indeed display most of these features strongly support the notion that AE2 abnormalities may be involved in the pathogenesis of PBC.

G17:

EVIDENCE FOR ELECTROLYTE TRANSPORT OF TWO-DIMENSIONAL SALIVARY GLAND ENGINEERED FROM HUMAN SUBMANDIBULAR TISSUE

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Reconstruction of damaged salivary gland tissue following cancer-demanded radiotherapy or Sjogren syndrome is an unresolved challenge for regenerative medicine. To restore lost salivary epithelial function, either acinar cell renewal should be achieved or the function of the remnant ductal cells should be altered from an absorbing epithelium into a secretory epithelium. Our aim was to prepare primary cultures of human submandibular gland and to provide optimal conditions for polarized secretory epithelial monolayers.

Cell cultures were prepared from dissected human submandibular glands (Tissue Eng: 14:1915-26,2008). Transepithelial electrolyte transport was estimated by short circuit current (I_{sc}) measurements in Ussing chamber. Monolayers in HepatoStim medium achieved transepithelial electrolyte movement as shown by I_{sc}. These currents were substantially but not fully inhibited by basolateral Cl^- and bicarbonate withdrawal indicating the involvement of basolateral-to-apical anion transport, or by the inhibition of apical eNaC activity by amiloride, indicating the involvement of apical-to-basolateral Na^+ transport. An almost complete inhibition was observed in response to simultaneous eNaC block and withdrawal of the two anions. I_{sc} was accelerated by apical ATP or basolateral carbachol application but not by forskolin, indicating the regulatory role of Ca^{2+}-activated, but not cAMP-activated regulatory pathways. Inhibition of basolateral NKCC1 by bumetanide reduced the response to ATP indicating the active involvement of this transporter as well. In conclusion, we successfully developed a human salivary secretion model, which shows mixed acinar/ductal phenotype. This model may serve to establish the basis for pharmacological or genetic interventions to correct salivary gland dysfunction.

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G18:

REGULATION OF NHE3, A STORY OF SIGNALING COMPLEXES AND CYTOSKELETON

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NHE3 is the epithelial brush border Na^+/H^+ antiporter and accounts for the majority of intestinal and renal Na^+ absorption. It is highly regulated as part of digestion, being inhibited upon eating followed by rapid stimulation; exaggeration of the inhibition contributes to all diarrheal diseases. Regulation involves the NHE3-C terminus and occurs by changes in trafficking involving changes in NHE3 endocytosis and/or exocytosis which involve multiprotein signaling complexes that form on the NHE3 C-terminus. NHE3 attaches to the microvillar cytoskeleton by two C-terminal domains; one directly attaching to ezrin and the other indirectly attaching to ezrin via the NHERF family of multi-PDZ domain containing scaffolds. The latter is part of a complex that includes NHERF1-4, CK2, CaMKII, CaM and PLCg. NHE3 can be regulated by trafficking and also is attached to the cytoskeleton because signaling that regulates NHE3 also transiently dissociates NHE3 from the cytoskeleton. The aspect of signaling that regulates NHE3/cytoskeletal association is separate from that regulating NHE3 trafficking. As an example, LPA acting via the apical LPA5 receptor acutely stimulates NHE3 exocytosis and transiently dissociates NHE3 from the microvillar cytoskeleton by dissociation NHE3 from NHERF2. These studies demonstrate that there are two aspects to signaling that regulates NHE3 activity in the apical membrane of polarized epithelial cells, an aspect that regulates trafficking and a second coordinated aspect that regulates association with the microvillar cytoskeleton.

G19:

TARGETING BILE ACIDS FOR TREATMENT OF INTESTINAL TRANSPORT DISORDERS

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Background: Cl^- secretion, the primary driving force for intestinal fluid secretion, can become dysregulated in disease conditions, leading to onset of diarrhoea. We investigated the potential for targeting the nuclear bile acid receptor, farnesoid X receptor (FXR), for treatment of diarrhoeal diseases.
Methods: GW4064 was used as a prototypical FXR agonist. Cl^- secretion across cultured epithelia and muscle-stripped mouse colon was measured in Ussing chambers. Protein expression was measured by RT-PCR and western blotting.
Results: Treatment of T_{84} cells with GW4064 (2 μM), induced nuclear translocation of FXR and inhibited Ca^{2+}- and cAMP-evoked Cl^- secretory responses to 55.6 ± 8.4 and 72.0 ± 4.0% of controls, respectively ($n = 7$; $p < 0.01$). Effects of GW4064 were concentration-dependent (0.1–10μM) and maximal after 24 hrs. To determine molecular target(s) of the antisecretory actions of GW4064, we analyzed the activity of transport proteins comprising the Cl^- secretory pathway. GW4064 significantly inhibited Na^+/K^+ ATPase pump activity and CFTR-mediated Cl^- currents. Furthermore, the FXR agonist reduced CFTR protein expression, but did not alter expression of the Na^+/K^+ ATPase pump. Intraperitoneal injection of GW4064 (100 mg/kg) to mice also attenuated agonist-stimulated Cl^- secretory responses across ex vivo colonic tissues and dramatically reduced the severity of diarrhoea in an allergic mouse model.
Conclusions: Our studies reveal a novel role for FXR in regulating intestinal fluid and electrolyte secretion. By virtue of their ability to inhibit multiple components of the Cl^- secretory pathway, agonists of FXR are good targets for the development of new anti-diarrhoeal drugs.

We acknowledge Science Foundation Ireland for financial support.

G20:

A NEW PARADIGM OF PATHOGENESIS OF PANCREATITIS

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Pancreatitis in both its acute and chronic forms causes significant morbidity, mortality and hospitalizations. In the US alone, over 300,000 patients are admitted each year for pancreatitis and more than 2 billion dollars are spent on their care. Despite this, there is currently no specific therapy for this disease, a situation that stems from our incomplete understanding of its pathobiology. Hence, there is an urgent need to gain a better understanding of the pathophysiology of pancreatitis so that novel and specific treatments could be developed. Intra-acinar trypsinogen activation has been observed consistently in the early stages of pancreatitis and is believed to initiate acinar cell injury and inflammation during acute pancreatitis. The associations of hereditary pancreatitis with mutations in trypsinogen that lead to persistent activation of trypsin, or with loss of function mutations in trypsin inhibitors that result in un-inhibited intra-acinar trypsin activation, further support the role of trypsin in pancreatitis. However, the evidence supporting the role of trypsin is correlational and circumstantial at best. Thus far, no study has shown that activation of trypsinogen in pancreatitis is causally responsible for its pathogenesis and conversely, some investigators have even suggested a protective role for trypsin in pancreatitis.
Even though the role of inflammation in acute and chronic pancreatitis is gaining greater appreciation, the relative contributions of inflammation and trypsin activation in pancreatic and systemic injury are unknown. Intriguingly, our recent data suggest that trypsin only partially contributes to acinar cell injury during acute

pancreatitis. Furthermore, our data also show that inflammation is activated independent of trypsin activation and could contribute to the trypsin-independent component of pancreatic injury during acute pancreatitis. Even more exciting are our data suggesting that repeated inflammatory episodes *in the absence of trypsinogen activation* can lead to chronic pancreatitis. These findings are seminal and challenge the decades old-dogma that trypsin is central to the pathogenesis of pancreatitis.

G21:

EARLY INTRAACINAR EVENTS IN THE PATHOPHYSIOLOGY OF ACUTE PANCREATITIS

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The pathogenesis of the early events of acute pancreatitis is unclear. Activation of trypsinogen, the proinflammatory transcription factor nuclear factor-κB (NF-κB) and mitochondrial damage in pancreatic acinar cells are commonly thought to play an important role in the development of acute pancreatitis. However, the sequence and relationship of these processes are matters of debate. Accumulating evidence suggests that acinar NF-κB and trypsinogen activation occur with similar time dependence, but are independent from each other. Recently, we have shown that mitochondrial injury in itself can also lead to the development of severe acute necrotizing pancreatitis. The latter process is likely to be mediated by the cyclophilin D-dependent opening of the mitochondrial permeability transition pore. Our results indicate that mitochondria are critical in determining pancreatic acinar cell fate and may be therapeutic targets in acute pancreatitis.

G22:

THE PATHOPHYSIOLOGY OF PANCREATIC DUCTAL BICARBONATE SECRETION

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The human pancreatic ductal epithelium secretes 1-2L of alkaline fluid every day which may contain up to 140mM $NaHCO_3$. Until the last couple of years most of the scientists beleived that the physiological function of this alkaline secretion is to wash digestive enzymes down the ductal tree and into the duodenum, and to neutralise acidic chyme entering the duodenum from the stomach. However, most recently we suggested that there are at least two other important physiological roles of bicarbonate inside the pancreas: i) to neutralise protons secreted by acinar cells and ii) to curtail trypsinogen autoactivation within the pancreatic ductal system. Therefore, derangements of bicarbonate secretion will decrease luminal pH which will promote the autoactivation of trypsinogen. Trypsin will further inhibit bicarbonate transport leading to a vicious cycle generating further falls in luminal pH and enhanced trypsinogen activation, which will favour the development of pancreatitis. There are of course other important lines of evidence supporting the idea that pancreatic ducts play part in the pathogenesis of pancreatitis: i) ductal fluid and bicarbonate secretion are compromised in acute and chronic pancreatitis, ii) one of the main endpoints of chronic pancreatitis is the destruction of the ductal system, iii) mutations in CFTR may increase the risk of pancreatitis, and iv) etiological factors for pancreatitis, such as bile acids or ethanol in high concentration, inhibit pancreatic ductal bicarbonate secretion. In summary, pancreatic ductal bicarbonate secretion is a very important field of research which needs much more attention.

G23:

NOVEL PATHOGENETICAL FACTORS IN IRRITABLE BOWEL SYNDROME

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Irritable bowel syndrome (IBS) is a chronic functional disorder characterized by abdominal pain and altered bowel habits, affecting 3-20% of the general population. According to Rome III criteria, IBS patients can be identified as diarrhea-predominant (IBS-D), constipation-predominant (IBS-C) or mixed (IBS-M). Although several factors have been described as contributors to the development of IBS, the pathomechanism is only partially understood and effective therapeutic approaches are lacking. Proteases are highly present in the gastrointestinal tract. Apart from digestion, proteases play a particularly important role as signaling molecules on protease-activated receptors (PARs).

Elevated fecal serine-protease activity in IBS-D patients cleaves PAR-2 and induces colonic paracellular permeability increase and colorectal hypersensitivity to distension in mice. Nevertheless, serine-proteases are not found elevated in feces of IBS-C patients, even though visceral hypersensitivity, as well as increased permeability are clearly present in this IBS subtype. In a second study, we found in IBS-C patients an enhanced fecal cysteine-protease activity, in comparison with healthy controls and IBS-D patients. Cysteine-protease levels were positively correlated with disease severity. Repeated application of IBS-C fecal supernatants in mice colon triggered increased permeability, linked to the enzymatic degradation of the transmembrane tight junction protein, occludin. This phenomenon is associated with enhanced visceral sensitivity to colorectal distension, independently upon PAR2 activation. Occludin levels were found decreased in colonic biopsies from IBS-C patients, and IBS-C fecal supernatants were able to degrade recombinant human occludin in vitro.

Taken together, our results suggest that luminal proteases may represent a new factor contributing to the genesis of symptoms in IBS.

G24:

FROM CHRONIC INFLAMMATION TO CANCER

Zsolt Tulassay

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Chronic inflammation is an important risk factor for the development of cancers. Based on findings from epidemiological, clinical to experimental molecular studies, the link between chronic inflammation and risk of developing cancer is now well established. At least 20% of all cancers arise in association with infection and chronic inflammation. Although the pathogenic impact for chronic inflammation has been proposed in tumor initiation, progression and metastatic process, the exact mechanism of this connection is still not completely elucidated. Inflammation and cancer are linked both along intrinsic (driven by genetic events causing malignancy) and extrinsic (driven by inflammatory conditions predisposing to tumor) pathways. In some tumors, a strong association with infection has been recognized (*Helicobacter pylori,* Hepatitis B and C), whereas in other tumors the etiology of inflammation is non-infectious (chronic pancreatitis, inflammatory bowel diseases, Barrett's esophagus).

H. pylori infection affects the mucosal as well as the systemic immune response by secretion of cytokines and the recruitment of distinct inflammatory cells. Consequently, eradication of *H. pylori* infection can prevent progression of severe gastritis into gastric cancer or even cure gastric MALT-lymphoma.

Patients with longstanding inflammatory bowel diseases (IBD) have an increased risk of colorectal cancer (CRC). The molecular pathway leading to CRC in IBD appears to differ from the well-known adenoma-to-CRC sequence, given the fact that these cancers appear to arise from either flat dysplastic tissue or dysplasia-associated lesions or masses.
Although the link between chronic inflammation and risk of developing cancer is now well established, several open questions remain.

G25:

INNATE AND ADAPTIVE IMMUNITY IN THE PATHOGENESIS OF COELIAC DISEASE

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Our current understanding of coeliac disease (CD) is that the barrier function of the small intestinal epithelium is damaged which leads to the leakage of the a cereal proteins across the intestinal epithelium and initiates inflammation in the lamina propria. Gliadin peptides, such as p57-p89 preferentially drive adaptive immune responses. Other gliadin peptides, such as p31-p43 elicit a direct innate immune response. This peptide could be recognised by TLRs of dendritic cells leading to increased synthesis of IL-15. In our recent work we found higher TLR2 and TLR4 mRNA expression and protein levels in the duodenal mucosa of children with treated and untreated CD compared to controls.
We also found that heat shock protein (HSP 72) mRNA and protein expression were increased in the small intestinal mucosa of both untreated and treated coeliac children compared to controls. We assume that the increased HSP72 expression helps to alleviate the symptoms caused by gluten toxicity.
In the peripheral blood of untreated coeliac children the numbers of Th1, NK (CD3-CD161+), NKT (CD3+ CD161+) and invariant NKT (CD3+ 6b11+) cells were decreased, while the number of activated CD4+ cells as well as the myeloid and TLR 2 and 4 positive dendritic cells were increased. The numbers of antigen presenting cells (APC) expressing TLR-2 and TLR4 were also expressed in freshly diagnosed coeliac children compared to controls.
All the presented results prove that innate and adaptive immune mechanisms are interrelated and both are decisive in the development of CD.

G26:

THE EFFECTS OF LAPAROSCOPIC NISSEN FUNDOPLICATION ON BARRETT'S ESOPHAGUS: LONG-TERM RESULTS

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Background: The aim of our study was a retrospective investigation of the efficacy of laparoscopic Nissen fundoplication in patients with Barrett's esophagus.
Method: A total of 78 patients with Barrett's esophagus underwent surgery. Patients were divided into three groups on the basis of the preoperative endoscopic biopsies: a non-intestinal group (n=63) with fundic or cardiac metaplasia, an intestinal group (n=18) with intestinal metaplasia, and a dysplastic group (n=7) with low-grade dysplasia. Clinical follow-up was available in the case of 64 patients at a mean of 42 ±16.9 months after surgery.
Results: Check-up examination revealed total regression of Barrett's metaplasia in 10 patients. Partial regression was seen in 9 cases, no further progression in 34 patients, and progression into cardiac or intestinal

metaplasia in 11 patients. No cases of dysplastic or malignant transformation were registered. Where we observed the regression of BE, among the postoperative functional examinations results of manometry (pressure of lower esophageal sphincter) and pH-metry were significantly better compared with those groups where no changes occurred in BE, or progression of BE was found.

Discussion: Our results highlight the importance of the cases of fundic and cardiac metaplasia, which can also transform into intestinal metaplasia.

Conclusions: Antireflux surgery can appropriately control the reflux disease in a majority of the patients who had unsuccessful medical treatment, and it may inhibit the progression and induce the regression of Barrett's metaplasia in a significant proportion of these patients.

O-G1:

NON-OXIDATIVE ETHANOL METABOLITES INDUCE INTRACELLULAR ATP DEPLETION AND INHIBIT PANCREATIC DUCTAL BICARBONATE SECRETION IN HUMAN PANCREATIC EPITHELIAL CELL LINE

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Introduction: Pancreatic ductal epithelial cells (PDEC) have important roles in the maintenance of pancreatic integrity and bicarbonate secretion. Excessive ethanol consumption is one of the most common causes of acute pancreatitis, but the effects of ethanol metabolites on PDEC are unknown.

Objectives: The aim of this study was to characterize the effects of ethanol and its non-oxidative metabolites on PDEC.

Materials & methods: In our experiments human pancreatic adenocarcinoma cell line was used (CAPAN-1). Changes of intracellular ATP level $[(ATP)_i]$, pH (pH_i) and Ca^{2+} concentration ($[Ca^{2+}]_i$) of CAPAN-1 cells were measured using microfluorometry.

Results: The administration of high concentration (100 mM) of ethanol and palmitoleic acid (POA) (100, 200 μM) induced $(ATP)_i$ depletion. Ethanol in low concentration (10 mM) induced Ca^{2+} spikes, however 100 mM ethanol had only moderate effect on $[Ca^{2+}]_i$. 200 μM POA induced sustained $[Ca^{2+}]_i$ elevation, which was significantly decreased by the administration of 20 mM caffeine and 10 μM ruthenium red. The plateau phase of the Ca^{2+} signal was inhibited by 1 μM gadolinium and abolished by extracellular Ca^{2+} withdrawal. Ethanol had dual effect on the activities of acid/base transporters (Na^+/H^+ exchanger, Na^+/HCO_3^- cotransporter, Cl^-/HCO_3^- exchanger (CBE)) of PDEC, 10 mM stimulated CBE however 100 mM inhibited them. The administration of high concentration of POA inhibited the acid/base transporters, this effect was abolished by BAPTA-AM preincubation.

Conclusion: These results suggest that ethanol and non-oxidative ethanol metabolites induce $(ATP)_i$ depletion, $[Ca^{2+}]_i$ elevation and inhibit pancreatic ductal bicarbonate secretion. The impaired bicarbonate secretion can contribute to the development of acute pancreatitis.

This work was supported by OTKA, MTA and NFÜ/TÁMOP.

O-G2

A NOVEL ROLE FOR BCL-2 IN REGULATION OF CELLULAR CALCIUM EXTRUSION

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Introduction: Bcl-2 plays important roles in Ca^{2+} signalling by influencing Ca^{2+} release from the ER through IP3Rs and regulating CICR.

Objectives: Here we have investigated whether Bcl-2 could also affect cellular Ca^{2+} extrusion.

Materials & methods: Cytosolic Ca^{2+} measurements were performed with Fluo-4 or Fura-2 and ER Ca^{2+} measurements with D1 plasmid.

Results: In the absence of external Ca^{2+}, elevation of the cytosolic Ca^{2+} concentration elicited by release of Ca^{2+} from internal stores, is followed by a slow restoration of the resting cytosolic Ca^{2+} level. In normal pancreatic acinar cells this is entirely due to operation of the plasma membrane Ca^{2+} pump. Cells lacking Bcl-2 were able to restore the cytosolic Ca^{2+} concentration, to the resting level observed prior to the intracellular Ca^{2+} release, much faster than the control cells. These cells also had a significantly reduced resting cytosolic Ca^{2+} level. The enhanced Ca^{2+} extrusion was not due to Na^+/Ca^{2+} exchange activity, as the Ca^{2+} extrusion rate was independent of the presence or absence of external Na^+. Overexpression of Bcl-2 in the pancreatic acinar cell line AR42J decreased the Ca^{2+} concentration in the endoplasmic reticulum, whereas silencing Bcl-2 expression (siRNA) had the opposite effect. Immunolocalisation and expression of Bcl-2-GFP in both pancreatic acinar and AR42J cells showed that a fraction of Bcl-2 co-localises with the plasma membrane Ca^{2+} pump, which might enable Bcl-2 to regulate its activity directly. Finally, loss of Bcl-2, while increasing Ca^{2+} extrusion and reducing cytosolic Ca^{2+} concentration rises due to release of Ca^{2+} from internal stores, dramatically decreases necrosis and promotes apoptosis induced by oxidative stress.

Conclusion: We have discovered a novel role for bcl-2 protein.

O-G3:

NA+/H+ EXCHANGER REGULATORY FACTOR-1 MEDIATES PANCREATIC DUCTAL FLUID AND BICARBONATE SECRETION BY AFFECTING CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR LOCALIZATION IN MICE

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Introduction: Na^+/H^+ exchanger regulatory factor-1 (NHERF-1) is a scaffolding protein which is responsible for the apical localization of cystic fibrosis transmembrane conductance regulator (CFTR), a key player in pancreatic ductal bicarbonate secretion.

Objectives: The aim of this study was to evaluate the role of NHERF-1 in pancreatic ductal localization of CFTR, and in bicarbonate and fluid secretion.

Materials & methods: The expression of CFTR was analysed by immunohistochemistry. Pancreatic juice was collected from anesthetized wild-type (WT) and NHERF-1 knock-out (KO) mice in basal and secretin-

stimulated conditions. We isolated intra/interlobular ducts from the pancreas of NHERF-1 WT and KO mice. Fluid secretion into the closed luminal space of the ducts was analysed using a swelling technique. Luminal anion exchange activity was determined by microfluorometry.

Results: Pancreatic ductal CFTR staining was more diffuse and less apical in the NHERF-1 KO vs. WT mice. The volume of pancreatic juice was significantly reduced in NHERF-1 KO vs. WT mice under both basal and secretin-stimulated conditions in vivo. Accordingly, the forskolin-stimulated fluid secretory rate was significantly lower in ducts from KO vs. WT mice in standard HCO_3^-/CO_2 solution in vitro. The reduction of ductal bicarbonate secretion in the NHERF-1 KO mice was confirmed by the alkali load and the inhibitor stop methods on basolaterally perfused ducts and by the luminal Cl^- removal technique on microperfused ducts.

Conclusion: Our results suggest that pancreatic ductal NHERF-1 is involved in CFTR localization and is essential for fluid and bicarbonate secretion.

This study was supported by OTKA, MTA/DFG and NFÜ/TÁMOP.

O-G4:

NON-CONJUGATED BILE ACIDS INDUCE ATP DEPLETION, MITOCHONDRIAL DAMAGE AND INHIBIT THE ION TRANSPORT MECHANISMS IN HUMAN COLONIC CRYPTS

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Introduction: Under pathophysiological conditions, such as short bowel syndrome, bile acids can reach the colon in high concentrations and can induce diarrhea.

Objectives: Our aim was to investigate whether impaired ion transport activities are involved in the pathomechanism of bile acid-induced diarrhea.

Materials & methods: Colonic biopsies were obtained from control patients (with negative colonoscopic findings) and from cholecystectomised/ileum-resected patients with/without diarrhea. Colonic crypts were isolated by collagenase digestion, and intracellular pH (pH_i) and ATP levels (ATP_i) were measured by microspectrofluorometry. Na^+/H^+ exchangers (NHEs), Na^+/HCO_3 cotransporter, Cl^-/HCO_3^- exchanger (AE) activities were determined. Intracellular mophological changes were analysed with transmission electronmicroscopy (TEM).

Results: The non-conjugated chenodeoxycholate (CDC) and the conjugated glycochenodeoxycholate (GCDC) caused dose-dependent acidosis in colonic crypts. The pHi decrease was significantly greater in case of CDC vs. GCDC. 0.3 mM CDC strongly inhibited the activities of acid/base transporters. 0.3-1 mM CDC significantly and irreversibly reduced ATP_i. TEM showed mitochondrial damage after 1 mM CDC-treatment, but no such alteration was detected in case of lower concentrations of CDC or 1 mM GCDC. Impaired NHE and AE activities were observed in cholecystectomised/ileum-resected patients suffering from diarrhea compared to control patients.

Conclusion: Non-conjugated bile acids cause intracellular acidosis, ATP_i depletion, mitochondrial damage and inhibit the ion transporters of colonic epithelial cells. These processes may reduce fluid and electrolyte absorption in the colon and promote the development of diarrhea.

This work was supported by OTKA, MTA and NFÜ (TÁMOP).

O-G5:

THE EFFECTS OF PANCREATITIS-INDUCING FACTORS ON DUCTAL FLUID SECRETION

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Introduction: Decreased fluid secretion by pancreatic ductal epithelial cells (PDECs) may play an important role in the development of acute pancreatitis (AP); however, the exact mechanism is unknown.
Objectives: Since epithelial water transport is mediated by aquaporin (AQP) water channels, the aim of this study was to characterize the effects of AP inducing factors and the proinflammatory mediator, tumor necrosis factor- (TNF-) on AQPs in a human pancreatic ductal cell line.
Materials & methods: CAPAN-1 cells were treated with ethanol (EtOH; 1-100 mM), chenodeoxycholate (CDC; 0.1-0.5 mM), glycochenodeoxycholate (GCDC; 0.1-0.5 mM) or TNF- (0.2, 20 ng/ml) for 6, 12, 24 and 48 hours and the expression of AQP isoforms (AQP1-12) was examined by real-time RT-PCR and immunocytochemistry. Water transport was characterized by the dye dilution technique.
Results: We showed the presence of all the 12 AQP isoforms in CAPAN-1 cell line. CDC and GCDC dosedependently decreased, whereas a 24-hour incubation with EtOH, increased the expression of AQPs. TNF- did not affect significantly the expression of AQPs. Notably, a 72-hour incubation in culture media restored the expression of AQPs in the 6- and 12-hour CDC- and GCDC treated groups and in the 24-hour EtOH-treated group. Immunocytochemical stainings were in accordance with the RT-PCR results. Functional investigation of AQPs showed, that CDC inhibited the water transport.
Conclusion: The role of AQP in the pathogenesis of AP needs further investigations.

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O-G6:

INFLAMMATORY CYTOKINES DOWNREGULATE THE NA^+/H^+ EXCHANGER 3 (NHE3) INTERACTING PDZ- DOMAIN PROTEIN PDZK1 IN ULCERATIVE COLTIS PATIENTS, COLITIS MICE AND IN CACO2BBE CELLS: LINK TO INFLAMMATION-ASSOCIATED NHE3 DYSFUNCTION.

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Introduction: We have previously reported a dysfunction of the major intestinal sodium absorptive transporter, the Na^+/H^+ exchanger NHE3, in the surface colonocytes of UC patients, despite normal expression and membrane localization.

Objectives: In this study, we searched for potential mechanism for this functional defect in NHE3 transport.
Materials & methods: Realtime PCRs, Western blotting, immunohistochemistry, ileal loop experiments and pH fluorometry using pH sensitive BCECF dye were done.
Results: In three immunologic mouse models for Colitis (Rag2 KO CD45RB^{high} and IL-10 KO) and Crohn's disease (TNF ^{ARE} +/-), and in biopsies from patients with active UC, we found no change in the mRNA expression of NHE3 or NHERF1, but a dramatic decrease in PDZK1, in the respective inflamed segments. Fluid absorption in vivo, and acid-activated NHE3 activity in vitro decreased significantly in the inflamed segments, despite normal immunohistochemical NHE3 staining in the brush border membrane. In PDZK1 heterozygote mice, where enterocyte PDZK1 protein content was reduced by >50%, acid-activated NHE3 activity decreased to a similar degree in colonocytes compared to inflamed mice, suggesting a causal relationship between PDZK1 downregulation and NHE3 dysfunction. In Caco-2bbe cells treated with cytokines, significant down regulation in PDZK1 mRNA and protein expression was observed.
Conclusion: The data demonstrate a marked decrease in the PDZ-adaptor protein, PDZK1, in inflamed murine and human intestine, and a dysfunction of NHE3 activity that is similar in inflamed enterocytes and in those with genetic downregulation of PDZK1. PDZK1 downregulation during inflammation may thus be one factor responsible for inflammation-associated NHE3 dysfunction.

O-G7:

ON THE MECHANISM OF HEREDITARY CHRONIC PANCREATITIS

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Introduction: Mutations in human cationic trypsinogen (serine protease 1, PRSS1) cause hereditary chronic pancreatitis, an autosomal dominant genetic disorder of the pancreas. Premature, intrapancreatic activation of trypsinogen mutants has been hypothesized to initiate the disease. Activation of cationic trypsinogen is proteolytically regulated by chymotrypsin C (CTRC), which increases the rate of autoactivation by processing the trypsinogen activation peptide to a shorter form but suppresses final trypsin levels by promoting trypsinogen degradation.
Objectives: Our aim was to identify the effect of PRSS1 mutations on the regulation of cationic trypsinogen by chymotrypsin C.
Materials & methods: Cationic trypsinogen mutants were produced recombinantly and purified to homogeneity. Trypsinogen activation was followed by activity assays and gel electrophoresis.
Results: Here we demonstrate that in the presence of chymotrypsin C, trypsinogen mutants associated with classic hereditary pancreatitis autoactivate at markedly increased rates and reach much higher trypsin levels than wild type cationic trypsinogen. The mechanistic basis of increased activation is mutation-specific and involves resistance to degradation and/or increased N-terminal processing by chymotrypsin C.
Conclusion: The observations indicate that hereditary pancreatitis is caused by chymotrypsin C-dependent dysregulation of cationic trypsinogen, which results in strongly increased trypsinogen activation.

O-G8:

PROTECTIVE ROLE OF CALMODULIN IN ALCOHOL-INDUCED TRYPSINOGEN ACTIVATION

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Introduction: Acute pancreatitis is generally initiated by premature trypsinogen activation in pancreatic acinar cells mediated by excessive intracellular calcium release from internal stores. One of the major causes of acute pancreatitis is excessive alcohol intake, but the molecular mechanism of this severe inflammatory disease is not completely understood.

Objectives: We now show that in permeabilized mouse pancreatic acinar cells even a relatively low ethanol concentration as well as its non-oxidative metabolite palmitoleic acid ethyl ester (POAEE) elicit calcium release from intracellular stores and also induce intracellular trypsinogen activation.

Materials & methods: Single pancreatic acinar cells and clusters of two or three acinar cells were isolated from the pancreas of adult CD 1 male mice. Cells to be permeabilized were loaded with calcium sensitive dye Fluo-5N AM and then transferred to poly-L-lysine coated coverslips in a flow perfusion chamber. Cells were permeabilized using a short pulse of high intensity two-photon laser beam. BZiPAR was used to continuously measure the activity of trypsin by adding the substrate to the perfusion chamber after cell permeabilization.

Results: Reintroducing the calcium sensor calmodulin (at a normal intracellular concentration) to the permeabilized cells dramatically reduced ethanol-induced calcium release and trypsinogen activation. Pre-incubation of cells with a calmodulin activator CALP-3 abolished the ethanol and POAEE effects in intact and permeabilized cells. The calmodulin-sensitive inositol trisphosphate receptor calcium release channels, that are responsible for normal pancreatic stimulus-secretion coupling, also play a major role in the toxic action of ethanol. Calmodulin provides a protective mechanism, regulating the sensitivity of the calcium release process.

Conclusion: The marked inhibition of ethanol-induced (or POAEE-induced) Ca^{2+} release and trypsin activation by CALP(s) suggests potential therapeutic benefits for treatment of pancreatitis and prompts for a search of more potent peptides and/or drugs. It has been also demonstrated that trypsin activation can be prevented by removing extracellular Ca^{2+}. Further studies of regulation of Ca^{2+} signalling mechanisms including Ca^{2+} entry and store replenishment would also be beneficial for our understanding of acute pancreatitis initiation and possible treatment of the disease.

O-G9:

HIGH RESTARTING RATE AMONG PATIENTS WITH CROHN'S DISEASE AFTER CESSATION OF ONE-YEAR TREATMENT PERIOD WITH BIOLOGICALS: RESULT OF NATIONAL RASH STUDY

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Introduction: Biological therapy proved to be effective in the treatment of Crohn's disease (CD) and ulcerative colitis (UC). One of the important questions about biological therapy is the duration of treatment and the relapse rate after discontinuation.

Objectives: The aim of the study was to assess the frequency and the time to restart anti-TNF therapy after one-year treatment period, and to evaluate predisposing factors.

Patients & methods: Data of 187 IBD patients were analyzed. 68.4% of the CD patients received infliximab, 31.6% adalimumab. 21.1% of the CD patients received previous episodic/continuous biological therapy.

Concomitant immunosuppressions at induction therapy were steroids in 62% and azathioprine in 81.8% of patients. Medical records were captured prospectively; data of the CD and UC groups were analyzed separately.

Results: 78.5% of the patients were in remission after a one-year treatment period. Dose intensification was needed in 13.8% of CD and in 11.4% of UC patients. Biological therapy had to be restarted because of clinical flare after remission in 45.9% of CD patients and in 28.6% of UC patients. Corticosteroid use at induction, previous anti-TNF- therapy and dose intensification were associated with the need for restarting biological therapy in CD.

Conclusion: Biological therapy had to be restarted in almost half of the CD patients after the discontinuation within a median of 8 months after the discontinuation, despite being in remission at one-year. Steroid use, previous biological therapy and dose intensification but not CRP was identified as predictors for the need for restarting biological therapy.

O-G10:

PLASMA METHYLATED SEPT9 IS A SCREENING MARKER IN BOTH LEFTAND RIGHT– SIDED COLON CANCER. COMPARISON TO FOBT AND CEARESULTS

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Introduction: Methylated SEPT9 (*m*SEPT9) DNA is a biomarker for colorectal cancer (CRC) from peripheral blood. The relationship of *m*SEPT9 to CRC location, FOBT andcarcinoembryonic antigen (CEA) are unknown.

Objectives: 1) Determine the sensitivity of *m*SEPT9 in the left-and right-sided CRC. 2)Compare *m*SEPT9 and FOBT in individuals with no evidence of disease (NED) and CRC. 3)Compare *m*SEPT9 and CEA in NED and CRC.

Materials & methods: Plasma samples were collected from NED (n = 92) and CRC (n =92). Qualitative determination was performed using Epi *pro*Colon kit 2.0 (Epigenomics AG). Samples for FOBT were used from NED (n = 17) and CRC (n = 22). Serum samples for CEA analysis were collected from NED (n = 27) and CRC (n = 27).

Results: *m*SEPT9 values were detected in 15.2 % (14/92) NED and 95.6 % (88/92) CRC. FOBT was positive for 29.4 % (5/17) NED and 68.2 % (15/22) CRC. Positive CEA results were detected in 14.8 % (4/27) of NED and 51.8 % (14/27) CRC. *m*SEPT9 was positive in 96.4 % (54/56) of left-sided and 94.4 % (34/36) of right-sided CRC. FOBT was positive in 83.3 % (10/12) of left-sided and 50 % (5/10) of right-sided CRC. CEA was positive in 60 %(9/15) of left-sided and 41.7 % (5/12) of right-sided CRC.

Conclusion: *m*SEPT9 has no difference between left and right side CRC. *m*SEPT9 showed higher sensitivity for CRC compared to FOBT. Plasma *m*SEPT9 showed higher sensitivity in both sides of CRC then blood CEA.

O-G11:

THE EVALUATION OF OESOPHAGEAL FUNCTION IN PATIENTS WITH DIFFERENT TYPES OF OESOPHAGEAL METAPLASIA

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Introduction: The increasing prevalence of oesophageal adenocarcinoma has focused the attention to the development of oesophageal metaplasia. Although gastrooesophageal reflux is thought to be an important factor in this process, detailed evaluation of the oeseophageal function is not performed.
Objectives: To evaluate the oesophageal function in patients with different types of oesophageal metaplasia and in cases with dysplasia on the basis of the Montreal definition of gastrooesophageal reflux disease.
Patients & methods: 270 consecutive patients (M/F 151/119, mean age 54.2 years) with endoscopic and histological evidence of oesophageal metaplasia were prospectively studied: patients with specialized intestinal metaplasia (SIM, n = 109) and patients without SIM (n = 161). Patients with SIM were subdivided into a dysplasia-positive (n = 34) and a dysplasia-negative (n = 75) group. All patients underwent reflux symptom analysis, oesophageal manometry, and simultaneous 24-hour pH and biliary reflux monitoring.
Results: Patients with SIM were significantly older and hada significantly higher body mass index than patients without SIM. A significant male predominance was observed in patients with SIM and dysplasia compared to the dysplasia-negative group. The clinical symptom spectrum and the prevalence of erosive oesophageal lesions were similar in all groups. Patients with SIM had longer metaplastic segments, which was further increased in thedysplasia-positive group. During oesophageal manometry, pH and biliary refluxmonitoring, patients with SIM had more severe alterations than patients without SIM, and these were further increased in patients with SIM and dysplasia.
Conclusion: Patients with SIM had more severe oesophageal function abnormalities than those with other types of oesophageal metaplasia (e.g. gastric). The oesophageal function was further impaired if dysplasia was present in the metaplastic mucosa.

O-G12:

METHYLATION-RELATED BIOMARKER IDENTIFICATION BY GENE EXPRESSION ANALYSIS OF LASER MICRODISSECTED COLONIC CELLS

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Introduction: Changes of the DNA methylation pattern is proven to be an important process during colorectal tumorigenesis.
Materials & methods: Our aim was to identify mRNA expression patterns using LCM samples to determine the underexpressed genes in colorectal adenoma and tumor compared to the healthy stage. Furthermore, we aimed to analyse the possible mechanisms of downregulation, such as cancer-related methylation using cell culture model. From 6 colorectal cancers and 6 adenomas, epithelial cells were collected from the healthy and the pathological regions by laser capture microdissection. After microarray analyses, downregulated genes

were identified. In parallel, HT29 colon adenocarcinoma cells were demethylated with 5-aza-2'deoxycytidine and the upregulated genes were determined. The selected genes were validated by RT-PCR. Bisulfite sequencing and immunohistochemistry for a selected gene (PTGDR) were performed.

Results: During the adenoma-carcinoma sequence 95 genes showed gradually decreasing expression and in the cell culture model 66 upregulated genes were identified after demethylation. Between these groups, there were 17 overlapping genes (eg.: CDKN2B, PTGDR, RASSF6), which were validated on independent samples by RT-PCR. Reduced expression of PTGDR was confirmed by immunohistochemistry along the adenoma-carcinoma sequence. In the promoter region of the gene three CpG islands were predicted, where the methylation status was determined.

Conclusion: The regulation of the identified genes showing decreased expression during the adenoma-carcinoma sequence progression can be associated with DNA methylation. On the basis of our results, the set of genes can be determined genome-widely, which can be key factors in the formation and the prognosis of the disease.

O-G13:

DETERMINATION OF GASTRIC EMPTYING, THE CURRENT GLUCOSE LEVELS AND NEUROPATHY IN PATIENTS WITH TYPE 1 DIABETES MELLITUS

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Introduction: The possible correlations between the severity of autonomic neuropathy (AN), the current glucose levels and gastric emptying are still not clearly explored.

Objectives: The aims of our study were to evaluate gastric emptying during a continuous glucose monitoring and to assess the severity of AN in patients with type-1 diabetes (DM).

Patients & methods: 17 patients with type 1 DM were included into the study (HbA1c: 8.3±0.2%, age: 34.9±2.2 years, duration of DM: 15.7±2.8 years; mean±SE). 9 subjects were healthy controls. Stomach motility was evaluated by a scintigraphic gastric emptying procedure. The subcutaneous glucose levels were measured by a continuous glucose monitoring system (CGMS, Medtronic Ltd) during the total interval of the gastric emptying. The five standard cardiovascular reflex tests were applied for the assessment of AN. Sensory nerve integrity was studied with a Neurometer (Neurotron Inc., Baltimore, MD)

Results: There was a trend for a longer gastric emptying in diabetic patients compared to healthy subjects ($T_{1/2}$: 80.1±9.8 vs 49.6±5.5 min., p=0.06, diabetic vs control). The ratio of abnormal gastric emptying among diabetic patients was 7/17. No correlations were found between the glucose levels recorded during the gastric emptying (the lowest and highest, the mean, the difference of highest and lowest glucose) and the gastric motility. Gastric emptying in groups of patients created by the different glucose parameters did not differ significantly. There was no correlation between HbA1c values and the gastric motility. Moderate to severe AN was found in diabetic patients (AN: 3.1±0.4 vs 0.3±0.2 p<0.001, heart rate response to beathing: 16.8±1.8 vs 26.3±2.3 beats/min, p<0.01; 30/15 ratio: 1.02±0.02 vs 1.21±0.04, p<0.01; handgrip: 14.6±2.8 vs 28.3±3.5 mm Hg, p<0.05; diabetic vs control). The current perception thresholds (CPT) on the peroneal nerve at 5 Hz of the patients differed from controls (CPT: 2.89±0.9 vs 0.68±0.07 mA, p<0.05 indicating hypaesthesia).

Conclusion: There was no correlation between gastric emptying and actual glucose levels assessed by continuous glucose monitoring. Slower gastric emptying was found in the presence of a moderate to severe

autonomic and sensory neuropathy in patients with a 15-year-long duration of type 1 diabetes. These data suggest that severity of autonomic neuropathy rather than the current glucose levels may have importance in the pathogenesis of delayed gastric emptying in diabetic patients.

O-G14:

GASTROINTESTINAL NEUROMUSCULAR DYSFUNCTION IN KLOTHO MOUSE MODEL OF AGEING

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Introduction: Disordered gastrointestinal motility particularly constipation, increase with ageing, but their underlying mechanisms are poorly understood due to lack of experimental models. Previously we established the progeric klotho mouse as a model of aging-associated anorexia and gastric dysmotility. We also detected reduced faecal output in these animals.

Objectives: The aim of this study was to investigate in vivo function and cellular make-up of the small intestinal and colonic neuromuscular apparatus.

Materials & methods: Klotho expression was studied by RT-PCR and immunohistochemistry. Motility was assessed by dye transit and bead expulsion. Smooth muscle and neuron-specific gene expression was studied by Western immunoblotting. Interstitial cells of Cajal (ICC) and precursors were analyzed by flow cytometry, confocal microscopy, and three-dimensional reconstruction. HuC/D+ myenteric neurons were enumerated by fluorescent microscopy.

Results: Klotho protein was detected in neurons, smooth muscle cells, and some ICC classes. Small intestinal transit was slower but whole-gut transit of klotho mice was accelerated due to faster colonic transit and shorter intestinal lengths, apparent only after weaning. Fecal water content remained normal despite reduced output. Smooth muscle myosin expression was reduced. ICC, ICC precursors, as well as nitrergic and cholinergic neurons maintained their normal proportions in the shorter intestines.

Conclusion: Progeric klotho mice express less contractile proteins and develop generalized intestinal neuromuscular hypoplasia mainly arising from stunted postweaning growth. As reduced faecal output in these mice occurs in the presence of accelerated colonic and whole-gut transit, it likely reflects reduced food intake rather than intestinal dysmotility.

O-G15:

DECREASED SOMATOSTATIN PRODUCTION IN COLORECTAL CANCERWITH UNCONTROLLED CELL PROLIFERATION, AS COMPARED TOCONTROLLED CELL GROWTH IN YOUNG AND ADULT COLONIC MUCOSA

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Introduction: Molecular background of controlled and uncontrolled cell proliferation in colonic mucosa is unknown. Somatostatin (SST) has anti-proliferative and pro-apoptotic effects. Production of SST is unexamined in colonic mucosa during aging and colorectal carcinogenesis.

Objectives: 1. Comparison of mitotic index (MI) in healthy colonic samples from children and adults, that can be characterized with controlled cell proliferation, contrary to colorectal cancers (CRCs) with uncontrolled cell growth. 2. Analysis of SST expression on mRNA and protein levels.

Materials/Patients & methods: Proliferation was detected by Ki-67 immunohistochemistry and SST producing cells with polyclonal antibody on colonic biopsy from healthy children (n1=14; n1=14), adults (n2=10; n2=20) and CRCs (n3=10; n3=23). After digital scanning, MI and ratio of SST producing cells were determined. Colonic samples were collected for the analysis of SST gene expression (n1=6; n2=41; n3=34), using HGU133plus2.0 microarrays; results were validated with real-time PCR. **Results:** MI was significantly higher in children colonic samples (0,34±0,07) and CRC samples (0,42±0,11) compared to healthy adults (0,15±0,06) ($p<0,05$). Ratio of SST producing cells was significantly higher in children (0,70%±0,79%) compared to CRC samples (0%±0%) ($p<0,05$). mRNA expression of SST did not alter during aging in healthy colonic mucosa, but decreased during carcinogenesis ($p\leq0,05$).

Conclusion: Colonic samples from healthy children and CRCs can be characterized with increased proliferative activity compared to healthy colonic samples from adults, although it is a well controlled process in childhood contrary to CRC. Local SST production decreases during colorectal carcinogenesis and it can contribute to the unregulated cell proliferation.

O-G16:

ENDOSCOPIC ACTIVITY AT THE TIME OF DIAGNOSIS DOES NOT PREDICT DISEASE COURSE IN CROHN'S DISEASE, WHILE ENDOSCOPIC FINDING IS WORSENED BY SMOKING

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Introduction: Endoscopy is essential for the diagnosis, the management and the prognosis of Crohn's disease (CD).

Objectives: Our aim was to evaluate whether the diagnostic endoscopic activity predicts the outcome of CD. We examined whether there are associations between diagnostic endoscopic activity and different parameters.

Patients & methods: The medical records and endoscopic findings of 201 recently diagnosed CD patients were assessed. Demographic data, endoscopic findings, extraintestinal manifestations, perianal lesions, surgeries and treatment types were recorded. The evaluation of endoscopic activity was based on the Simplified Endoscopic Activity Score for Crohn's Disease (SES-CD).

Results: 533 endoscopies were performed in 201 patients with CD. Endoscopic relapse occurred in 66 patients. 38 of them were smokers. Of the 65 patients with severe endoscopic lesion, 18 showed endoscopic relapse during the follow-up period. Statistically, smoking was shown to be a risk factor for endoscopic relapse ($p=0.04$). Diagnostic SES-CD values did not correlate with surgical interventions. Severe stenosis at the diagnostic colonoscopy predisposed to ileal ($p=0.05$) or colonic ($p=0.0002$) surgeries during the course of the disease. Biological therapy did not decrease the need for surgery in patients with severe stenosis. None of the further examined factors were associated with endoscopic relapse.

Conclusion: Our results did not reveal association between the SES-CD values at the diagnosis and in the outcome. Considering patient-specific data smoking was the only factor predicting a more severe endoscopic activity. Although endoscopy remains a very important diagnostic tool in CD, our results confirm that quitting smoking is a crucial goal of therapy.

O-G17:

ASSOCIATION BETWEEN AUTOIMMUNE PANCREATITIS AND SYSTEMIC AUTOIMMUNE DISEASES

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Introduction: In 50-63% of the cases autoimmune pancreatitis (AIP) is associated with systemic autoimmune diseases (SAIDs), suggesting that it may be a systemic disorder.
Objectives: The aim of the present study was to investigate the association of AIP and SAIDs by measurement of serum immunoglobulin-G4 (IgG4).
Patients & methods: The serum level of IgG4 was measured in 61 patients with SAIDs of different types who had not yet participated in glucocorticosteroid treatment. Patients with an elevated IgG4 level were examined by abdominal ultrasonography (US) and in some cases computer tomography (CT).
Results: Elevated serum IgG4 levels (919±996 mg/l) were detected in 17 (28%) of the 61 SAID patients. 10 patients had Sjögren's syndrome (IgG4: 590±232 mg/l), 2 of them in association with Hashimoto's thyroiditis, and 7 patients (IgG4: 1388±985.5mg/l) had systemic lupus erythematosus (SLE). The IgG4 level in the SLE patients and that in patients with Sjögren's syndrome were not significantly different from that in AIP patients (783±522 mg/l). Abdominal US and CT did not reveal any characteristic features of AIP among the SAID patients with an elevated IgG4 level.
Conclusion: The serum IgG4 level may be elevated in SAIDs without the presence of AIP. The determination of serum IgG4 does not seem to be suitable for the differentiation between IgG4 related diseases and SAIDs.

O-G18:

RARE CATIONIC TRYPSINOGEN MUTATIONS FOUND IN PATIENTS WITH CHRONIC PANCREATITIS ARE HARMLESS VARIANTS

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Introduction: Mutations in the cationic trypsinogen gene (PRSS1) are causative for hereditary pancreatitis or risk factors for chronic pancreatitis. The mutations result in a gain of function manifested by increased autoactivation or resistance to degradation. To date, a large number of rare or private PRSS1 mutations have been identified in subjects with idiopathic chronic pancreatitis.
Objectives: Our aim was to establish the possible clinical relevance of published rare PRSS1 mutations on the basis of their phenotypic characteristics.
Materials & methods: Wild-type cationic trypsinogen and 6 mutants were produced recombinantly and purified to homogeneity. Trypsinogen activation, trypsinogen/trypsin degradation and enzyme kinetic

parameters were investigated by activity assays and gel electrophoresis. Cellular expression of trypsinogens was assessed by SDS-PAGE and trypsin activity assays of conditioned media from transfected HEK 293T cells.

Results: We found that none of the investigated mutants exhibited a gain of function, in contrast to the common pancreatitis-associated mutations. Surprisingly, four mutants showed loss-offunction characteristics either due to reduced secretion (K92N, G83E and V123M) and/or increased degradation by chymotrypsin C (G83E, V123M and I88N).

Conclusion: Rare cationic trypsinogen mutations observed in patients with chronic pancreatitis are innocuous variants with no functional consequence or they cause loss of function and may even be protective against pancreatitis. This study reinforces the concept that clinical relevance of rare PRSS1 variants should not be assigned on the basis of a perceived analogy with genuine disease-causing PRSS1 mutations, but should be evidence-based.

P-G1:

FUNCTIONAL CHARACTERIZATION OF THE P.L104P HUMAN CATIONIC TRYPSINOGEN VARIANT

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Introduction: Mutations in the *PRSS1* gene encoding human cationic trypsinogen are associated with hereditary pancreatitis. A number of rare *PRSS1* variants have been identified in subjects with sporadic idiopathic chronic pancreatitis. The clinical significance of rare *PRSS1* variants is unclear; therefore, functional studies are required to determine possible disease association.

Objectives: Our aim was to examine the functional properties of the rare p.L104P variant.

Methods: Activation, degradation, enzyme activity and inhibitor binding of recombinantly expressed and purified wild-type and mutant cationic trypsinogen were studied by enzymatic assays and SDS-PAGE. Secretion of trypsinogen was studied using transiently transfected HEK 293T cells.

Results: Mutation p.L104P alters the S2 subsite of the substrate binding site on trypsin. Accordingly, the mutant trypsin exhibited decreased activity on a small peptide substrate and on chymotrypsinogen. Binding of the physiological trypsin inhibitor SPINK1 was also decreased by 20-fold. Remarkably, however, autoactivation of p.L104P trypsinogen was not impaired. Secretion of the p.L104P mutant from HEK 293T cells was 20-fold lower than that of wild type due to intracellular retention and degradation.

Conclusion: Although the markedly decreased inhibitor binding of p.L104P trypsinogen suggests that this variant autoactivates faster in the presence of SPINK1 than its wild-type counterpart, the severe secretion defect points to an overall loss-of-function phenotype inconsistent with disease-association. On the other hand, further studies are required to examine whether intracellular retention of the p.L104P might cause endoplasmic reticulum (ER) stress and whether ER stress might be a pathogenic factor in chronic pancreatitis.

P-G2:

ACTIVATION OF HUMAN CHYMOTRYPSINOGEN ISOFORMS

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Introduction: The human pancreas secretes four different isoforms of the digestive proenzyme chymotrypsinogen: CTRB1, CTRB2, CTRC and CTRL1. Physiological activation of chymotrypsinogen is catalyzed by trypsin in the duodenum. During the activation process trypsin cleaves off a 13-15 amino-acid long N-terminal propeptide from chymotrypsinogen, which remains attached to the active chymotrypsins through a disulfide bond.

Objectives: The aim of the present study was to characterize the activation and enzymatic activity of human chymotrypsins in a comparative manner and to elucidate the functional role of the disulfide-attached propeptides.

Materials & methods: Human digestive proenzymes were produced recombinantly and purified to homogeneity. Cysteine residues responsible for anchoring the cleaved propeptides were mutated to alanine. Chymotrypsinogen activation and chymotrypsin activity were followed by enzymatic assays and SDS-PAGE.

Results: Trypsin activated CTRL1 at a higher rate (ranging from 2-fold to 9-fold) than CTRB1, CTRB2 or CTRC. When comparing human trypsin isoforms, anionic trypsin activated CTRB2 and CTRC approximately 4-fold better than cationic trypsin. Activated CTRB2 lacking a disulfide-linked propeptide exhibited accelerated autolytic degradation. All four chymotrypsins readily cleaved peptide substrates after phenylalanine and tyrosine amino-acid residues, while only CTRB1 cleaved after tryptophan and CTRC cleaved after leucine and methionine.

Conclusion: The preferential activation of CTRL1 suggests that this isoform may be the first chymotrypsin activated in the duodenum. The disulfide-linked activation peptides are required to stabilize chymotrypsins against autolysis. Finally, human chymotrypsin isoforms exhibit both overlapping and unique substrate specificities which permit efficient digestion of a diverse set of peptide bonds in dietary proteins.

P-G3:

DETECTION OF METHYLATION PROFILE CHANGES OF CFDNA FRACTIONS IN PATIENTS WITHCOLORECTAL CANCER COMPAIRED TO ADENOMA AND HEALTHY CONTROLS

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Introduction: During the progression of colorectal cancer the methylation profile changes can be detected with evaluation of the circulating cell free DNA.

Objectives: Our aim was to develop a method for separation, quantification of the methylated and nonmethylated cfDNA fractions of healthy, colorectal adenoma and cancer patients.

Materials & methods: We were investigated 10-10-10 patients with adenoma, colorectal cancer and healthy controll. We have been isolating the cfDNA from plasma with QIAamp Circulating Nucleic Acid Kit (Qiagen) and performed optimised fragmentation with DNaseI nuclease enzyme. For DNA capturing paramagnetic beads were used (MethylMiner_ Methylated DNA Enrichment Kit, Invitrogen). Then the methylated fragments were eluted based on the degree of methylation by increasing NaCl concentration. For the examination of the fractions PicoGreen and real-time PCR were applied by means of methylated and non-methylated artificial DNA control. For further analysis we used new generation sequencing based on methylation profile.

Results: We have used 100 ng input DNA, and fragmented each sample 20 minute long using1.66U/ml Dnase enzym concentration to get the ideal 200 – 1000 bp size. We established a real-time PCR method to identify

the optimal 850 mM NaCl concentration to extract the methylated fraction. We have assessed that the of cfDNA is methylated in patients with colorectal cancer 39,49% in adenoma 41,39% and 43,56% in healthy cases. With sequncing we could detect the hypermethylation of ALDH1A3, SFRP1, MAL and SLIT2 genes.
Conclusion: The detection of epigenetic changes from peripheral blood can be important step to develop screening and diagnostic assays.

P-G4:

LIVER MITOCHONDRIAL RESPIRATORY FUNCTION IS DECREASED IN SENESCENT RATS

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Introduction: Mitochondria play a central role in myocardial energy metabolism and are also key regulators of longevity. Functional impairment of mitochondria is underlying the aging process.
Objectives: The present study was aimed at characterizing the effects of ageing on mitochondrial respiration.
Materials & methods: Respiratory rates were measured in liver mitochondria isolated from senescent (18-20 months) vs. adult (4-6 months) Wistar rats. Liver mitochondria were isolated by differential centrifugation technique. Mitochondrial respiration was measured using a Clark-type oxygen electrode (StrathkelvinLtd.) at 37°C in the presence of the following sequence of additions:glutamate/malate (NAD^+-linked substrates) or succinate (FAD-linked substrate in the presence of rotenone). Basal (state 2) andADP-stimulated (state 3) respiratory rates were recorded and expressed as natoms O_2 /min/mg protein and respiratory control ratio (RCR) was calculated.
Results: State3 respiratory rates showed a clear decline in the mitochondria isolated from old rats (n=8) compared with adult rats (n=7) when using either NAD^+(100.7 ±2.1 vs. 124.8 ±5.6, p =0.0013) or FAD (124.3 ±1.9 vs. 144.0 ±5.9, p=0.0085) linked substrates with no differences from controls in state 4 rates, resulting in a decreased RCR for both substrates (6.08 ±0.42 vs. 7.68 ±0.35, p=0.0098; 3.27 ±0.12 vs.3.85 ±0.19, p=0.0298), respectively.
Conclusion: Impairment of active respiration in liver mitochondria is associated with ageing in healthy animals.

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P-G5:

ETHANOL AND ITS NON-OXIDATIVE METABOLITES INHIBIT CFTR ACTIVITY IN GUINEA PIG PANCREATIC DUCT CELLS

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Introduction: The mechanism by which ethanol (EtOH) induces acute pancreatitis (AP) is not completely understood; however it is suggested that the non-oxidative metabolites of EtOH play a crucial role in the

development of AP. Recent studies indicated that impaired ductal fluid secretion can lead to the development of AP.

Objectives: Since cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel plays a crucial role in maintaining pancreatic fluid secretion, our aim was to investigate the effect of EtOH, and its non-oxidative metabolites on basal and forskolin-stimulated CFTR currents in pancreatic ductal cells.

Materials & methods: We used the patch clamp techniqueto study the effects of EtOH (1, 10 and 100 mM), palmitoleicacid (POA) and palmitoleic acid ethyl ester (POAEE; 10, 100 and 200 μM) on whole cell CFTR currents in single, guinea pig pancreatic duct cells.

Results: Administration of 100 mM EtOH increased the basal and reversibly inhibited the forskolinstimulated CFTR currents. In contrast, 1 and 10 mM EtOH had no effect on the CFTR currents. POA at higher concentrations (100 and 200 μM) significantly decreased both the basal and forskolin-stimulated currents, whereas, POAEE was able to block the forskolin-stimulated currents onlyat 200 μM concentration.

Conclusion: The effects of EtOH and its non-oxidative metabolites in the pathomechanism of AP needs further investigations.

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P-G6:

SUBSTANCE P INHIBITS DUCTAL BICARBONATE SECRETION IN GUINEA PIG PANCREATIC DUCTS VIA NEUROKININ RECEPTORS 2 AND 3

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Introduction: Substance P (SP) is a well-known neuropeptide, which exerts its effect via neurokinin (NK) receptors. Previously, we have shown that SP inhibits secretin-stimulated fluid secretion in intact guinea pig pancreatic ducts and that this inhibitory effect of SP can be relieved by spantide, an NK receptor antagonist.

Objectives: In this study our aim was to investigate which NK receptor(s) mediate(s) the inhibitory effect of SP.

Materials & methods: We isolated intra/interlobular ducts from guineapig pancreas after treatment with collagenase. The rate of HCO_3^- secretion was estimated by the alkali load technique using microfluorometry. Expressionand localization of NK receptors were examined by immunocytochemistry.

Results: Basolateral administration of 10 nM secretinsignificantly increased HCO_3^- secretion, which was completely blocked by application of 20 nM SP. The NK1 antagonist RP67580 (10 μM) did not influence the inhibitory effect of SP. However, the NK2 antagonist, MEN10376 (10 μM) and the NK3 antagonist, SB218795 (10 μM) significantly reversed the inhibitory effect of SP by 42.5 ± 2.1 % and 68.1 ± 3.5 %, respectively. The NK1 receptors were localized to the luminal membrane, while the NK2 and NK3 receptors were identified both on the lateral and luminal membranes of the intra/interlobular ducts.

Conclusion: In this study we characterized the localization of NK receptor subtypes in the guinea pig pancreas and showed evidence that SP inhibits HCO_3^- secretion via the laterally expressed NK receptors, namely the NK2 and 3.

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P-G7:

THE POSSIBLE ROLE OF MITOCHONDIAL INJURY IN L-LYSINE-INDUCED ACUTE PANCREATITIS

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Introduction: Large intraperitoneal (i.p.) doses of basic amino acids, such as L-arginine, L-ornithine or L-lysine, have been shown to induce acute pancreatitis.
Objectives: Our aim was to investigate the mechanisms through which L-lysine damages the pancreas.
Materials & methods: Male Wistar rats were injected intraperitoneally with 2 g/kg L-lysine and sacrificed 0-168 h afterwards. Biochemical and histological parameters of pancreatitis were determined. We characterized the kinetics of L-lysine-induced mitochondrial injury, trypsinogen and nuclear factor-κB (NF-κB) activation which are commonly thought to play an important role in the development of acute pancreatitis. The morphology of isolated rat pancreatic acinar cells was assessed by electron microscopy after incubation of cells with 20 mM L-lysine for 2h.
Results: Intraperitoneal administration of L-lysine induced severe acute necrotizing pancreatitis. L-lysine administration caused early pancreatic mitochondrial damage (from 1 h) that preceded the activation of trypsinogen (12-48 h) and NF-κB (24-168 h). Serum amylase and lipase activities were significantly increased, whereas pancreatic amylase activity was decreased. L-lysine administration also induced pancreatic oxidative stress and heat shock protein 72 expression. Elevated pancreatic myeloperoxidase activity was detected from 18 to 72 h. Large concentrations of L-lysine significantly inhibited the rate of mitochondrial membrane potential recovery after consumption of ADP in isolated pancreatic, but not liver, mitochondria.
Conclusion: Our data suggest that L-lysine administration impairs the function of isolated pancreatic, but not liver, mitochondria. Early pancreatic mitochondrial injury caused by large doses of L-lysine may lead to the development of acute pancreatitis.

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P-G8:

THE INCREASING APPEARANCE OF EPITHELIAL-TO-MYOFIBROBLAST TRANSITION IN LINE WITH TRANSFORMING GROWTH FACTOR BETA II RECEPTOR AND TOLL-LIKE RECEPTOR 9 PROTEIN EXPRESSION DURING COLORECTAL CARCINOGENESIS

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Introduction: The epithelial-to-myofibroblast transition (EMyT) may be a source of the colorectal cancer-associated microenvironment. The main regulator of EMyT is transforming growth factor (TGF)-βvia its receptor (TGF-βRII) activation. During adenoma-carcinoma sequence (ACS) the enhanced stromal TGF-β level may associate with Toll-like receptor (TLR)-9 activation by increased cancer cell originated circulating free DNA motifs.

Objectives: Examine intraepithelial EMyT by detecting the frequency of α-smooth muscle actin (SMA)+/ cytokeratin (CK)+ cells in relation to TLR-9 and TGF-βRII protein expression during ACS.

Materials & methods: Histologically healthy (n=8), adenoma (n=8) and CRC (n=8) biopsy samples were included into tissue microarrays. Slides were immunostained with anti-α-SMA, anti-CK, anti-TLR9 and anti-TGF-βRII antibodies, then digitalized and analyzed with virtual microscopy.

Results: Intraepithelial α-SMA immunoreaction showed dot-like expression patterns mainly in perinuclear location. The proportion of intraepithelial α-SMA+/CK+ cells was significantly higher in CRC samples (3,34±1,01%) as compared to healthy (1,94±0,69%) or adenoma (1,62±0,78%) ones ($p<0,01$). This change was closely correlated with TGF-βRII expression. We found significantly increased ($p<0,05$) cytoplasmic TLR-9 expression both in CRC epithelium (68,25±24,33%) and adenoma (70,34±2,49%) as compared to healthy samples (32,92±8,314%).

Conclusion: In the course of tumor progression, we found an increased appearance of EMyT events in line with enhanced intraepithelial TGF-βRII expression. The dot-like α-SMA staining in CK positive cells may refer to the initial phase of EmyT. The increased expression of TLR-9 in adenoma without the increasing number of intraepithelial α-SMA+/CK+ cells, is probably due to the deficiency of TLR-9 ligands of CRC origin.

P-G9:

THE ANION TRANSPORTER SLC26A6 (PUTATIVE ANION TRANSPORTER-1) REGULATES CO_2/ HCO_3^- INDUCED MURINE SMALL INTESTINAL FLUID ABSORPTION

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Introduction: Slc26a6 (PAT-1), a multi anion transporter, is strongly expressed in the apical membrane of the upper small intestine. Data on its function are equivocal.

Objectives: To investigate if, under which conditions, and by what mechanisms, Slc26a6 contributes to intestinal fluid absorption in vivo.

Materials & methods: Small intestinal fluid absorption was assessed by single pass perfusion of 3 cm long, vascularly perfused jejunal segments in anesthetized acid/base status controlled mice with prewarmed, isotonic solutions of different ionic compositions but constant pH of 7.4. The volume and pH of the inflowing and outflowing solution was measured.

Results: WT jejunum absorbed fluid and acidified the lumen. Slc26a3 (DRA)-deficient jejunum absorbed less fluid than WT, and acidified the lumen more strongly, consistent with its action as a Cl^-/HCO_3^- exchanger. Slc26a6 (PAT-1)-deficient jejunum also absorbed less fluid, but resulted in less luminal acidification. Switching the luminal solution to a 5% CO_2/HCO_3^- buffer strongly augmented fluid absorption in a PAT-1- and NHE3- but not DRA-dependent manner. Removal of luminal Na^+ abolished fluid absorption in luminal NaCl but not in CO_2/HCO_3^-, where the absorption became completely dependent on PAT-1 expression. In the absence of luminal Cl^-, luminal CO_2/HCO_3^- also augumented fluid absorption, but in this case, the increase in absorption was dependent on both PAT-1 and NHE3 expression, and was markedly increased in DRA-deficient jejunum.

Conclusion: The results suggest that Slc26a6 (PAT-1) strongly augments CO_2/HCO_3^- induced jejunal fluid absorption, likely operating in a 2 HCO_3^-/1 Cl^- as well as 2 HCO_3^-/1 HCO_3^- mode.

P-G10:

THE ROLE OF ION TRANSPORTERS IN THE PATHOGENESIS OF ULCERATIVE COLITIS

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Introduction: The absorption of water and ions is an important function of colonic epithelial cells in physiological and pathophysiological conditions.

Objectives: The aim of our study was to characterize the segmental differences of ion transport mechanisms (Na+/H+ exchangers [NHE1-3], the epithelial sodium channel [ENaC] and the SLC26A3 Cl^-/HCO_3^- exchanger downregulated in adenoma [DRA]) in human colonic epithelial cells and to examine the activities of these transporters in ulcerative colitis (UC).

Materials & methods: Colonic biopsies were obtained from each patient (n=128 for control, n=69 for UC) undergoing colonoscopy. The expression and function of NHE1-3, ENaC and DRA were determined by using microfluorimetry, patch clamp and real time RT PCR techniques.

Results: The activities of electroneutral (via NHE3) and the electrogenic Na^+ absorption (via ENaC) were in inverse ratio to each other in the proximal anddistal colon. No differences were detected in the activity of NHE2 in different segments of the colon. Surface cell Cl^-/HCO_3 exchange was more active in the distal vs. the proximal part of the colon. Both sodium and chloride absorptions were damaged in UC, whereas, NHE1 activity (which has been shown to promote immune response) is up-regulated by 6-fold.

Conclusion: Our results demonstrate differences in the ion transport mechanisms between the different parts of the colon. Both sodium and chloride transports are damaged, whereas NHE1 is up-regulated in UC. The selective inhibition of NHE1 and/or stimulation of NHE3, ENaC and DRA may open up new therapeutical targets in UC.

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P-G11:

TOXICITY AND ABSORPTION ENHANCER PROFILE OF SURFACTANTS ON A HUMAN INTESTINAL BARRIER MODEL

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Introduction: Overcoming biological barriers, including the gastrointestinal epithelial barriers for drug delivery is a great challenge. There is a need for new absorption enhancers with more favorable profile. There are reports on the potential use of sucrose esters in pharmaceutical formulations as excipients, however no data are available on their toxicity profile and their usefulness as absorption enhancers in epithelial models.

Objectives: Our aim was to investigate the toxicity and absorption enhancer properties of sucrose esters on in vitro model of intestinal absorption.

Materials & methods: Laurate (D-1216), myristate (M-1695), and palmitate (P-1695) sucrose esters and Cremophor RH40 and Tween 80 reference absorption enhancers were tested on human Caco-2 epithelial monolayers. Toxicity was measured by MTT and lactate dehydrogenase release assays, and real-time cell microelectronic sensing. Barrier integrity was monitored by permeability and resistance measurements, and immunostaining for junctional proteins ZO-1 and claudin-4.

Results: Treatment with sucrose esters at 300 μg/ml concentrations for 1h resulted in 50% toxicity, while the reference molecules were toxic at doses above 10000 μg/ml. Untreated monolayers formed a good barrier as reflected by transepithelial resistance (722±80 Ωxcm^2) and positive immunostaining for claudin-4 and ZO-1. Sucrose esters in non-toxic concentrations significantly reduced resistance and increased the permeability of fluorescein, P-glycoprotein substrates and hydrophilic drugs in Caco-2 cells. Using microelectronic sensing we could demonstrate that the permeability enhancing effect of sucrose esters was quick and reversible, and Cremophors at high doses induced delayed cell death.

Conclusion: Our results indicate the possible use of sucrose esters as absorption enhancers in oral formulations.

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P-G12:

ORIGINAL ESOPHAGEAL SURGICAL PROCEDURES

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Introduction: The poor results of conventional treatment of esophageal disruptions demand multimodality assessment of this major emergency.

Objectives: The value of the new esophageal surgical techniques in some unusual esophageal injuries and pathological conditions is evaluated in this study.

Patients & methods: Among the 564 esophageal interventions performed by one of the authors, in a right-sided spontaneous esophageal rupture, 2 days after left pneumonectomy, to avoid contamination of the pneumonectomised left thoracic cavity, transhiatal primary repair with drainage and gastrostomy was performed. In a 15 cm long, 9-day intrathoracic esophageal tear, decortication, suture and Urschel-type esophageal diversion was used. In a 6 week-old empyema due to iatrogenic esophageal injury, Urschel- Ergin type esophageal exclusion with tube thoracostomy was useful. In a 68 year-old patient with lower- posterior mediastinal tumor and associated complicated hiatal hernia, to reduce the magnitude of intervention, transhiatal simultaneous tumor resection and hiatal hernia repair was decided.

Results: All operations proved to be useful, but one patient was lost 2 weeks later due to a „kissing" duodenal ulcer.

Conclusion: In some special instances, when conventional esophageal surgical approaches or methods are contraindicated or has a high risk, the new alternative procedures may considered the best option.

P-G13:

FUNCTIONAL CHARACTERIZATION OF HUMAN OESOPHAGEAL EPITHELIAL CELLS

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Introduction: Oesophageal epithelial cells (OEC) are exposed to gastric and/or bile acids during gastroesophageal reflux disease. Therefore, acid-extruding mechanisms through ion transporters may play an important role in the protection against acid injury.

Objectives: The aim of this study was to characterize the ion transport processes of the human CRL-4027 OEC which were derived from a region of non-dysplastic metaplasia of Barrett's esophagus.

Materials & methods: OEC were grown to confluent monolayers and loaded with the fluorescent dye, BCECF-AM, to monitor changes in intracellular pH (pH_i).

Results: The resting pH_i of CRL-4027 was 7.32 ± 0.031 (n=4). Removal of Na^+ from the standard HEPES solution caused a reversible intracellular acidosis; in addition the recovery from acidosis after an NH_4Cl pulse was completely abolished in Na^+-free solution. These pHi changes confirm the presence of functionally active Na^+/H^+ exchangers (NHE) in OEC. The administration of HCO_3^-/CO_2 rapidly and greatly decreased pHi which was followed by a pH_i recovery. Notably, the recovery from acidosis after an NH_4Cl pulse was significantly faster in the presence of HCO_3 indicating the presence of a functionally active Na^+/ HCO_3^- cotransporter (NBC). Removal of Cl^- from a HCO_3^-/CO_2^- containing solution caused alkalisation in OEC.

Conclusion: Our results indicate two sodium dependent acid-extruding mechanisms (NHE and NBC) and a Cl^- dependent HCO_3^- secretory mechanism in OEC. Both mechanisms could play important roles in the oesophageal defence against acid injury.

This work was supported by the Hungarian Scientific Research Fund, the Hungarian Academy of Sciences and the National Development Agency (TÁMOP).

I1:

PEPTIDE THERAPY IN SEPSIS AND INFLAMMATION: A NOVEL STRATEGY TO SUPPRESS INFLAMMATION.

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Each peptide should have specific structure determined by its amino acid sequence which may react with its antisense peptide. To generate candidates of complementary peptide (C-pep) reactive to a target amino acid sequence based upon the sense-antisense amino acid relationship. We invented an evolutionary computer program that generates C-pep sequences that have a potential to interact with a target peptide. We generated C-peps targeted to C5a anaphylatoxin which is considered to be an effective target for treatment of hyperinflammation since C5a stimulates generation of TNFa and other inflammatory cytokines. Antibodies to C5a was effective in treating experimental primate models of sepsis indicating that C5a inhibitors should be useful for treatment of sepsis.

Amino acids 37 to 53 of C5a (RAARISLGPRCIKAFTE) is an antisense peptide to the antisense homology boxes of the C5a receptor. C-pep to this region was termed PepA (ASGAPAPGPAGPLRPMF), and acetylated PepA (AcPepA) was more effective in animal experiments. AcPepA was effective in Cynomolgus monkeys intravenously infused with a lethal dose of l LPS (4 mg/kg) destined to die. The monkeys were rescued by intravenous administration of 2 mg/kg/h of AcPepA. The excellent therapeutic effect of AcPepA is likely to be due to restriction of high mobility group box 1 (HMGB1) surge induced by the effect of C5a on C5L2, which is the second C5a receptor, since the released HMGB1 has the capacity to stimulate TLR4 as an endogeneous ligand resulting in further activation of inflammatory cells to release inflammatory cytokines forming positive feedback circuit of inflammation.

I2:

GUT MICROBIOTA AND IMMUNE DEVELOPMENT AND FUNCTION

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A trillion bacteria, assembled in the gastrointestinal system, constitute an important organ with important functions. The gut microbiota plays an essential role in the development and function of the immune system; maturation of gut-associated lymphatic tissue, secretion of IgA and the production of antimicrobial peptides. Another important microbiota function is to ferment indigestible food substances and release simple sugars, absorbable nutrients, and short-chain fatty acids (SCFAs), important nutrients for the microbiota but also for the eukaryotic body cells. Especially the SCFAs are essential for important trophic and developmental functions, especially on the intestinal mucosa. It is increasingly obvious that, despite that the majority of the microbiota-constituting microbes yet remains to be identified, reductions in number of and diversity of these important bacteria, almost always present in Westerners, and referred to as dysbiosis, is accompanied by a number of serious pathological conditions; often obesity and metabolic disturbances in the body, sometimes serious metabolic diseases such as diabetes but also malignant diseases. Recent studies show greater than expected differences in microflora between Westerners and those living a rural life in Africa and consuming a diet rich in polysaccharides the African microbiota being significantly richer in Gram-positive microorganisms. It is suggested that reduction in Gram-positives, and relative increase in Gram-negatives are associated with

high intake of saturated fats, deleterious to the Gram-positives, and insufficient intake of minerals especially magnesium, essential for reproduction and function of the Gram-positives. The obvious consequence is increased production of endotoxin, leaky gut, endotoxemia, metabolic disturbances and chronic diseases. Attempts to permanently correct dysbiosis without greater changes in lifestyle has this far failed.

I3:

THE ROLE OF THE ENDOCANNABINOIDS IN THE REGULATION OF INFLAMMATION

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The emerging endocannabinoid system (ECS; comprising the endogenous lipid mediators endocannabinoids, produced in virtually all tissues; their metabotropic and ionotropic cannabinoid receptors; biosynthetic pathways and metabolizing enzymes) has been implicated in multiple regulatory functions of the human body. Recent studies have intriguingly suggested the existence of a functional ECS in the skin, the largest organ of the integumentary system. Indeed, the ECS has been implicated in various cutaneos biological processes (e.g. proliferation, growth, differentiation, apoptosis). Of further importance, similar to other organs (e.g. airways, gastrointestinal tract) the ECS appears to be fundamentally involved in skin inflammation as well. Indeed, both classical endocannabinoids (anandamide, 2-arachidonoylglycerol) and "indirect" endocannabinoids (palmitoylethanolamide) were shown to suppress various forms of dermatitis induced in mice. Likewise, genetic ablation of the major endocannabinoid degrading enzyme fatty acid amid hydrolase, which intervention resulted in the elevation of the cutaneous concentration of ananamide, also attenuated skin inflammation. Intriguingly, we have recently shown that locally produced endocannabinoids "tonically" limit excessive activation and maturation of human cutaneous mast cells, key players of skin inflammation. These findings collectively suggest that the cutaneous endocannabinoid "tone" constitutively control the proper and well-balanced processes of immune competence and/or tolerance. Therefore, targeted manipulation of activities/levels of elements of the ECS offers a novel, promising strategy for the future management of human skin inflammation (and most probably other, closely related dermatological conditions).

I4:

SEX STEROIDS AND RECEPTOR ANTAGONISTS FOR IMPROVING IMMUNE FUNCTIONS AND DECREASING MORTALITY FROM SEPSIS FOLLOWING TRAUMA-HEMORRHAGE

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Introduction: The preponderance of trauma victims are young males; their mortality rate after trauma is not only higher compared to females but they are also more susceptible to subsequent sepsis. However, the mechanism responsible for the lower mortality/sepsis in females remains unclear.

Objectives: To determine if: 1) gender dimorphism in immune response exists following trauma-hemorrhage; 2) if administration of sex steroids/receptor antagonists after trauma improves immune functions and decreases mortality from subsequent sepsis.

Material & methods: A mouse model of tissue trauma and major blood loss (60% blood loss; mean blood pressure ~35 mmHg for 90 min) followed by fluid resuscitation was used; studies were conducted at various intervals after resuscitation. Therapeutic agents, when used, were administered intravenously during resuscitation.

Results: Proestrus females demonstrate maintained immunological functions as opposed to markedly altered responses in males after trauma. The increased synthesis and decreased catabolism of 5α-dihydrotestosterone is responsible for the loss of T cell functions in males while increased 17β-estradiol (E2) synthesis in T cells of proestrus females after trauma prevents immunosuppression. Treatment of animals after trauma with E2, flutamide, prolactin, metoclopramide, or DHEA restores immune cell functions following trauma in males and ovariectomized females and also decreases mortality rates from subsequent sepsis.

Conclusions: Thus, E2, flutamide, DHEA, prolactin or metoclopramide, which are readily available clinically, appear to be safe and novel immunomodulating agents for the treatment of immune depression following severe blood loss and for decreasing mortality from subsequent sepsis in males and females, respectively (USPHS grant RO1 GM37127).

I5:

INDIVIDUALIZED GOAL-DIRECTED THERAPY FOR TRAUMA-INDUCED COAGULOPATHY

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Uncontrolled blood loss together with major brain injury remain the primary causes of early trauma-related mortality. Up to one third of all trauma patients are coagulopathic at admission to the emergency room (ER). Thus, the early diagnosis of the underlying coagulation disorder is highly important. Standard coagulation tests, such as the prothrombin time (PT), the international normalized ratio (INR), and the activated partial thromboplastin time (aPTT), are widely used. Recent data suggest that whole-blood viscoelastic tests, such as thromboelastometry (ROTEM®) or thrombelastography (TEG®) portray trauma induced coagulopathy (TIC) more accurate and substantially faster than standard coagulation tests.

The "theragnosticconcept" of an individualized, early and goal-directed coagulation therapy is based on a combination of viscoelastic tests and targeted coagulation therapy. There is increasing evidence that low maximum amplitude (=low clot strength) of the clot is predictive of blood product transfusion requirements, amplitude is also directly influenced by plasma expanders (shown (pre) and clinically). Based on these findings, goal-directed coagulation therapy aims primarily on the maintenance or restoration of clot strength by early transfusion of fibrinogen containing fluids, such as fibrinogen concentrate, cryoprecipitate or fresh frozen plasma. Thrombin generation is not substantially affected in the very early stages of TIC. The CT in ROTEM or the r-time in TEG serves as surrogate parameter for impaired thrombin generation. Profibrinolytic pathways are activated in severe shock and tissue trauma. Thus, tranexamic acid should be considered for the treatment of trauma induced hyperfibrinolysis. This concept potentially reduces the blood product requirement and improves outcomes.

I6:

HEPATIC ISCHEMIA-REPERFUSION INJURY: PATHOMECHANISMS AND THERAPEUTIC STRATEGIES

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The complex functions of the liver in biosynthesis, metabolism, clearance and host defense are tightly dependent on an adequate microcirculation. To guarantee hepatic homeostasis, this requires not only a sufficient nutritive perfusion and oxygen supply, but also a balanced vasomotor control and an appropriate cell-cell communication. Deteriorations of the hepatic homeostasis, as observed upon warm or cold ischemia and reperfusion (I/R), are associated with a high morbidity and mortality. During the last three decades, experimental studies have demonstrated that hepatic microcirculatory disorders are determinants for postischemic organ dysfunction and failure. Disorders include (i) a dysregulation of the vasomotor control with deterioration of the endothelin-nitric oxide balance, sinusoidal constriction and shutdown of the microcirculation, as well as (ii) an overwhelming inflammatory response with microvascular leukocyte accumulation, platelet adherence and Kupffer cell activation. Within the sequelae of events, pro-inflammatory mediators, such as reactive oxygen species and TNF-alpha, are the key players, causing the microvascular dysfunction and perfusion failure. Tissue injury is characterized by different modes of cell death, including apoptosis, necrosis and aponecrosis. The presentation will cover the microvascular, cellular and humoral pathomechanisms underlying I/R-induced liver injury and the therapeutic targets to attenuate tissue injury and organ dysfunction. It will also indicate future directions to translate the knowledge achieved from experimental studies into clinical practice.

I7:

VASCULARIZATION IN TISSUE ENGINEERING: ANGIOGENESIS VERSUS INOSCULATION

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The key challenge in tissue engineering is the establishment of efficient vascularization strategies for tissue constructs, which guarantee their long-term survival and function after implantation. Several strategies have been proposed to stimulate the ingrowth of new blood vessels into tissue constructs. These include the modification of the chemical composition and architecture of scaffolds, their bioactivation by incorporation of growth factor delivery systems or by cell seeding as well as the stimulation of stem cell recruitment. However, because angiogenesis is a time-consuming process, all of these approaches cannot prevent ischemic cell death within larger three-dimensional tissue constructs during the initial phase after implantation. To overcome this problem, in vitro or in situ prevascularization has emerged as a novel concept in tissue engineering. This bears the advantage that preformed microvascular networks within tissue constructs simply have to inosculate with the host microvasculature at the implantation site to get completely blood-perfused within a short period of time. Thus, during the last years considerable progress has been made in the establishment of promising vascularization strategies in tissue engineering. Particularly the inosculation of preformed microvascular networks has the great potential to markedly improve the survival of tissue constructs after implantation. The optimization of this vascularization strategy may pave the way for a broad clinical use of tissue engineering applications in the future.

I8:

HYPOXIA-INDUCED NON-MICROBIAL METHANE GENERATION: MECHANISM AND SIGNIFICANCE

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Introduction: Gastrointestinal methanogenesis was earlier considered to be an exclusive indicator of carbohydrate fermentation by the anaerobic flora.

Objectives: We have suggested that the methyl groups of the ubiquitous phosphatidylcholine molecule are potential electron acceptors during temporary oxygen deprivation, and in consequence of this, methane may be liberated (FASEB J 2003). We have demonstrated the possibility of non-microbial methane formation (1) in model chemical reactions with choline and its metabolites in the presence of hypoxia and oxygen radical generation, (2) in rat liver mitochondria and mitochondrial subfractions under hypoxic conditions, (3) in endothelial cell cultures with or without oxygen, and (4) in experimental animal models of vascular occlusion and reperfusion. In other studies significant methane generation was demonstrated in plants also, after abiotic stress. In line with this, it was not known whether non-asphyxiating amounts of methane have any impact on the mammalian physiology or pathophysiology.

Materials & methods: We set out to characterize the effects of methane-oxygen respirations on the process of in vivo inflammatory events arising after intestinal ischemia and reoxygenation. Assay systems were used to test the in vitro bioactivity potential of different gas concentrations.

Results: Methane administration reduced the magnitude of the tissue damage, decreased the superoxide and nitrotyrosine levels after reperfusion, and specifically inhibited leukocyte activation in vitro.

Conclusion: Methane generation is a consequence of transient oxygen deprivation in aerobic cells. Methane modulates leukocyte activation and affects key events of ischemia-reperfusion-induced oxidative and nitrosative stress, and is therefore of potential therapeutic interest in inflammatory pathologies.

I9:

ANTIBODIES AND ANTIBODY RECEPTORS, STRUCTURES AND APPLICATION FOR THERAPY

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The immune response depends on the binding of opsonized antigens to cellular Fc receptors and the following initiation of cellular effector functions of the immune system. The crystal structures of a soluble Fc gamma receptor, an Fc fragment, and their complex explain a wealth of functional data of the system. They also provide a rationale for the modulation of effector activities by changes of the domain arrangement of the Fc fragment caused by altered glycosylation.

Myelin oligodendrocyte glycoprotein (MOG) is a major autoantigen in multiple sclerosis. The crystal structures of MOG and its complex with a specific autoantibody provide a basis for new diagnostic and therapeutic strategies against the pathogenic autoantibody response to MOG. These molecular structures offer multiple ways to interfere with the cellular immune response in autoimmune diseases:

a) by small molecules disrupting the Fc receptor-Fc contact;

b) by antibodies directed against the contact area;

c) by soluble Fc receptors as antagonists;

d) by mimetics of the MOG epitop.

Animal experiments following strategies b) and c) have successfully completed for three model autoimmune diseases, SLE, arthritis, and EAE.

A company SUPPREMOL has been founded to pursue these strategies on a commercial basis

I10:

COMPLEMENT - BRIDGING INNATE AND ADAPTIVE IMMUNITY

Anna Erdei

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Cells and molecules of the innate immune system which encounter pathogens first, play instructive role in subsequent events of immune responses. Among the humoral factors, the alternative and lectin-dependent pathways of complement activation play an important role not only by lysing several microbes, but via generating activation fragments which interact with cells involved in initiating the adaptive response. We show that the activation fragment of the most abundant complement protein C3, via its covalent interaction with antigen presenting cells, significantly enhances the proliferation of antigen-specific T lymphocytes, which express complement receptors upon triggering. CR1 and CR2 expressed by B lymphocytes are known for long to be involved in antibody responses. Our results show that in contrast to mice - where these receptors are the product of the same gene and both are involved in the enhancement of the antibody response -, on human B cells ligand binding to CR1 - in contrast to CR2 - *inhibits* B cell proliferation induced by BCR cross-linking. This finding points to significant differences between immune reactions of mice and men, where complement is involved. The importance of C3 in shaping the adaptive response is further supported by the very recently described role of T-cell derived C3-fragments.

I11:

THE INTERPLAY OF SIGNALLING CASCADES INITIATED BY MEMBRANE AND CYTOSOLIC PATTERN RECOGNITION RECEPTORS IN HUMAN DENDRITIC CELLS

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Introduction: Dendritic cells (DC) derive from hematopoietic precursors. As coordinators of innate and adaptive immunity they display characteristic sets of pattern recognition receptors (PRR) and depending on specific signals, they exhibit multiple functional activities.
Objectives: Compartmentalization and expression of PRR, combination of their ligands, and the activated signalling cascades result in different outcomes.
Materials & methods: Monocytes generated from PBMC of healthy blood donors were subjected to FACS, QPCR, immunoblot and ELISA analysis.
Results: Activation of plasmacytoid DC (pDC) by TLR7 or TLR9 ligands induces the expression of interferon genes but in combination it results in decreased response. These stimuli also upregulate cytosolic RIG-like receptor (RLR) expression but the type I interferon response is concomitantly inhibited. Conventional DC do not respond to MDP, but IFNg sensitizes the cells for IL-1b secretion. NOD2-RIP2-IKKg/NEMO complex together with the activation of stress kinases result in NF-kB and AP-1 activation, whereas caspase-1 activity is induced by IFNg. The cytokine-triggered differentiation of human monocytes to DC gives rise to subsets that differ in TLR and RLR expression, cytokine secretion and T-cell polarization depending on the delicate balance of the NF-kB- and IRF3-mediated signalling cascades. The two-component adjuvant IC31® targets the vesicular compartments of moDC and induces type I interferon responses without activating NFkB. In the presence of IC31® differentiation and cytokine secretion of CD1a+ DC was inhibited, whereas the IFNb response was increased.
Conclusion: Cross-talk of PRR-induced recognition, signaling and effectors within and among DC offers a unique platform for fine tuning innate and adaptive immune responses.

I12:

PROINFLAMMATORY ALARMINS PROMOTE HOST DEFENSE

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Recent studies have identified a group of structurally diverse multifunctional host proteins that are rapidly released following pathogen challenge or cell injury and, most importantly, are able to both chemotactically recruit and activate dendritic antigen-presenting cells. These potent immunostimulants, including defensins, cathelicidin (LL37), eosinophil-derived neurotoxin (EDN), lactoferrin (LF), granulysin, high-mobility group box protein 1 (HMGB1) and HMGN1 serve as early warning signals to activate innate and adaptive immune systems. They interact with chemokine-like receptors and activating receptors on host cells. For example, some beta defensins, LL37, HMGB1 and EDN mimic chemokine and cytokine activities by interacting with CCR6, FPRL-1, RAGE and Toll-like receptors (TLR2) respectively. Defensins (HBD2 and HBD3), like cathelicidin (LL3T), by binding to DNA also promote DNA uptake and activation of TLR9 in plasmacytoid dendritic cells, resulting in markedly augmented production of IFN alpha. All these proteins also are antimicrobial peptides (AMP's) and are constitutively produced and released by leukocytes, but can also be induced by injurious stimulants and cytokines. In addition they are produced by keratinocytes and epithelial cells. These AMP's all induce the maturation of myeloid dendritic cells and have in vivo immunoadjuvant effects. We have highlighted the unique activities of these proteins by classifying them as "alarmins", in recognition of their role in rapidly mobilizing the immune system in response to infections and injurious danger signals.

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I13:

THE ROLE OF IMMUNOLOGICAL FACTORS IN THE PATHOGENESIS OF ACNE

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Acne is the most common inflammatory skin disease. Genetic factors, abnormal follicular differentiation, increased cornification, enhanced sebaceous gland activity hyperseborrhea and bacterial hypercolonization, as well as inflammation and immunological host reactions are the main pathogenetic factors in the development of the disease. Hyperproliferation of the follicular epithelium leads to formation of microcomedones, which are the initial acne lesions and can be found in normal-looking skin. Inflammation in acne is usually associated with abnormal ductal colonization of *P.acnes*. Recent studies have shown that *P.acnes* triggers antimicrobial peptide and cytokine secretion of keratinocytes and sebocytes *in vitro*. *P.acnes* genotyping revealed that different strains of *P.acnes* exist, such as typeIA, typeIB, typeII and typeIII and the profile of *P.acnes* induced proinflammatory cytokines/chemokines and antimicrobial peptides in human keratinocytes are in correlation with the genotypes. Recent data suggest that *P.acnes* might also be involved in the ductal hyperproliferation of keratinocytes, as the bacteria influences the proliferation and differentiation of the cells. In addition to differences in the *P. acne* strains, genetic polymorphisms of genes altering innate immune responses and the regulation of inflammatory processes might also be involved in the pathogenesis of the diseases.

I14:

RELEVANCE OF DEFENSINS IN MULTIFACTORIAL DISEASES

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Introduction: Defensins-antimicrobial and immunomodulatory peptides- can also be regarded as alarmins. The genetic polymorphisms of human defensin - alpha, and human defensin- beta may be responsible for individual differences in expression and level of these mediators.

Objectives: The relevance of single nucleotide polymorphisms (SNPs) and the copy number variation (CNV) in human beta defensin genes in multifactorial diseases such as gastrtis, Crohn's disease, pancreatitis, diabetes and stroke were investigated, as both inflammation and infectious agents play important role in the pathogenesis of these diseases. Additionally, the genetic characteristics of alpha defensin was analysed in diabetic patients.

Patients & methods: Three SNPs of DEFB1 (encoding human beta-1 defensin); G-20A, C-44G, G-52A were genotyped by Custom Taq Man genotyping assays. Gene copy numbers of the inducible human beta-2 defensin gene (DEFB4) and the alpha defensin genes (DEFA1/3) were determined by means of quantitative real time PCR assays

Results: SNPs in DEFB1 gene may be one of the factors that contribute in individual differences for the susceptibility to the colonic form of Crohn's disease, or *H. pylori* induced gastritis and pancreatitis. A higher frequency of a lower (<4) copy number of DEFB4 gene was observed in patients with severe acute pancreatitis and also in patients with ischaemic stroke. The high alpha defensin levels however in diabetic patients did not correlate with the DEFA1/3 copy numbers.

Conclusion: The variations in the levels of human alpha and beta defensins may influence the host immune response and might be therefore risk factors in multifactorial diseases.

I15:

TUMOR RESISTANCE .

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This work departs from the notion that humans are a relatively low cancer species (2 of 3 people never get cancer) because we are protected by a multigene system against tumor development, fixed by evolution. Part of the protection against incipient tumor development and against the metastatic growth of disseminated tumor cells is acting directly at the tissue level. Our choice for the experimental study of one potentially relevant system fell on "neighbour suppression", the inhibitory effect of normal fibroblasts on established tumor cell lines, studied in a high throughput microwell system in vitro. We have found that normal skin fibroblasts are highly inhibitory for prostatic carcinoma cells, hernia fibroblasts are not, or much less inhibitory, and fibroblasts obtained from cancerous prostates are non inhibitory. Inhibition is contact dependent and leads to a reduction in tumor cell multiplication and migration. We have shown the importance of fibroblast monolayer architecture for the inhibitory effect. To study clinical relevance, a coded blind study is being performed with skin fibroblasts of prostatic carcinoma patients whose tumors have progressed, or remained quiescent.

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I16:

LANGERHANS CELLS: NEW FACES – NEW FUNCTIONS

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Langerhans cells form a network of dendritic leukocytes within the mammalian epidermis. They have long been considered to be the major sensitizing cells in the skin by initiating productive immunity in naïve resting T cells.

While this may well be the case, new experimental data have challenged this concept. These include the description and functional characterization of immunostimulatory dendritic cells in the dermis which, after epicutaneous application of contact allergens, arrive in the regional lymph nodes earlier than Langerhans cells do. Under certain experimental conditions, the genetically driven removal of the murine Langerhans cell population may result in increased T cell immunity against haptens and infectious agents.

These findings are complemented by the observation that glucocorticosteroid-treated, i.e. immature, human Langerhans cells preferentially activate regulatory T cells. New experimental approaches are needed to fully elucidate the biological role of this particular member of the dendritic cell family.

I17:

CHEMOKINES – FROM INFLAMMATION TO METASTASIS

Bernhard Homey

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Chemokines represent a family of small cytokine-like proteins (n=45 in the human) that bind to 19 different seven transmembrane spanning G protein-coupled receptors. Next to their critical role in leukocyte trafficking and inflammation, chemokines take on complex functions in the tumor microenvironment. Increasing evidence supports the concept that chemokines not only provide signals mediating the host anti-tumor response but may potentially act as facilitators of tumor progression and metastasis by modulating proliferation, survival, migration, invasion and angiogenesis. Here, the role of chemokines in organ-specific leukocyte trafficking during inflammation as well as their role in tumor progression and metastasis will be discussed.

I18:

ROLE OF TISSUES IN IMMUNOREGULATION AND IMMUNE TOLERANCE TO ALLERGENS

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In chronic allergic inflammation, dermis in the atopic dermatitis skin and submucosa in the asthmatic lung act like a peripheral lymphatic organ, where dendritic cells, T cells and B cells contact each other. This is followed by a second step of antigen-presentation and activation in the inflamed tissue. Immune system cells and their cytokines interact with resident tissue cells, which leads to a series of tissue events leading to proinflammatory cytokine and chemokine release from both sides. Some of these events seem to be part of the immune pathology, such as basement membrane thickening, epithelial death, desquamation and spongiosis, however, they also act as mechanisms that control the severity of tissue inflammation. These can be listed as: keep away effects; wash away effects and immune suppression. "Keep away" effects play a role in allergen ignorance by decreasing the allergen burden. They are lamina reticularis thickening and allergen-specific secretory IgA, in addition to cough and mucociliary activity. "Wash away" effects that decrease the intensity of inflammation are epithelial apoptosis, spongiosis, leakage and wash of proinflammatory cytokines and inflammatory cells away from the tissues towards the lumen. Direct "suppression" is taking place by allergen-specific B and T regulatory cells as well as "regulation" of B cells in the direction of non-inflammatory antibody isotypes (more IgG4 and IgA, less complement activating antibodies and IgE).

I19:

T AND B REGULATORY CELLS

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Introduction: The pivotal role of Treg cells in inducing and maintaining immune tolerance has been demonstrated during the last 15 years, where their adoptive transfer was shown to prevent or cure several T-cell mediated disease models, including asthmatic lung inflammation, autoimmune diseases and allograft rejection.

Objectives: Regulatory B cells expressing IL-10 suppress immune responses and the lack or loss of regulatory Bcells leads to exacerbated symptoms in experimental autoimmune encephalitis and other autoimmune diseases.

Patients & methods: Another B cell-related immune regulatory response restricted to humans is induction of non-inflammatory IgG4 antibodies, which is characteristic for high dose antigentolerance models. Several molecules including CD25 and PD-L1 were upregulatedin IL-10producing B cells.

Results: Br1 cells potently suppressed antigen-specific CD4+T cell proliferation whereas other B cells did not. Furthermore we demonstratethat human Br1 cells show selectively increased production of IgG4. B cellsspecific for the major bee venom allergen phospholipase A2 that were isolatedfrom beekeepers had increased expression of IL-10 and IgG4.

Conclusion: HumanBr1 cells may regulate humoral and cellular immunological tolerance throughsuppression of T cells responses and production of anti-inflammatory IgG4antibodies.

I20:

THE ROLE OF HISTAMINE IN THE REGULATION OF ANTI-TUMOR IMMUNITY

András Falus

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Introduction: The availability of molecular databases, high-throughput molecular technologies and robust bioinformatic/bibliomic and pathway networking methodologies provided a novel input in histamine research, as well.

Objectives: The lecture attempts to summarize transcriptomics and proteomics of enzymes involved in histamine metabolism in tumor research.

Materials & methods: Transgenicmice with targeted histidine decarboxylase and in vitro transfection technologies were combined with other genomic methods.

Results: 1. The role of histamine is demonstrated in tumor (melanoma) growth using mouse melanoma cells manipulated via stable transfection with sense mouse HDC mRNA, a mock control and an antisense HDC RNA segment, respectively. Gene expression profiles and in silico pathway analysis of transgenic mouse melanomas, secreting different amounts of histamine show a histamine H1 receptor dependent inhibition of the tumor suppressor insulin-like growth factor II receptor and the antiangiogenic matrix protein fibulin-5.

2. HDC-knockout mice show a high rate of colon and skin carcinogenesis. HDC is expressed primarily in immature myeloid cells (IMCs) that are recruited early on in chemical carcinogenesis. In addition, mouse CT26 colon cancer cells directly downregulate HDC expression at epigenetic manner through promoter hypermethylation of HDC gene and inhibit myeloid cell maturation. These data indicate key roles for HDC and histamine in myeloid cell differentiation and IMCs in early cancer development.

Conclusion: Overexpression of histidine decarboxylase (HDC) results in increased melanoma formation through inhibition of an antiangiogenetic factor. Cancer cells modify epigenetic elements, such as hypermethylation of HDC gene.

I21:

LINKING INNATE AND ADAPTIVE IMMUNITY IN PSORIASIS

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Psoriasis is the end result of a dysregulated immune response, whereby an increased number of activated T cells, dendritic cells and an increased production of cytokines induce abnormal keratinocyte differentiation and proliferation and clinically visible skin lesions. Arguments in favour of innate involvement are involvement of the IL-1 system, highly upregulated epidermal antimicrobial peptides, the involvement of toll-like receptors (TLR), type I IFN-related transcription factors, (NK) T and gammadelta T cells in the pathogenesis of psoriasis. The pro-inflammatory response of keratinocytes together with dermal IFN-α induce myeloid DC to produce large quantities of TNF-α, IL-12 and IL-23. IL-12 stimulates Th1 cells whereas IL-23 stimulate Th17 cells and activates and maintains the production of IL-17A, IL-17F, IL-20, IL-21 and IL-22. Experiments in interleukin-17, IL-20, IL-21, IL-22 and IL-23 in knock-out mice has shown that especially IL-20, IL-21 and IL-22 cause the epidermal profileration and acanthosis. This spectrum of cellular and soluble players indicate that in psoriasis pathophysiology innate as well as adaptive are activated. Whether innate or adaptive immune mechanisms are primary in this disease is a matter of debate. The occurrence of clonal T cell populations in chronic stages of psoriasis could be the result of priming by lesional skin-derived DC's, loaded with self proteins released from skin damage induced by innate inflammation. We argue that innate immune mechanisms are important in initial phases of psoriasis, whereas adaptive autoimmune mechanisms contribute to its chronicity and to disease maintenance.

I22:

MICRORNAS: NOVEL REGULATORS IN PSORIASIS

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MicroRNAs are a growing class of small, endogenous non-coding RNAs, which suppress the expression of their target genes through sequence-specific interactions. MicroRNAs are thought to regulate the majority of human genes and they are involved in most biological processes. The importance and varied functions of microRNAs are illustrated by the diverse phenotypes, including disease, that arise when microRNAs are mutated or improperly expressed.

Recently, we showed the deregulation of miRNAs in inflammatory diseases, exemplified by psoriasis and atopic eczema. Psoriasis lesions show a characteristic miRNA expression profile distinct from that of normal skin and atopic eczema. The keratinocyte-specific miRNA miR-203 is specifically up-regulated in psoriasis lesions and it is critically involved in keratinocyte differentiation as well as inflammatory pathways. Another miRNA, miR-125b, is down-regulated in psoriasis lesions and regulates keratinocyte proliferation and differentiation. In addition, we have shown that miR-21 suppresses apoptosis in activated T cells and thus its overexpression may contribute to psoriatic skin inflammation. Recently, we found that miR-146a, a negative regulator of the NF-κB pathway, is suppressed in psoriasis keratinocytes, suggesting that its loss may contribute to the elongation/maintenance of inflammation.

Our results suggest that miRNA deregulation contributes to the dysfunction of both resident skin cells and infiltrating immune cells in psoriasis. MicroRNAs are likely to be involved in the regulation of all steps of psoriasis pathogenesis and further investigation is needed to better understand their specific roles. Since microRNAs regulate gene networks rather than single genes, drugs targeting them may be efficient in treatment of psoriasis.

I23:

NEW ASPECTS OF THE SKIN NERVOUS SYSTEM

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Receptor end organs and free nerve endings in the skin are the peripheral sentinels of the sensorial nervous system encoding for touch, temperature and pain. Using a novel approach to analyse the outermost nerves of the skin we visualized for the first time the distinct micro-anatomical structure of the superficial skin nervous system. We could demonstrate that terminal, non-myelinating Schwann cells are present in high numbers in direct vicinity to the dermo-epidermal junction. In this respect we have developed a robust method to isolate large quantities of Schwann cells from split thick skin and to propagate and expand them in vitro. Furthermore we could analyse for the first time the architecture of the touch dome within human hairy skin in detail and demonstrate that it represents a distinct anatomical unit. Concerning the nerves supplying the touch domes we found, unexpectedly, that besides Aß-fibers also Aδ- and C-fibers were regularly present suggesting that the receptive qualities of human touch domes exceed mechanosensation and that they may serve as multifunctional nerve end organs in human skin. We suggest that the skin is an excellent source for cell

I24:

WHY SOME GET PSORIASIS WHILE OTHERS DON'T - UNDERSTANDING THE KOEBNER PHENOMENON

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Psoriasis is a common complex genetic disease characterized by hyperplasia and inflammation in the skin; however, the contributions of epidermal cells and the immune system to disease susceptibility and manifestation remain unclear. Linkage studies have defined a psoriasis susceptibility locus (PSORS4) on 1q21, the epidermal differentiation complex, which includes genes for small S100 calcium binding proteins. These proteins are involved in extra- and intracellular signaling during epithelial host defense, linking innate and adaptive immunity. Inflammation-prone psoriatic skin constitutively expresses elevated concentrations of S100A7 (psoriasin)/S100A15 (koebnerisin) in the epidermis. Both human S100 proteins share a common mouse homolog, named mS100a7a15. Genetically modified mice expressing elevated amounts of doxycycline-regulated mS100a7a15 in skin keratinocytes demonstrated an exaggerated inflammatory response when challenged by exogenous stimuli (Koebner phenomenon). This immune response is characterized by immune cell infiltration and elevated concentrations of Th1 and Th17 proinflammatory cytokines, which have been linked to the pathogenesis of psoriasis and were further amplified upon challenge. Both inflammation priming and amplification required mS100a7a15 binding to the receptor of advanced glycated end products (RAGE). mS100a7a15 potentiated inflammation by acting directly as a chemoattractant for leukocytes, further increasing the number of inflammatory cells infiltrating the skin. Taken together, this study provides a pathogenetic psoriasis model using a psoriasis candidate gene to link the epidermis and innate immune system in inflammation priming, highlighting the S100A7A15-RAGE axis as a potential therapeutic target.

I25:

TRANSGLUTAMINASES IN SKIN PATHOLOGY

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Introduction: Patients with pathological, reduced or missing protein functions present themself with disease phenotypes indicating us the physiological role of these proteins.

Objectives: This overview of three transglutaminases (TG), the keratinocyte TG (TG1), tissue TG (TG2) and epidermal TG (TG3) will cover inherited and autoimmune skin diseases related to cutaneous TG pathologies.

Patients & methods: Clinical, immunological and genetic data of our patients, and skin barrier studies in experimental animal models will be included in the overview

Results: Lamellar ichthyosis and congenital ichthyosiform erythroderma both caused by recessive TG1 mutations in the TGM1 gene will be demonstrated in patients with enhanced transepidermal water loss and consecutive cutaneous inflammation as two different barrier defect phenotypes. Epigenetic factors further modifying these clinical pictures will be also presented in patients with bathing suit ichthyosis and in a particular collodium baby phenotype. In the talk TG3 will be introduced as a new skin barrier molecule, proven by our data obtained in TG3 knockout mice. We will also discuss how IgA type TG2 autoimmunity

is leading by epitope spreading to the induction IgA type TG3 autoantibodies in patients with mild celiac disease and to the development of an autoimmune blistering skin disease called dermatitis herpetiformis. The loss of gluten tolerance and the induction of TG2 and TG3 autoimmunity are in the background of the skin bound IgA-TG3 immuncomplexes, and in the accumulation of neutrophils in the skin symptoms of dermatitis herpetiformis patients.

Conclusion: TG 1,2 and 3 pathologies are responsible for very different cutaneous diseases, indicating their physiological role in skin barrier and integrity.

I26:

T CELLS IN ATOPIC DERMATITIS

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Atopic dermatitis (AD) is an inflammatory skin disease with a prevalence of 10-20% in children and 1-3% in adults. The pathogenesis of the disease is complex, as a combination of skin barrier dysfunction as well as distorted innate and adaptive immune responses lead to the development of the characteristic clinical picture.

Soon after Mosmann and Coffman described the division of CD4+ T cells into functional Th1 and Th2 subsets, based on their cytokine secretion profile, AD has been recognized as a Th2 disease. In the last years more subpopulations of CD4+ T cells joined the Th1/Th2 duo. The exact roles of the recently described effector T cell populations, the Th17 and Th22 cells, in AD are still controversial.

Regulatory T cells (Tregs) has also been investigated in AD, but not to the same extent as that of effector cells. Although their number is elevated, their ability to suppress T cell activation seems to be deficient in the presence of staphylococcus aureus.

Unconventional T cells are far less uncovered in AD, although in the last decade, human invariant natural killer T (iNKT) cells have entered into the focus of immunological investigations. The unique ability of iNKT cells to produce high amounts of both Th1-type (IFNγ) and Th2-type (IL-4) cytokines suggested the possibility that they may have a pathogenic role in allergic inflammation. The lecture summarizes our knowledge about the effector, regulatory and innate-like T cells in AD.

I27:

PSORIASIS SUSCEPTIBILITY FACTORS

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Psoriasis is a chronic inflammatory skin disease that besides inflammation shows characteristic changes in the epidermal and dermal compartments of the skin. Our research focuses on investigating whether immune disregulation in the disease is driven at least partially by abnormal tissue homeostasis. Our data indicate that one of the key molecules in the psoriatic altered epidermal response is the $EDA^{+}Fn$. Fibronectin increases cell cycle entry among uninvolved psoriatic skin derived keratinocytes compared to keratinocytes of healthy skin. The fibronectin receptor a5 is overexpresssed in psoriatic uninvolved keratinocytes and psoriatic keratinocytes exhibit an abnormal hyperproliferative response to T cell lymphokines, among them gIFN. Our results indicate that one important step in psoriasis pathogenesis could be the IFNg induced abnormal regulation of IL23A

in psoriatic keratinocytes. Also, we found that in *in vivo* psoriatic uninvolved skin KGF, KGFR, EDA^{+}Fn, and a5 integrin, factors closely related to cutaneous wound healing, cell proliferation and differentiation are highly expressed compared to normal skin. Comparison of the uninvolved psoriatic epidermis with the normal by differential display has lead to the identification of a non-coding RNA. This ncRNA proved to be stress inducible in various cells therefore, we called it PRINS (Psoriasis-susceptibility Related RNA Gene Induced by Stress). Surprisingly PRINS expression decreases when the uninvolved epidermis develops the lesion. Our results confirm that the psoriatic uninvolved skin has an abnormal homeostatic state which can be important in the induction and maintenance of chronic inflammation.

O-I1:

BACTERIAL LIPOPOLYSACCHARIDES DAMAGE DEFENSE MECHANISMS AT THE BLOOD-BRAIN BARRIER

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Introduction: The blood-brain barrier (BBB) is the most important defense structure protecting the nervous system from insults. Brain endothelial cells form the BBB and participate in host responses to infections. Microbial pathogens may trigger inflammatory responses in brain endothelial cells, which express Toll-like receptors, defensins and are affected by alarmins according to recent studies.

Objectives: The effects of lipopolysaccharides (LPS), cell wall components of Gram-negative bacteria and main pathogenic factors in sepsis were studied on the integrity and functions of the BBB.

Materials & methods: LPS and potential protective molecules were investigated on mouse *in vivo,* rat and human *in vitro* BBB models.

Results: LPS-treatment resulted in sickness behaviour and increased BBB permeability for fluorescein and albumin in mice. In LPS-treated cultured brain endothelial cells a dose- and time-dependent decrease in the integrity of monolayers, reduced intensity and altered pattern of immunostaining for junctional proteins ZO-1, claudin-5 and beta-catenin, inhibition of P-glycoprotein efflux pump activity, increased production of reactive oxygen species, nitric oxide, and matrix metalloproteinases were measured. It was demonstrated for the first time that unconjugated bilirubin aggravated LPS-induced damages in brain endothelial cell morphology and functions. Serum amyloid P component (SAP), a modulator of host reactions during infections alleviated the symptoms of septic shock, and reduced BBB permeability in LPS-treated mice. Polysaccharide pentosan, a heparinoid reduced the damaging effects of LPS in brain endothelial cells.

Conclusion: LPS and bilirubin are toxic and induce BBB dysfunctions. SAP and pentosan counteract the effects of LPS and can be of therapeutical significance in bacterial infections affecting the BBB.

O-I2:

THE ROLE OF INFLAMMATION FACTORS IN THE DEVELPOMENT OF RESTENOSIS AFTER CAROTID ENDARTERECTOMY

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Introduction: Early restenosis following surgical vascular vessel reconstructions is an infalmmatory process leading to myointimal hyperplasia. The role of different inlammatory factors in the development of restenosis is not clear yet.

Objectives: to clarify the different mechanisms leading to neointima hyperplasia we performed a prospective study with patients operated on carotid artery. Acute phase reactants, complement system, growths factors and gene polymophrisms were examining, correlated with ultrasound findings in the follow up period.

Patients & methods: A total of 117 consecutive patients who underwent carotidendarterectomy (CEA) were included in the study. Blood samples were collected pre-and post surgery cone year long. Ultrasound imaging were carried out 6 weeks, 6 months and 12 months after surgery. The results were correlated and statistically analised.

Results: During the follow- up highly significant ($p<0.0001$) drop occured in the serum concentration of hs-CRP and fibrinogen. Significant increase in the frequency of mannose binding lectin (MBL) variant genotypes was observed in patients not experiencing restenosis compared with the patients with restenosis ($p<0.007$). Complement C3 levels increased during follow-up and correlated with the percentage of restenosis in MBL normal genotype carriers. Significant increase in VEGF and PDGF prediicted restenosis in patients homozygous for normal MBL genotype.

Conclusion: During vascular operations due to the clamping manoevers an ischaemiareperfusion injury occures, which indicates the increase of acute phase reactants followed by elevation of growths factors resulting hyperplasia of smooth muscle cells which is the pathological manifestation of early restenosis. Based on our experiences the above mentioned process is related to an intact lectin pathway.

O-I3:

DECIPHERING THE MECHANISM OF THE COMPLEMENT LECTIN PATHWAY ACTIVATION

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Introduction: The lectin pathway of complement activation is an important component of the innate immune defense. Activation of the lectin pathway is triggered by carbohydrate arrays on the surface of micro-organisms. Multimolecular complexes consisting of pattern recognition molecules (MBL and ficolins) and zymogen serine proteases (MASP-1,-2 and -3) initiate the complement cascade. Until now the autoactivating mannose-binding lectin-associated serine protease-2 (MASP-2) has been considered as the autonomous initiator of the proteolytic cascade. The role of the much more abundant MASP-1 protease was controversial.

Objectives: Our aim was to reveal the exact role of MASP-1 and MASP-2 in the activation of the lectin pathway.

Materials & methods: Highly specific inhibitors were developed against MASP-1 and MASP-2 by means of phage display. We used naturally occurring inhibitor-scaffolds in the in vitro selection procedures.

Results: Inhibition of MASP-2, as expected, inhibited the lectin pathway in normal human serum. Surprisingly however, selective inhibition of MASP-1 also completely blocked the activation of the lectin pathway including the activation of MASP-2. Moreover, we also demonstrated that 60% of the C3-convertase complexes contain C2a generated by MASP-1.

Conclusion: Using unique, monospecific inhibitors against MASP-1 and MASP-2 we revealed a completely novel mechanism of lectin pathway activation. In normal human serum MASP-2 activation strictly depends on MASP-1. MASP-1 activates MASP-2 and moreover, inhibition of MASP-1 prevents autoactivation of MASP-2. Transient inhibition of MASP-1 may protect from the harmful consequences of ischemia-reperfusion injury which should be an attractive new therapeutic approach.

O-I4:

CKIP-1 INTERFERES WITH TNF REVERSE SIGNALING

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Introduction: Reverse signaling of TNF superfamily members (intracellular signaling elicited by interaction with receptors) seems to be a fine-tuning mechanism regulating many aspects of innate and adaptive immunity.

Objectives: We reported earlier that the TNF is serine phosphorylated in mTNF expressing cells. Interaction with its receptor was shown to trigger rapid dephosphorylation of mTNF, Ca^{2+} signaling, MAPK pathway and caspases are involved in downstream signaling events.

Materials & methods: We used the N-terminal domain of TNF as bait in a yeast two hybrid assay to find CKIP-1. This protein also interacts with c-Jun, ACP and, ATM, Akt, IFP35, Nmi and Smurf-1. Interaction of CKIP-1 and mTNF was corroborated in pull down assays, expression of CKIP-1 was significantly elevated in myeloid cells exposed to LPS. CKIP-1 activated the TNF promoter and overexpression of both c-Jun and CKIP-1 activated the TNF promoter.

Results: Infliximab (anti-TNF monoclonal) or soluble receptor-elicited reverse signaling or expression of the N terminal fragment of TNF antagonized the effects of CKIP-1. CKIP-1 increased CD86 surface expression, TNF reverse signaling inhibited it. N-terminal fragment of mTNF influenced the localization of CKIP-1 and drastically inhibited the effect of CKIP-1 and c-Jun on TNF promoter activation.

Conclusion: Infliximab induced TNF reverse signaling elicited translocation of CKIP-1 from the plasma membrane into intracellular regions. The shuttling of CKIP-1 was found to be governed by the N-terminal PH domain and C-terminal auto-inhibitory region of CKIP-1. TNF reverse signaling elicited opposing effects on different cell lineages. TNF reverse signaling proved to determine life or death of activated immune cells.

O-I5:

OUR EQUILIBRATED COEXISTENCE WITH THE LIFE ENDANGERING EPSTEIN BARR VIRUS, EBV.

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Introduction: Humans are carriers of EBV. The main target of EBV is the B lymphocyte. Its fate is determined by the expression of virally induced genes. This is under the influence of cellular genes. The EBV encoded EBNA-2 and LMP-1 are the key proteins in the growth promoting program expressed in a defined differentiation window of the B cells. The EBV carrying proliferating B cells are life threatening, they could grow to lymphomas. Normally, with well functioning immune system, the transformed cells are recognized and eliminated. The syndrome of Infectious Mononucleosis is the manifestation of the prompt innate immune response to the primary EBV infection. While it can be accompanied by severe symptoms, it always subside. It is followed by the virus carrying state and the development of adaptive immunity.

Objectives: Analysis of the cell mediated immunological control mechanisms in EBV infection.

Materials & methods: EBV infected B lymphocytes, Transformed cell lines, Activated T lymphocytes Immunoblot, Immunofluorescence.

Results: The CD4 T cell produced lymphokines IL21 and CD40 ligand modify the expression of EBV encoded protein expression in viral genome carrying B cells. They dowregulate EBNA-2 and upregulate LMP-1 expression.

Conclusion: Modification of the expression of EBV encoded genes counteract the proliferation inducing effect of B cells due to the downregulation of EBNA-2. Nevertheless, these "nonproliferating" virus carrying B cells still represent a danger. B cells with restricted viral gene expression produce factors that modify the microenvironment and express receptors for growth promoting lymphokines. These events are thought to be involved in the development of Hodgkin´s lymphoma.

O-I6:

EPIGENETIC REGULATION OF THE LAMIN A/C PROMOTER IN EBV-POSITIVE B CELL LINES

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Introduction: Lamin A/C and lamin B proteins form a supporting network at the nuclear periphery that serves as a docking platform for nuclear processes (transcription, splicing). Lamin A/C proteins are usually absent from hemopoietic cells, whereas lamin B proteins are expressed in all mammalian cell types studied so far.

Objectives: We wished to study the expression and epigenetic regulation of the genes encoding lamin A/C in B cell lines harbouring strictly latent Epstein-Barr virus (EBV) genomes.

Materials & methods: Western blotting and RT-Real-Time-PCR was used to assess Lamin A/C and lamin B1 expression. Bisulfite sequencinng was used to map methylated CpG dinucleotides at the lamin A/C promoter.

Results: In Burkitt lymphoma cells of EBV latency type I, lamin A/C expression was absent or hardly detectable. In contrast, lymphoblastoid cell lines of EBV latency type III lamin A/C was highly expressed. Lamin B1 was present in the cells independently of EBV latency type. The 5' regulatory sequences of lamin A/C were unmethylated independently of promoter activity, whereas a CpG-rich region 3' from the promoter was unmethylated at active, but highly methylated at silent promoters.

Conclusion: The activity of lamin A/C promoter depends on the phenotype and EBV latency type of EBV positive B cell lines. We propose that the lamin A/C promoter is demethylated and switched on during EBV-mediated B cell transformation (immortalization). We suggest that the induction of lamin A/C proteins in EBV-positive lymphoblastoid cells may create a new environment for important nuclear regulatory processes.

O-I7:

MRNA SEQUENCING OF THE CHLAMYDIA TRACHOMATIS INFECTED AND INTERFERON-GAMMA TREATED HUMAN NEUTROPHIL GRANULOCYTE TRANSCRIPTOM

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Introduction: The neutrophil granulocyte is a key cell type involved in the Chlamydia trachomatis (C. trachomatis) mediated inflammation. Interferon-gamma (IFN-gamma) is the major cytokine in the elimination of chlamydia infection. Even though the importance of neutrophil granulocytes, the impact of C. trachomatis infection and IFN-gammaon the neutrophil gene expression has not been studied.

Objectives: Exploration of the gene expression changes in C. trachomatis infected and/or IFN-gamma treated human granulocyte cell line HL-60.

Materials & methods: New generation sequencing was used to discover the impact of the Chlamydia trachomatis D infection and IFN-gamma on the transcriptome of human neutrophil granulocyte cell line HL-60.

Results: Experimental data showed that C. trachomatis infection altered the expression of host genes involved in inflammation and innate immunity. These genes included matrix metalloproteinases, complement cascade members and different proinflammatory cytokines. Interestingly, we found that several adaptive immunity related genes were also induced including members of the antigen processing and presentation machinery and the T-cell chemokines MIG, I-TAC, IP-10 and RANTES. Protein level measurement of MHC-I, CXCL9 and CXCL10 expression supported the mRNA sequencing data.

Conclusion: Our experiments support the role of neutrophil granulocytes in the C. trachomatis mediated inflammation, and also highlight their involvement in the antigen presentation, T-cell recruitment and activation.

O-I8:

PROGNOSTIC ROLE OF CD68, CD163 AND CD1A EXPRESSION IN HUMAN PRIMARY CUTANEOUS MALIGNANT MELANOMA

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Introduction: Metastatic potential of cutaneous malignant melanoma (CMM) depends not only on the tumor cell properties, but also on the effectiveness of anti-tumor host immune response. Accordingly, it has to be also determined to characterize precisely the prognosis and suitable therapy of CMM.

Objectives: To investigate immunological features of primary CMM without metastasis (n= 23) and with hematogenic metastasis (n= 23) using tissue microarray (TMA) analysis.

Patients & methods: In this retrospective immunohistochemical study staining with monoclonal antibodies against CD68, CD163, DC-SIGN and CD1a was performed on paraffin sections derived from TMA.

Results: We observed significant differences between the groups of metastatic and nonmetastatic CMM concerning the presence of tumor infiltrating $CD68^+$ and $CD163^+$ macrophages and peritumoral $CD1a^+$ dendritic cells. Presence of $CD68^+$ and $CD163^+$ macrophages infiltrating the tumors correlated with metastasis formation ($p= 0.001$), while $CD1a^+$ dendritic cells surrounding the tumors were associated with lower risk of hematogenic spread ($p= 0.003$).

Conclusion: Our results confirm that tumor microenvironment might play crucial role in melanoma progression and the selected markers are able to characterize a part of the anti-tumor host immune response. The immunohistochemical analysis of the increased or decreased level of CD68, CD163 and CD1a immunoreactivity in the tumor can contribute to better prediction of prognosis.

O-I9:

NOVEL ANTIBODY PROFILE ANALYSIS BASED ON TRACKING B LYMPHOCYTES IN MELANOMAS AND BREAST CARCINOMAS IS AN ASSET FOR THE NEW IMMUNOLOGICAL SCORE FOR CANCER THERAPEUTICS

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Introduction: This study is focused on a major question of tumorimmunology, that is to reveal the potential role and capacity of immunocompetent cells found in solid tumors.

Objectives: We aimed to develop a new immunoglobulin profile analysis based on our previous findings on B cells (Tumor infiltrating B lymphocyte, TIL-B) infiltrating malignant melanomas and breast carcinomas.

Patients & methods: Expressed heavy and light chain immunoglobulin variable region gene (VH-JH and Vk-Jk) usage was analysed at DNA level from various melanoma and breast cancer tissues. Hundreds of cloned and sequenced heavy and light chain immunoglobulin variable region genes were sequenced and comparatively analysed at DNA and amino acid levels with Vector NTI Advance 11, Bioedit 7.0 Alignment editor, ClustalX2.0.11, TreeView 1.6.6 programs. Tissue samples of melanomas and breast cancers were processed for tissue microarrays (TMA Master for MIRAX Viewer 1.12, 3D Histech) and unique sialilated glycosphingolipides were tested by immunohistochemistry.

Results: Comparative sequence data analysis revealed a pattern of immunoglobulin variable region genes with tumor associated antigen binding potentials. Immunohistochemistry showed GD3 ganglioside expression on melanomas and some breast carcinomas.

Conclusion: Our data indicate internationally as first that tumor associated sialilated glycosphingolipides are among the target molecules of these TIL-B immunoglobulines. The novel antibody profile analysis reveals important aspects of the patients' cancer related potential anti tumor humoral immuneresponse.

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O-I10:

REACTIVE OXYGEN SPECIES GENERATED BY NADPH OXIDASES IN RAGWEED SUBPOLLEN PARTICLES ACTIVATE HUMAN MONOCYTE-DERIVED DENDRITIC CELLS

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Introduction: It has previously been reported that ragweed (Ambrosia artemisiifolia) pollen grains release subpollen particles (SPPs) of respirable size upon hydration. These SPPs contain allergenic proteins and NADPH oxidase of ragweed pollen.

Objectives: In this study we have examined whether exposure to SPPs induces maturation and activation of dendritic cells (DCs).

Materials & methods: To test this assumption, human monocyte-derived DCs were treated with ragweed SPPs.

Results: Analysis with confocal laser-scanning microscopy demonstrated that DCs are capable of phagocytosing SPPs. Phagocytosis of SPPs triggers an increase in the production of IL-8, TNF-alpha and IL-6. Flow cytometric analysis revealed that treatment with SPPs initiates activation of DCs by up-regulating the expression of CD40, CD80, CD86 and HLA-DR. Furthermore, SPP-treated DCs have an increased ability to stimulate proliferation of naive T cells. SPP-exposed DCs co-cultured with allogeneic $CD3^+$ pan-T cells promote elevated secretion of IFN-gamma and IL-17 mainly from T cells of non-allergic subjects, whereas trigger production of IL-4 exclusively from T cells of allergic individuals. The observed phenotypic and functional changes of SPP-treated DCs are mediated, at least partly, by intrinsic NADPH oxidase activity of SPPs.

Conclusion: Collectively; our data indicate that inhalation of ragweed SPPs may lead to maturation/activation of DCs in the airways initiating both innate and adaptive immune responses.

O-III:

CHEMOTACTIC DRUG TARGETING – A NOVEL APPROACH OF TARGET CELL DEPENDENT DRUG DELIVERY

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Introduction: In the last decade development of new families and techniques in drug targeting (DT) was one of the most promising ways of selective delivery of drugs. Classification and complexity of the identical groups of DT (passive - liposome, active - antibody, molecular – biologics) indicates that DT conceals a number of new options including its feasibility. Chemotactic drug targeting (CDT) offered by us represents a special, reciprocal way of DT which potential advantages are derived from the moiety that not the drugs are delivered by different ways to the target cells but chemotaxis directed responsiveness of target cells is used to find the drug containing conjugates.

Objectives: Our objective is to give a summary on characterization of the CDT ligands and responsiveness of model cells ranging from ciliate level to cell lines representing different tumors.

Materials & methods: The tested parameters were chemotaxis (NeuroProbe), cell adhesion (xCELLigence), cytotoxicity (CASY), internalization and intracellular signaling in Tetrahymena and tumor cell lines (THP1, MM6, LM2/3).

Results: Due to the definition the main structural elements of the CDT conjugates are: (i) carrier; (ii) chemotactically active, directing ligand(s); (iii) drug and (iv) spacer-sequence. The 10 year research of CDT has proved that, there is a high variability in the mainly peptide constructs as well as there are significant differences in target cell specificity. Among carrier structures oligotuftsins and polylysines were the most effective; their phagocytosis or cell adhesion enhancer effects were also advantageous in CDT. Among several

chemotactic key ligands investigated formyl peptides, SXWS and tuftsin sequences were the most effective, while GnRH-IIIs worked as combined carrier-attractants in CDT. Enzyme-labile spacer sequences (GFLGC) made CDT cell compartment specific, while conjugation of drugs (methotrexate, daunorubicin) do reduce significantly cytotoxicity in tumor targets.

Conclusion: In summary, our results indicate that CDT, is an effective, target cell specific candidate of DTs which may have significance in treatment of solid tumors and leukemias.

O-I12:

SILENCING SUPPRESSOR ACTIVITY REMODELLED

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Introduction: RNA silencing is a sequence specific cellular process that leads to RNA degradation, inhibition of translation of mRNAs and heterochromatin formation. RNA silencing has several functions, among them one of the most important is to counteract with molecular parasites, such as viruses and transposons. Viruses evolved silencing suppressor proteins to inhibit RNA silencing. We have previously identified the P1 protein of the Sweet potato mild mottle virus (SPMMV) as a silencing suppressor that is able to counteract with active RISC. P1 of SPMMV binds Argonaute via its 3 N-terminal WG/GW motifs and inhibits RISC activity by the suppressor domain located in the middle of the protein.

Objectives: The closest homolog of the SPMMV P1 is the P1 protein of the Sweet potato feathery mottle virus (SPFMV).

Materials & methods: We have isolated the cDNA for the SPFMV P1.

Results: Although the Argonaute binding domain of SPFMV P1 shows significant homology to the corresponding domain of the SPMMV P1, it only contains 1 WG/GW domain. We found that the SPFMV P1 did not have silencing suppressor activity. However, reconstruction the missing 2 WG/GW motifs by mutagenesis resulted in silencing suppressor activity and Argonaute binding.

Conclusion: To our knowledge this is the first case, when a viral protein with unknown function have gained silencing suppressor function by targeted in vitro remodeling.

O-I13:

FASCIOLA HEPATICA TEGUMENTAL ANTIGENS SUPPRESS TH1-PROMOTING MAST CELLS

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Introduction: Mast cells can drive Th1 inflammatory responses that contribute to the clearance of bacterial infections and Th1 mediated autoimmune diseases such as arthritis. The parasitic worm Fasciola hepatica and the products it release suppress Th1 immune responses to bystander bacterial infections and prevent the initiation of inflammatory disorders like autoimmune disease. We have previously shown that F. hepatica tegumental coat antigen (FhTeg) has antiinflammatory effects on dendritic cells.

Objectives: This study investigated if FhTeg exhibits anti-inflammatory effects on Th1 promoting mast cells.

Materials & methods: Bone marrow-derived mast cells and peritoneal-derived mast cells were stimulated with LPS and whole cell antigen of *Bordetella pertussis* and mast cell proliferation, deregulation, cytokine

secretion and cell surface marker expression were measured. Mast cells were also co-cultured with $CD4^+$ cells to examine the impact upon adaptive immunity. Protein was obtained to measure FhTeg's impact upon the TLR signalling pathway in mast cells.

Results: FhTeg inhibited the secretion of pro-inflammatory cytokines and cell surface marker expression on mast cells stimulated with bacterial ligands. FhTeg treated mast cells failed to promote Th1 immune response when co-cultured with $CD4^+$ cells. FhTeg suppressed the expression of transcription factors in the TLR signalling pathway which explains why there is a decrease in activation of mast cells.

Conclusion: In conclusion FhTeg impairs mast cells ability to drive Th1 immune responses. Further studies will help us to identify the mechanism involved that may lead to potential identification of novel antiinflammatory therapeutic targets.

O-I14:

CR3 IS THE MAIN PHAGOCYTIC RECEPTOR FOR IC3BOPSONIZED PARTICLES ON DENDRITIC CELLSWHILE CR4 PLAYS SUPPORTING ROLE

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Introduction: Opsonization with C3 complement fragments is a powerful tool of the innate immunity to enhance phagocytosis of antigen. iC3b is the main fragment of C3 that mediates this function, its receptors are CR3 (CD11b/CD18) and CR4 (CD11c/CD18). The function of CR4 has not been clarified yet. CR4 binds the same ligand as CR3 hence it is accepted that CD11c also takes part in iC3b mediated phagocytosis. Both receptors belong to the family of beta2 integrins which are present on several human cells including dendritic cells (DCs), that's function is to orchestrate the immune response. Our aim was to analyze the function of these two receptors of DCs.

Objectives: Since both CR3 and CR4 bind the same ligand -iC3b- we studied the contribution of the two receptors to phagocytosis of iC3b coated particles.

Materials & methods: We analyzed the phagcytosis of iC3b opsonized yeast and bacteria by human monocyte-derived DCs (MDCs) by flowcytometry and confocal microscopy using antibody blocking and RNA silencing techniques.

Results: We proved that uptake of iC3b opsonized yeast or bacteria can be blockedby anti CD11b antibodies, but not by anti-CD11c and using siRNA we confirmed our findings. We also found that upon iC3b mediated phagocytosis CD11b was internalized into the cells at significantly more extent than CD11c.

Conclusion: Our results show that CR3 is the main receptor of MDCs that mediates the uptake of iC3b opsonized particles, CR4 has only a supporting role in this process.

P-I1:

REDUCED PLASMA BIG-ENDOTHELIN LEVEL AFTER COMPLEMENT C5A ANTAGONIST TREATMENT ACCOMPANIED BY IMPROVED SMALL INTESTINAL MICROCIRCULATION IN EXPERIMENTAL MODEL OF CARDIAC TAMPONADE

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Introduction: Cardiogenic shock due to cardiac tamponade (CT) leads to circulatory redistribution with severe impairment of the gastrointestinal (GI) microcirculation. We hypothesized that activation of complement cascades plays a central role in endothelial dysfunction with subsequent activation of endothelial cell-derived mediators including the endothelin system.

Objectives: Our aim was to investigate the effects of complement C5a antagonist (C5aA) treatment on endothelin synthesis and GI microcirculatory changes in a large animal model of CT.

Materials & methods: In anaesthetized, ventilated and thoracotomized minipigs (n=7) CT was induced for 60 min by intrapericardial fluid administration, meanwhile mean arterial pressure (MAP) was kept between 40-45 mmHg. Group 2 was treated with C5aA at the 45th min of CT (n=6), a further group (n=6) served as sham-operated control. Macrohemodynamics were monitored for 240 min, the microcirculatory pattern of the small intestine was evaluated by intravital videomicroscopy. Plasma big-endothelin (bigET) changes were measured with ELISA before CT, before administration of C5aA and at the end of post-tamponade phase.

Results: Microperfusion was oscillating and redblood cell velocity (RBCV) was reduced during CT. After relief of CT, cardiac output was normalized but MAP was decreased despite the increased bigET level. The C5aA treatment abolished microcirculatory oscillation, increased RBCV (320±48 *vs* 650±11 m/s) and reduced plasma bigET concentration (5.2±3.8 *vs* 26±0.8 fmol/ml) significantly.

Conclusion: Release and elevation of plasma bigET is important component of the development of splanchnic microcirculatory derangement after CT. The complement system plays a pathophysiologic role in endothelin signaling in this condition.

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P-I2:

THE ROLE OF LYMPHANGIOGENESIS IN WOUND HEALING

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Introduction: Angiogenesis plays a pivotal role in the healing of acute wounds. However, effects of lymphangiogenesis on wound healing are still not clarified. It is known that vascular endothelial growth factor receptor-3 (VEGFR-3) is primarily characteristic of lymphatic vessels.

Objectives: Our aim was to examine the effects of VEGFR-3 blocking antibodies on wound healing and microcirculatory parameters of wound edges. Changes induced by this treatment were compared to those evoked by VEGFR-2 antibodies (inhibition of angiogenesis).

Materials & methods: Experiments were performed on male SKH-1 hairless mice. In the dorsal region a skin fold was formed and fixed with two symmetric, fenestrated titanium frames. A circular wound was prepared on one side of the skin fold. In group 1 (control, n=6) sterile phosphate buffered saline was injected into the wound edges. In group 2 and 3 (both n=6) VEGFR-2- and VEGFR-3-inhibiting antibodies were administered, respectively. The observation period was 20 days. Photographs were taken in order to determine the decrease of wound area. Intravital videomicroscopy was used for the assessment of microcirculation. Tissue samples were collected and analyzed with routine histology.

Results: Epithelialization rate was significantly lower in group 2 and 3 than in control group. VEGFR-2 inhibition led to a retarded wound healing as compared to blocking of VEGFR-3. Angiogenesis and vascular maturation was decreased by both antibodies, but group 2 (VEGFR-2-blockade) displayed considerably lower angiogenesis than group 3.
Conclusion: Inhibition of lymphangiogenesis interferes with wound healing. Thus, lymphangiogenesis may be an important factor of wound healing.

P-I3:

CELL PHYSIOLOGICAL APPLICATIONS OF IMPEDIMETRY WITH A SPECIAL RESPECT TO CELL ADHESION AND MIGRATION

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Introduction: Impedimetry is a widely used technique based on measurement of electric impedance in AC. Due to the insulator behavior of cells coated by phospholipid membranes impedimetry is a dedicated tool to analyze series of cell physiological responses (e.g. cell adhesion, migration, proliferation). Recently several homemade equipments were built up to characterize cellular samples, however, the electric cell impedance sensing (ECIS) technology developed by Giaver and Keese (1984) represent a milestone using impedimetry in cell biology.
Objectives: Our objectives are to give a short coverage in the light of our previous results about
(i) advantages of electrode designs of arrays and (ii) main groups of detected cell physiological indices as well as potential applications.
Materials & methods: The summary is based on experiments carried out by two main families of equipments: ECIS (Applied BioPhysics) and xCELLigence (Roche) using different designs of arrays; while data of a third tool CASY analyzer (Innovatis-Roche) working on electronic pulse area analysis was also used as a close relative in physical backgrounds. The list of the cells analyzed is ranging from healthy cell (e.g. hESC, HUVEC) to tumor cell lines (e.g. melanoma-HT168M1) representing different levels of metastatic behavior and ciliates as well.
Results: The design of array provides significantly different choices as the small surface of the point shaped electrode offers sensible measurement of e.g. cell migration while the set pectinate electrodes offers a more robust measurement of cell adhesion. Development of arrays resulted more appropriate designs like flow arrays, electrode furnished filters of Boyden chambers and HTS systems for pharmacological tests. In respect of applications we have to underline sensibility of impedimetry to migratory processes (micro-motion) and the new wound-healing assays. Among the applications there are new assays of implantation of the zygote, quantitative analysis of creeping in ciliates, evaluation cardiomyocytes and electric noise based prediction of malignancy.
Conclusion: In conclusion, authors call attention to impedimetry as a new tool of cell biology which is suitable to use in combination with other high-tech instruments too.

P-I4:

EFFECTS OF HYPERCHOLESTEROLEMIA INDUCED INFLAMMATION AND INSULIN RESISTANCE ON POSTCONDITIONED RATS

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Introduction: High cholesterol level increases the risk of coronary heart disease, stroke, peripheral arterial diseases and other pathologic conditions.

Objectives: Investigation was focused on the effects of cholesterol rich diet (HF) on insulin sensitivity, inflammation, and the consequences of kidney ischemia/reperfusion with or without post-conditioning (PC) in rats.

Materials & methods: Two groups of rats were fed for 12 weeks with standard rat chow (I. Group), or a diet contained 2% cholesterol (II. Group). Oral glucose tolerance test (OGT) was performed at the 5th and 12th weeks. Serum glucose, insulin and TNF-alpha levels were measured. Finally both goups were devided further and median laparotomy was performed in the anaesthetized animals and both renal pedicles were closed for 90 minutes, than reperfused for 4 hours, with or without of four 15 seconds PC cycles, in 8-8 animals/subgroups. Serum glucose, insulin, TNF-alpha levels, and PMA-induced free radical production in blood samples were measured before and after the intervention.

Results: In HF group hyperlipidemia developed until the 5th, and increased further until 12th week resulting in glucose intolerance, insulin resistance and TNF-alpha release in OGT. In the course of renal ischemia, PC decreased the surgery induced elevation of glucose and free radical levels, and increased insulin levels in healthy animals, but not in animals were kept in HF.

Conclusion: Glucose load induces not only insulin, but TNF-alpha release too. High serum cholesterol, triglyceride and TNF-alpha levels induced by HF diet may be responsible for glucose intolerance, insulin resistance and impairments in postconditioning.

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P-I5:

PRIMARY CHARACTERIZATION OF A NOVEL IMMORTALIZED CELL LINE FOR STUDYING KERATINOCYTE INNATE IMMUNE FUNCTIONS

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Introduction: Normal human keratinocytes (NHK) have a restricted availability and a short lifespan because of their limited proliferative capacity in culture. HaCaT, a spontaneously immortalized keratinocyte cell line is widely used as a model of keratinocyte function, however these cells exhibit limitations for some applications.

Objectives: In this study we characterized a novel HPV-E6 oncogene immortalized human keratinocyte cell line (HPV-KER).

Materials & methods: The HPV-KER cells were synchronized and the expression of proliferation- and differentiation-related genes were followed by real time RT-PCR.

Results: The differentiation-related K1, K2 and K10 genes showed the highest expression in the contact inhibited cells and their expression dropped right after the cells were released from cell quiescence. The K14, K17, Ki67 and alpha 5 integrin genes showed a peak expression 12-24 hours after cells were released from cell quiescence then gradually decreased. The expression of involucrin and loricrin genes could not be detected in

this system. Next we investigated whether the stress-induced processes of HPV-KER cells resembled that of the immortalized HaCaT cell line or NHKs. The expression of p53 was studied after UV-B irradiation and the TNFA gene expression was followed in *Propionibacterium acnes*-treated cells. The results of these experiments revealed that the investigated stress induced processes of HPV-KER resembled those seen in NHKs and did not show similarities with HaCaT cells.

Conclusion: The established novel human keratinocyte cell line has functional characteristics similar to NHKs, therefore it may serve as a better *in vitro* model than HaCaT cells for studying certain keratinocyte functions.

P-I6:

HUMAN CD1A$^+$ DENDRITIC CELLS MEDIATE EFFICIENT ANTI-VIRAL IMMUNE RESPONSES VIA RIG-I AND MDA5 SIGNALING

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Introduction: Cytosolic RIG-I-like helicases (RLH) are pattern recognition receptors involved in type I interferon production and antiviral immunity. The heterogeneous population of dendritic cells (DCs) act as professional antigen presenting cells in both lymphoid and non-lymphoid tissues to coordinate innate and adaptive immunity.

Objectives: A comparative analysis of the expression, functional activities and signaling cascades associated to RLH in the in vitro generated and previously characterized CD1a$^+$ and CD1a human monocyte-derived dendritic cell (moDC) subtypes.

Materials & methods: Human monocyte-derived DCs were sorted by FACS DiVa. Relative mRNA expressions were analyzed by Q-PCR. Dendritic cell activation was induced by polyI:C, and influenza virus. Protein level expressions were measured by Western blot and ELISA. ELISPOT assay was used for the examination of T cell priming by activated DCs.

Results: The expression of RLH genes and proteins as well as the activity of the coupled signaling pathways were significantly higher in the CD1a$^+$ subset than in its developmentally related but phenotypically and functionally distinct CD1a$^-$ counterpart. Specific activation of RLH induced the secretion of IFN via IRF3. Ligand-induced RLH-mediated signaling in CD1a$^+$ moDC was shown to be indispensable for both priming naïve CD8$^+$ T lymphocytes and inducing potent influenza virus-specific cellular immune responses as demonstrated by RIG-I/MDA5 silencing that abrogated these functions.

Conclusion: Our results revealed the DC subset-specific activation of RLH and due to their specialized functional activities identified CD1a$^+$ moDC as potent antigen presenting and highly inflammatory cells. The specific targeting of CD1a$^+$ DC subpopulation may offer new tools for improving the efficacy of anti-viral vaccines.

P-I7:

CONSECUTIVE TREATMENT OF HUMAN MELANOMA CELLS BY ATRA AND POLYI:C RESULTS IN DISTINCT INFLAMMATORY CYTOKINE AND CHEMOKINE RESPONSES VIA TLR3 AND MDA5

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Introduction: In the last two decades the incidence of melanoma has increased worldwide and no effective treatment modalities have yet been developed. ATRA and polyI:C are known as strong inducers of TLR3 and MDA5 expression, and polyI:C triggers TLR3/MDA5 signaling specifically causing cell death in melanoma cells in vitro.

Objectives: We addressed the question whether ATRA pre-treatment could enhance the efficacy of polyI:C and if so, had it any additional effects.

Materials & methods: mRNA/protein expressions in WM35 and WM983A human melanoma cells were monitored by Q-PCR, Western blot, and ELISA. Dendritic cells (moDCs) and macrophages (moMACs) were differentiated from human blood-derived monocytes. CD1a+ moDCs were sorted byFACS, migration assays were performed in transwell chambers.

Results: Doubletreatment by ATRA+pI:C strongly increased the expression of TLR3 and MDA5 in both cell lines. Treated cells exhibited significantly higher mRNA and secreted levels of IL-1beta, IFNbeta, CXCL1, CXCL8/IL-8, CXCL9 and CXC10 than single ATRAor polyI:C treated ones. Silencing of MDA5 by siRNA prevented this effect on IFNbeta secretion, whereas TLR3 knock down interfered with CXCL(s) and IL-1beta production,only. Supernatants of ATRA+polyI:C activated cultures increased the migration of both moMACs and CD1a+ moDCs significantly as compared to single ATRA or pI:C treatment, in a TLR3-dependent manner.

Conclusion: Consecutive treatment with ATRA and polyI:C results in strong, TLR3/MDA5 mediated inflammatoryand chemokine responses in *in vitro* cultured human melanoma, which triggers a functional response in professional antigen-presenting cells. This novel mode of concomitant activation may offer a more efficient treatment option in future melanoma therapy.

P-I8:

ANTI-INFLAMMATORY EFFECTS OF L-ALPHA GLYCERYLPHOSPHORYLCHOLINE TREATMENT IN A RAT MODEL OF MESENTERIC ISCHAEMIA- REPERFUSION INJURY

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Introduction: Phosphatidylcholine (PC) and PC metabolites (i.e. choline, N,N-dimethylethanolamine and N-methylethanolamine) inhibit the production of reactive oxygen species (ROS) both in vitro and in vivo, and the effectiveness is proportional to the amount of methane generation and the number of methyl groups in the compound (Cell Physiol Biochem 2008, Shock 2008).

Objectives: Our aims were to examine whether the anti-inflammatory effects of PC are linked to the fatty acid parts or to the head group, hence we repeated the crucial in vivo experiments with L-glycerylphosphorylcholine (GPC), the deacilated polar derivative of PC, on the inflammatory consequences of ischaemia-reperfusion (IR).

Materials & methods: Anesthetized rats were divided into control (n=8), mesenteric IR (n=8), IR with GPC pre-treatment (n=8) or IR with GPC post-treatment (n=8) groups. Macrohaemodynamics were measured, intestinal microhaemodynamic parameters were registered by intravital videomicroscopy using OPS technique. Biochemical inflammatory markers (tissue superoxide, xanthine oxidoreductase (XOR) activity) and liver ATP contents were determined after 45 min ischemia and 180 min reperfusion period.

Results: Mesenteric IR increased the vascular resistance (MVR) and tissue superoxide production, and reduced liver ATP content significantly. GPC pre-treatment alleviated the elevation of MVR and decreased the tissue ROS production, while GPC post-treatment decreased both XOR activity and ATP depletion, and normalized the intestinal microcirculatory dysfunction.

Conclusion: Exogenous GPC counteracts local formation of ROS after intestinal ischemia and reoxygenation. The mechanistic details of this pathway suggest that the anti-inflammatory efficacy of PC is linked to the polar part of the PC molecule.

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P-I9:

THE EFFECTS OF KV1.3 AND IKCA1 POTASSIUM CHANNEL INHIBITION ON CALCIUM INFLUX OF HUMAN PERIPHERAL T LYMPHOCYTES IN RHEUMATOID ARTHRITIS

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Introduction: The transient increase of the cytoplasmic free calcium level plays a key role in lymphocyte activation. Kv1.3 and IKCa1 potassium channels are important regulators of the maintenance of calcium influx during lymphocyte activation and present a possible target for selective immunomodulation.
Objectives: We aimed to characterize the effects of lymphocyte potassium channel inhibition on short-term peripheral blood T-lymphocyte activation in major lymphocyte subsets of patients recently diagnosed with rheumatoid arthritis (RA).
Patients & methods: We took peripheral blood samples from 10 healthy individuals and 9 RA patients receiving no anti-rheumatic treatment. We evaluated calcium influx kinetics following activation with phytohemagglutinin in CD4, Th1, Th2 and CD8 cells. We also assessed the sensitivity of the above subsets to specific inhibition of the Kv1.3 and IKCa1 potassium channels.
Results: In healthy individuals, inhibition of the IKCa1 channel decreased calcium influx in Th2 and CD4 cells to a lower extent than in Th1 and CD8 cells. However, inhibition of Kv1.3 channels resulted in a larger decrease of calcium entry in Th2 and CD4 cells. No difference was detected between Th1 and Th2 or CD4 and CD8 cells in the sensitivity to IKCa1 channel inhibition among lymphocytes of RA patients. However, specific inhibition of the Kv1.3 channel acts differentially on calcium influx kinetics in RA lymphocyte subsets. Th2, and in particular CD8 cells are inhibited more dominantly than Th1 and CD4 cells.
Conclusion: The inhibition of Kv1.3 channels does not seem to be specific enough in peripheral RA lymphocytes, since anti-inflammatory Th2 cells are also affected to a noteworthy extent.

P-I10:

THE EFFECTS OF EXOGENOUS METHANE INHALATION ON MACRO-AND MICROCIRCULATORY CHANGES DURING INTESTINAL ISCHEMIA- REPERFUSION IN RATS

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Introduction: Following its release, methane is widely regarded as biologically inactive.
Objectives: Our objectives were to investigate the effects of methane inhalation on the macro-and microcirculatory changes and inflammatory processes during intestinal ischemia-reperfusion (IR) in rats.
Materials & methods: Male Sprague-Dawley rats were randomized to control (n=7), IR without methane (IR, n=7) and IR with methane (2.5% added to 21% oxygen, n=7) groups. The superior mesenteric artery

(SMA) was occluded for 45 min, which was followed by 120 min reperfusion. Methane treatment was started during the last 10 min of ischemia and lasted for 5 min during reperfusion. Systemic and mesenteric haemodynamics were monitored, the microcirculation of the serosa of the terminal ileum was observed with intravital videomicroscopy using OPS technique. The tissue generation of reactive oxygen species (ROS) was detected by a chemiluminescence assay, myeloperoxidase (MPO) activity was assessed by fluorimetric analysis, nitrotyrosin level was determined by ELISA assay.

Results: The significant microcirculatory dysfunction (as evidenced by IVM) after IR was remarkably improved by methane treatment, a significantly decreased ROS production and MPO activity was detected in contrast with IR group. Furthermore methane treatment highly reduced the elevated nitrotyrosin level after ischaemia.

Conclusion: Exogenous methane improves the perfusion deficiency of the mucosa after IR, and this effect may be linked to ROS scavenging or inhibition of leukocyte activation in the reperfused intestinal tissues.

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P-I11:

DIETARY PHOSPHATIDYLCHOLINE PROTECTS AGAINST INFLAMMATORY ACTIVATION IN EXPERIMENTAL COLITIS IN THE RAT

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Introduction: Local increase in mucus phosphatidylcholine (PC) content may decrease inflammatory activity in ulcerative colitis patients (Stremmel W et al., Gut 2005).

Objectives: Our aims were to characterize the anti-inflammatory properties of oral PC treatment regimens in acute and resolving phases of a rodent model of 2,4,6-trinitrobenzene-sulfonic acid (TNBS)-induced colitis.

Materials & methods: In series I rats were monitored 1 day after colitis induction, while in series II on the 6th day following TNBS enema. In both series the animals were randomized to colitis, control or colitis with PC-pretreatment groups (n=6, each). In the acute series PC-pretreated animals received 2% PC-enriched diet for 6 days before TNBS enema, and for 3 days before and 3 days after TNBS treatment in series II. Pro-inflammatory cytokines (TNF-alpha, IL-6) were measured in plasma, the severity of colonic mucosal damage was monitored with *in vivo* confocal laser endomicroscopy. FITC-dextran iv was used to evaluate the microvascular structure and topical acridine orange was applied to observe the mucosal architecture.

Results: Cytokine levels were significantly elevated in both series with colitis. Disruption of the capillary network and lack of epithelium were observed in series I (score: 7.0), while morphological damages were moderate in series II (score: 4.3). The PC-pretreatment decreased significantly the cytokine levels and histological scores in both series (acute phase: 4.0; resolving: 1.33) and increased the number of mucus-producer goblet cells in the resolving phase.

Conclusion: PC-pretreatment is able to inhibit the progression of inflammatory changes in colitis through decreasing the cytokine levels and preserving the epithelial structure.

P-I12:

ALPHA5-INTEGRIN AND ITS LIGAND, THE ONCOFETAL FIBRONECTIN (EDA$^+$FN) ARE DIFFERENTIALLY EXPRESSED IN PSORIATIC UNINVOLVED AND HEALTHY SKIN

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Introduction: To better understand the pathomechanism of psoriasis we compared gene and protein expression responses to mechanical stress in psoriatic uninvolved and in healthy skin. Previously we found striking differences between psoriatic uninvolved and healthy skin in Keratinocyta Growth Factor (KGF), Keratinocyta Growth Factor Receptor (KGFR) mRNA and protein levels, indicating abnormal skin homeostasis in the disease.

Objectives: In this work we studied the effect of mechanical stress on the expression of α5-integrin and its ligand the oncofetal fibronectin (EDA$^+$FN).

Materials & methods: We applied tape stripping on uninvolved skin of psoriatic patients and on normal skin of healthy subjects. Real-Time RT-PCR and immunofluorescent staining were carried out to detect mRNA and protein expressions in treated and non-treated skin samples.

Results: Both the alpha5-integrin and EDA$^+$FN protein expressions were elevated in psoriatic uninvolved skin even without tape stripping. Tape stripping induced the expressions of alpha5-integrin and EDA$^+$FN mRNAs both in psoriatic uninvolved skin as well as in healthy skin, similarly. We detected enhanced alpha5-integrin and EDA$^+$FN protein expressions after tape stripping in both psoriatic uninvolved skin and healthy skin, the increase in protein expressions was higher in the psoriatic uninvolved tissue.

Conclusion: These data confirm that abnormalities exist in the psoriatic uninvolved skin which can contribute to disease pathomechanism.

P-I13:

MELANOMA CELL-DERIVED EXOSOMES ALTER MACROPHAGE AND DENDRITIC CELL FUNCTIONS IN VITRO

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Introduction: Previous studies showed that cultured human tumor cell lines release exosomes, i.e., microvesicles of 20-100 nm diameters, bearing molecular markers of the tumor cells' plasma membrane. The immunomodulatory activities of tumor exosomes are poorly understood, with reported activating and inhibitory effects.

Objectives: We used B16F1 melanoma cell derived exosomes (mcd-derived exosomes) to clarify immunogen properities of them.

Materials & methods: We tested how mcd-exosomes influence the $CD4^+$ T cell proliferation induced by bone marrow derived allogeneic dendritic cells; we quantified the NF-B activation in mature macrophages stimulated with mcd-exosomes, and compared the cytokine profile of LPS-stimulated and mcd-exososome treated macrophages.

Results: We observed that mcd-exosomes helped the maturation of dendritic cells, and they also activated macrophages, as measured by NF-B activation. Compared to macrophages activated by LPS, the cytokine profile of mcd-exosome activated macrophages showed a type 2 bias.

Conclusion: Taken together, we demonstrated that melanoma derived exosomes interact with macrophages as well as dendritic cells, although the mixed biological effects observed in our experiments may contribute not only to anti-tumor immune response but also to tumor immune escape. The fact that, in the absence of therapeutical interventions, the tumor microenvironment harbors mostly type 2 tumor associated macrophages and suppressive dendritic cells indicates that melanoma derived exosomes might typically skew the balance in the direction of immune suppressive mechanisms.

P-I14:

NEONATAL BLUE LIGHT PHOTOTHERAPY AND MELANOCYTIC NEVI: A TWIN STUDY

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Introduction: Blue light phototherapy has been widely and successfully used for the treatment of neonatal jaundice to reduce the plasma concentration of bilirubin and hence to prevent kernicterus. Only a few and controversial data are available in the literature as to how neonatal blue light phototherapy influences melanocytic nevus development.

Objectives: Our aim was to perform a twin study to evaluate the effect of neonatal blue light treatment on nevus development.

Patients & methods: Fifty-nine monozygotic and dizygotic twin pairs were enrolled into this cross-sectional study. One of the twin members received, whereas the other did not receive neonatal phototherapy. Thedensity of melanocytic skin lesions and the prevalence of benign pigmenteduveal lesions was evaluated. A standardized questionnaire was used to assess data relating to constitutional, sun exposure and other variables. To search for possiblegene-environmental interactions involved in the appearance of pigmented lesions, MC1R variants and the I439V polymorphism of HAL genes were also determined in the enrolled twins.

Results: Neonatal blue light phototherapy was associated with a significantly higher prevalence of both cutaneous and uveal melanocytic lesions. No association was found between the examined gene polymorphisms and the number of pigmented alterations in the study group.

Conclusion: Our data suggest that neonatal bluelight phototherapy could well be a risk factor for melanocytic nevus development. Phototherapy with blue-light lamps is a standard and essential therapeutic modality in neonatal care; therefore, additional in vivo and in vitro studies are necessary to establish its potential long-term adverse effects.

P-I15:

INTERLEUKIN-1 RECEPTORS ARE DIFFERENTIALLYEXPRESSED IN NORMAL AND PSORIATIC T LYMPHOCYTES

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Introduction: Psoriasis is a common inflammatory skin disease. Psoriatic plaque formation is initiated and maintained by components of the skin innate and acquired immune system. Our previous results showed that regulatory T cells are functionally defective in psoriasis, however, the reasons are unknown.

Objectives: In this study we investigated the possible role of interleukin-1 receptors in psoriasis pathogenesis, with emphasis on regulatory and effector T cells.

Patients & methods: Effector ($CD4^+CD25^-$, Teff) and regulatory ($CD4^+CD25^+CD127^-$, Treg) T cells were isolated from normal and psoriatic blood. Expression of type 1 (signal-transmitting) and type 2 (decoy) interleukin-1 receptors, and IL-1R antagonist (IL-1RN) mRNAs were determined by real-time RT-PCR. Cell surface, intracellular and soluble protein expression was investigated by flow cytometry and ELISA.

Results: The proportion of regulatory, memory, naive and naive Treg cell population within the CD4 T cell pool is similar in psoriatic and healthy individuals in peripheral blood. Members of the interleukin-1 receptor family are differentially expressed in psoriatic and normal peripheral blood T cells. The psoriatic T cell population (both Tregs and Teffs) express higher levels of the functional and decoy IL-1 receptors upon activation than normal cells. Furthermore, our results provide additional information on effector/regulatory T cell characteristics, showing that there are fundamental differences between the expression of the signal transmitting and decoy IL-1 receptors on Treg and Teff cells.

Conclusion: Our data suggest that differential expression of genes in the interleukin-1 pathway in normal and psoriatic T lymphocytes may result in functional differences in these cells, and may have a role in psoriasis pathogenesis.

P-I16:

COP1, A P53 INTERACTING PROTEIN, IS STRONGLYEXPRESSED BY PROLIFERATING KERATINOCYTES, ITS EXPRESSION DECREASES AS CELLS DIFFERENTIATE AND AFTER UVB IRRADIATION

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Introduction: UVB irradiation has been shown to trigger a broad range of changes in gene expression in human skin; however, factors governing these events are still not well understood.

Objectives: In this study, we investigated changes in the human constitutive photomorphogenic protein-1 (huCOP1), which is an E3 ligase, and the human analogue of Arabidopsis thaliana (AtCOP1), the central negative regulator of plant photomorphogenesis.

Materials & methods: Our data show that huCOP1 protein is expressed both in the nucleus and in the cytoplasm of cultured keratinocytes, UVB reduces the levels of the huCOP1 mRNA and protein, and induces changes in the subcellular localization of huCOP1 protein.

Results: The gene-specific silencing of huCOP1 induces the accumulation of the tumor suppressor p53 protein, which is further increased after UVB irradiation. Our aim was also to investigate changes of huCOP1 protein in normal skin after UVB-irradiation and to elucidate the effect of differentiation on huCOP1 protein expression in human skin as well as in an in vitro differentiating keratinocyte model.
Conclusion: Our results suggest that huCOP1 participates in the orchestration of UVB response in keratinocytes and human skin, and besides UVB-irradiation the proliferation-differentiation states of keratinocytes also regulate huCOP1 expression in human skin.

P-I17:

THE ROLE OF MYELOID DENDRITIC CELLS IN THEPOLARIZATION OF EFFECTOR T CELLS IN ATOPIC DERMATITIS

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Introduction: Atopic dermatitis (AD) is a chronic inflammatory skin disease which is characterized by highly active adaptive immune responses. Different effector T helper (Th) subgroups are responsible for the development of the skin lesions, Th2 and recently Th22 and Th17 cells were implicated.
Objectives: Our aim was to investigate whether peripheral blood myeloid dendritic cells (mDC) from AD patients differ from control mDCs in their cytokine expression profile without any stimuli or in culture milieu characteristic to atopy.
Patients & methods: 12 untreated AD patients and 8 healthy individuals were enrolled in this study. CD1c/BDCA1$^+$ mDCs were isolated from PBMC and they were stimulated with Staphylococcal enterotoxin B (SEB) and thymic stromal lymphopoietin (TSLP). After assessing the maturation status of mDCs, their intracellular cytokine production (IL-12, IL-2, CCL17, IL-10, TGF-ß, IL-6, TNF-, IL-23) was also measured using laser scanning cytometer.
Results: Myeloid DCs of AD patients were more sensitive to skin-specific stimuli, as significantly elevated CD83 expression was detected after SEB and TSLP stimulation compared to healthy controls. Under unstimulated conditions mDCs from AD patients and controls did not differ significantly. On the other hand after SEB and TSLP stimuli the Th2 and Th22 polarizing cytokine pattern was prominent in mDCs from AD patients.
Conclusion: We suggest that the effector T cell polarizing ability of circulating mDCs is not impaired primary in AD, but after special environmental stimuli in AD skin they gain their characteristic polarizing cytokine pattern.

P-I18:

EXPRESSION OF HUMAN CONSTITUTIVE PHOTOMORPHOGENIC PROTEIN-1 (COP1) IN MELANOCYTIC AND NON- MELANOCYTIC TUMOURS. AN IMMUNOHISTOCHEMICAL STUDY.

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Introduction: Consitutive photomorphogenic protein (COP1) has proved to be a key regulator in light dependent plant development. We have previously shown that human COP1 (huCOP1) plays an important role in the UVB-induced signalling of human keratinocytes as a negative regulator of p53.
Objectives: There is less evidence about the function of huCOP1 in human UV-related skin carcinogenesis. Therefore our aim was to gain preliminary huCOP1 expression data using tissue microarray technique on routine human paraffine skin samples.
Materials & methods: HuCOP1 and p53 immunohistochemistry was performed on paraffine embedded samples of acanthoma, seborrhoeic and actinic keratoses, Bowen's disease, squamous and basal cell carcinomas, nevi and different types of melanoma (>500 cases).
Results: Normal and most perilesional epidermis exhibited consistent huCOP1 expression in basal keratinocytes, p53 expression in normal epidermis was strictly restricted to basal keratinocytes. As non-melanocytic lesions considered, acanthomas showed similar huCOP1/p53 topography, in actinic keratosis huCOP1/p53 expression was extended parallel with hyperproliferation of basal keratinocytes, however, Bowen's keratosis did not alter the pattern of huCOP1. In basal and squamous cell carcinoma, variable expression of huCOP1 and increased p53 expression were detected. In melanocytic lesions, nevi showed variable huCOP1 expression compared to normal epidermis. In malignant melanomas, the intensity of huCOP1 expression and the number of positive cells were variably increased compared to their benign counterparts.
Conclusion: The expression data confirm our previous preliminary results that huCOP1 expression is highly lesion-specific, displaying both parallel and antiparallel changes with p53, suggesting lesion specific alternative pathways of UVB-induced skin carcinogenesis.

P-I19:

IN VIVO EXAMINATION OF CORNEAL LANGERHANS CELLS IN SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) WITH CONFOCAL MICROSCOPY

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Introduction: Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease of unknown etiology, which can manifest in inflammation of various ocular tissues, including cornea.
Objectives: Aim of the study was to investigate Langerhans cells (LC) – an indicatior of inflammation in cornea -, and dry eye related parameters in SLE.
Patients & methods: Prospective consecutive case series of 32 SLE patients and 34 age-matched control (C) subjects. Lid paralell conjuctival folds (LIPCOF), tearbreak up time (TBUT), Schirmer test, ocular surface disease index (OSDI) have been evaluated, then LC densities were investigated with Heidelberg RetinaTomograph –Rostock Cornea Module (HRT-RCM) confocal microscopy in the central and in the peripheral regions of the cornea. The right eye of each patient was examined. For the comparison of control and SLE groups Mann-Whitney U test was performed ($p<0.05$ was considered significant).
Results: Between SLE and control groups significant differences were detected in three out of four dry eye related parameters: TBUT C: 11.09±3.37s SLE: 6.53±3.45s($p<0.001$), Schirmer test C: 11.67±3.21mm,

SLE: 7.47±9.50 mm (p=0.003), OSDI C: 11.06±7.18,SLE: 29.56±22.09 (p<0.001). In LIPCOF no significant difference was found between control eyes (1.24±0.54), and SLE patients (1.53±0.76; p=0.171). Central LC density was greater in SLE patients (42.5±45.7 cell/mm2) than in controls (20.57±19.18cell/mm2, p=0.027). Peripheral LC density was not different (C: 78.00±39.51cell/mm2, SLE: 119.94±155.21 cell/mm2; p=0.212).
Conclusion: A marked increase of LCs could be demonstrated by in vivo confocal corneal microscopy in the central cornea, which suggests, that SLE alters corneal homeostasis and might contribute to the development of dry eye.

P-I20:

DETERMINATION OF T-CELL EPITOPE REGIONS OF PROTEIN DESMOGLEIN 3 USING SYNTHETIC OLIGOPEPTIDES: DESIGN, SYNTHESIS AND IN VITRO ACTIVITY

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Introduction: Pemphigus vulgaris (PV), an autoimmune bullous skin disorder, is characterized by mucosal lesions, mucocutaneous blisters and erosions caused by autoreactive serum antibodies (IgG1 and IgG4) against desmosomal adhesion proteins, desmoglein 1 (Dsg1) and desmoglein 3 (Dsg3). Autoantigen-specific T-cells also play a crucial role in the initiation and perpetuation of Dsg3/Dsg1-specific T-cell responses. Involvement of $CD4^+$ T-lymphocytes in the pathogenesis of PV has been suggested by the strong association with MHC class II alleles.
Objectives: Identification of the functional T-cell epitopes of Dsg3 and Dsg1 proteins has an outstanding importance in immunopathological research, development, and the design of novel diagnostic tools.
Materials & methods: Different lengths of oligopeptides, designed from potential T-cell epitope regions of the Dsg3 protein sequence, have been synthesized, characterized and their *in vitro* cytotoxicity was tested. Peptides were used for *in vitro* stimulation (at 25.0 μM and 12.5 μM concentration) of the peripheral blood monomorphonuclear cells (PBMC) isolated from PV patients and healthy controls. After 24 and 48 hours of incubation the produced IFN-gamma was determined from the supernatants by ELISA.
Results: Synthetic Dsg3 oligopeptides induced different *in vitro* IFN-gamma production rate on PBMC obtained from PV patients and healthy controls determined by ELISA.
Conclusion: Epitope regions were localized according to the IFN-gamma production rate initiated by oligopeptides. Our approach identified promising oligopeptide candidates as diagnostic tools.

P-I21:

THE ROLE OF KERATINOCYTE ACTIVATION IN ACNE PATHOGENESIS

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Introduction: The activation of keratinocytes upon external stimuli plays an important role in various pathogenic conditions of the skin, including wound healing and psoriasis.

Objectives: Our aim was to investigate whether similar processes also play a role in the pathogenesis of acne.

Materials & methods: mRNA expression changes of activated keratinocyte markers were analyzed in response to *Propionibacterium acnes* (*P. acnes*) treatment using Real-Time PCR method. A newly established human papilloma virus E6/16 protein immortalized human keratinocyte cell line (HPV-KER) was treated with various *P.acnes* clinical isolates, and mRNA expression changes of TNFα, IL-1α, TGFα, KRT6, KRT16, ICAM1 and VCAM1 were measured.

Results: TNFα and IL-1α mRNA expressions exhibited a rapid and transient increase with the highest levels around 6-12 h after the bacterial treatment, which disappeared by 24 h. The TGFα and KRT6 mRNA expressions also increased but showed a delayed kinetics, which correlates with previous results suggesting that the expression of these genes is under the control of IL-1α and TNFα. A low level but steady increase of ICAM1 mRNA expression was also noted.

Conclusion: Our results suggest that *P. acnes* treatment alone initiates a cellular program marked by the increased expression of cytokines, growth factors, signaling molecules and extracellular matrix components in HPV-KER cells. The nature of these factors and the timing of these events propose that this skin-colonizing commensal *P. acnes* bacterium may play a role in the initiation of atypical keratinocyte functions, observed in acne pathogenesis.

P-I22:

INVESTIGATION OF TRANSPORTER INTERACTIONS OF ANTIMALARIALS IN VITRO

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Introduction: Options to control spread of malaria are increasingly limited due to emergence of parasites resistant to widely used antimalarials, therefore, discovery of novel antimalarials appears crucial as ever. However, animal experiments are too expensive and laborious for the pharmacokinetic characterization of large number of compounds. The fate of administered drugs may largely depend on their interactions with transporter proteins, which are present in all major pharmacologically relevant barriersand in plasmodiums as well.

Objectives: The aim of this study was to examine whether the high-throughput cell and membrane-based transporter assays can be applied to characterize the transporter interactions of candidate antimalarials.

Materials & methods: Reference antimalarials, such as artemisinin, chloroquine, etc, have been tested for their interaction with the ABC-transporters MDR1, MRP1 and BCRP using the Solvo PredEasy ATPase kits and the interaction with the SLC family members OCT1 and OCT2 uptake transporters in cell-based assay. Measured IC50 values were correlated with the clinical observations on the tested antimalarials.

Results: In many case our data are the first proof for transporter interaction of these clinically important drugs. Artemisinin is a substrate for MDR1, chloroquine is inhibitor of the MDR1 and substrate for the MRP1 and BCRP, mefloquine is substrate for the MDR1 but at higher concentrations is a not specific inhibitor of all the transporters and quinine is substrate for the MDR1. These results corresponded exactly to the clinical data on the antimalarials tested.

Conclusion: We conclude that the membrane- and cell-based HTS in vitro assays can be applied to facilitate the ADME characterization of candidate antimalarials.

P-I23:

REAL-TIME MONITORING OF THE INTERACTION OF KERATINOCYTES AND PROPIONIBACTERIUM ACNES BACTERIUM

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Introduction: Acne is a common multifactorial inflammatory skin disease of the pilosebaceous follicles in which the Gram-positive *Propionibacterium acnes* (*P. acnes*) is an important pathogenic factor. Previously we have shown that selected P. acnes strains (889 and ATCC 11828) belonging to various subgroups within the species differentially affected the proliferation and viability of cultured normal human keratinocytes.

Objectives: To further investigate the interaction between keratinocytes and *P. acnes*, we performed a real-time experiment monitoring the effect of three *P. acnes* strains (889, 6609, ATCC 11828) on the proliferation and viability of immortalized human keratinocytes.

Materials & methods: We used a novel, impedance (Z) measurement based technology that allowed real-time, label-free, kinetic monitoring of cell proliferation and viability. Based on the measured Z values a cell index (CI) was calculated for each well.

Results: After *P. acnes* treatment CIs constantly increased at 24 hours post-treatment. In cases of the *P. acnes* 889 and *ATCC* 11828 treated keratinocytes the CIs rapidly and significantly decreased after 30-36 hours post-treatment, suggesting that the bacterial infection might induce the death of these cells. No such changes were measured in response to *P. acnes* 6609 treatment. These results correlate with the known differential pathogenicity of the different bacterial strains.

Conclusion: These results suggest that assorted *P. acnes* isolates exhibiting altered clinical pathogenicity have different effect on keratinocyte proliferation and viability. The exact nature of this mechanism requires further investigations. Real-time monitoring of the interaction of microbial pathogens and host cells can enhance our understanding of keratinocyte-pathogen interactions and subsequently of acne pathogenesis.

P-I24:

THE OCCURRENCE OF AUTOANTIBODIES TARGETING MUTATED CITRULLINATED VIMENTIN IN PATIENTS WITH PSORIATIC ARTHRITIS AND PSORIASIS

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Introduction: The presence of autoantibodies against mutated citrullinated vimentin (anti-MCV) is demonstrated as a highly sensitive marker in the early diagnosis of rheumatoid arthritis (RA). Other anti-citrullinated peptide antibodies are present in up to 15% of patients with psoriatic arthritis (PsA), while the prevalence of anti-MCV in PsA patients has been poorly investigated.

Objectives: We decided to examine the occurrence of anti-MCV antibodies in our patients with PsA and in psoriasis patients without joint inflammation. Our aim was to find any association between the presence of anti-MCV antibodies and the patients' clinical features.

Materials & methods: Anti-MCV antibodies were measured in 48 patients with PsA, 40 patients with psoriasis but no arthritic disease, 139 patients with RA (reference group) and 25 healthy controls using ELISA test. The association between the anti-MCV positivity (cut off level 20 U/ml) and patients' clinical characteristics was investigated by statistical method (Fischer exact test).
Results: Eleven (22.9%) patients with PsA had anti-MCV antibodies (mean value: 102 U/ml). In contrast, all 40 psoriatic patients without arthritis proved to be anti-MCV negative. We did not find an exclusive clinical feature which could characterize the anti-MCV positive PsA group, but the frequence of nail involvement was significantly higher (p=0.003) in those PsA patients who had anti-MCV antibodies.
Conclusions: According to our results, anti-MCV antibodies can be detected in a small proportion of patients with PsA, but their pathological significance needs further investigations.

M1:

ORIGINS OF HUMAN POPULATIONS

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Introduction: Fossil distribution and molecular genetics together illuminate human origins. The first hominins to leave Africa were Homo erectu,s about 2 Mya. A distinct form, Homo neanderthalis, appeared later in Europe. In Africa, modern man, Homo sapiens, also evolved about 200 Kya: around 70 Kya, these spread across the world, displacing all previous hominins.
Objectives: Does genetics confirm this story?
Materials & methods: Mitochondrial and Y chromosome sequencing: later, whole genome sequencing, even from ancient bones.
Results: Sequences frommitochondria and Y chromosomes show great diversity within Africa, lessoutside, consistent with a small population leaving and spreading. *Neanderthalis* mitochondrial DNA matchesno modern DNA. But *neanderthalis* hasleft traces in modern European or Asian, but not African nuclear DNA, as if *sapiens* had interbred with *neanderthalis* in western Asia. *Neanderthalis* had the modern allele ofFOXP2, associated with grammatical language; and up to 40% of the modernEurasian *sapiens* HLA-A alleles may be*neanderthalis.* Remains from Denisovain the Altai mountains yielded mitochondrial DNA, neither *sapiens* nor *neanderthalis*:and many Denisovan nuclear sequences survive in the genomes of modern humans inIndonesia and eastward. Again, the local Denisovan HLA-A antigens wereadvantageous: in south China, half or more of HLA-A may be Denisovan.
Conclusion: Are there othernon-*sapiens* elements in the moderngenome? Modern Chinese have shovel-shaped incisors, shared with Chinese Homo *erectus* from at least 500 Kya. Several alleles appear to have come into themodern lineage from an archaic ancestor quite recently. One such allele, in themicrocephalin-1 gene that affects brain development, may have come into theEurasian genome about 37Kya. West African and Bushman genomes may also containancient elements.

M2:

ARCHEOGENETICS; TO STUDY THE HUNGARIAN PAST

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In order to study the continuity in maternal and paternal lineages between ancient and modern Hungarian populations, polymorphisms in the mitochondrial DNA and binary Y chromosomal markers were analyzed from ancient (10th–11th centuries), and recent Hungarian, and Hungarian speaking Sekler samples.
This study is also concerned with the mitochondrial control region genotypes of archaeological horse remains, excavated from pre-conquest Avar and post-conquest Hungarian burial sites, dating from the 6th to the 10th century.
As far as maternal lineages are concerned the ancient Hungarian samples analyzed according to apparent social status, commoners show a predominance of mtDNA haplogroups, common in west Eurasia, while high-status individuals, show a heterogeneous haplogroup distribution, with some Asian affinities. Recent Hungarian-speaking populations seem to be specifically European.
The Y chromosome Tat C allele is distributed in all the Finno-Ugric-speaking populations, except recent Hungarians, whereas out of the seven ancient skeletal remains, two possess it. This indicates a Siberian lineage of the invading Hungarians which later disappeared. Recent Hungarian-speaking populations, share similar Y chromosome haplogroups described for other European populations, except high frequency of haplogroup J and the presence of the haplogroup P* in Szeklers.
Phylogenetic relationships among horse mtDNA haplotypes indicated a separation between horses of the Avars and the Hungarians and genetic relationship was found between early Hungarian and Akhal teke horses. Our results show that the ethnic changes induced by the Hungarian Conquest were accompanied by a corresponding change in the stables of the Carpathian Basin.

M3:

FOUNDATION PRINCIPLES OF INTERACTIONS OF NANOPARTICLES WITH CELLS AND BIOLOGICAL BARRIERS (INCLUDING THE BLOOD BRAIN BARRIER)

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Nanoscale materials can interact with living organisms in a qualitatively different manner than small molecules. Crucially, biological phenomena such as immune clearance, cellular uptake and biological barrier crossing are all determined by processes on the nanometer scale. Harnessing these endogeneous biological processes (for example in creation of new nanomedicines or nanodiagnostics) will therefore require us to work on the nanoscale. This ensures that nanoscience, biology and medicine will be intimately connected for generations to come, and may well provide the best hope of tacking currently intractable diseases.
Whilst nanoparticle size is important, the detailed nature of the nanoparticle interface is key to understanding interactions with living organisms. This interface may be quite complex, involving also adsorbed protein from the biological fluid (blood, or other), leading to a sort of 'protein corona' around the nanomaterial surface. We discuss how this corona is formed, and how it may be a determining feature in biological interactions.
We give examples of these interactions relevant to several systems, including the blood brain barrier (NeuroNano FP7) where some significant outcomes seem affected by the role of the protein layer.

M4:

GENE THERAPY FOR INHERITED DISEASES OF THE HEMATOPOIETIC SYSTEM: FROM BENCH TO THE BEDSIDE

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Thousands of rare diseases are caused by a Mendelian genetic error. So far, more than 1800 genes associated with rare diseases have been identified and, in many cases, their expression patterns and functions have been unravelled. This information is a prerequisite for development of a therapeutic strategy. Depending on the disease's severity, and by assessing feasibility and treatment alternatives, gene therapy can be viewed as an option in some instances.

Genetic disorders are phenotypically very heterogeneous. In considering the appropriate form of gene therapy, three basic parameters need to be established: whether a mutation leads to a loss or gain of function; whether or not a gene product's function affects cell survival or development; and the disease gene's tissue specificity.

The first clinical successes of gene therapy were noted more than 20 years after initiation of research into this technique show that the approach is feasible. Advances in vector design, stem-cell biology, and the prevention of immunogenicity are likely to prompt relevant clinical trials for genetic diseases in the future. Gene therapy should be considered as one strategy among others to combat genetic disease. In this context, it can be regarded as scientifically sound and worth developing.

This will be illustrated by the presentation of the gene therapy innovative protocols for the treatment of inherited diseases of the hematopoietic system conducted in Hospital Necker, Paris, France.

M5:

STEM CELL-BASED MODELS OF NEURODEGENERATIVE DISEASE

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The derivation of neurons from human pluripotent stem cells provides exciting prospects for modelling neurological disorders. A prerequisite for such studies are robust protocols that efficiently yield standardized populations of neural cell types. We have established the derivation of long-term self-renewing neuroepithelial stem (lt-NES) cells from human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSC). These lt-NES cells provide a continuous source of human neurons, which can be used to study key steps of neurodegenerative diseases such as Alzheimer's disease-associated amyloid formation or protein aggregation associated with polyglutamine (polyQ) disorders. We applied this strategy to Machado-Joseph Disease (MJD), a dominantly inherited late-onset neurodegenerative disorder caused by expansion of polyQ-encoding CAG repeats in the MJD1 gene. Proteolytic cleavage of the MJD1 gene product, ataxin-3 (ATXN3), is believed to trigger the formation of ATXN3-containing aggregates, the neuropathological hallmark of MJD. Our data show that L-glutamate-induced excitation of MJD-iPSC-derived neurons initiates Ca^{2+}-dependent proteolysis of ATXN3 followed by the formation of SDS-insoluble aggregates. This phenotype can be abolished by calpain inhibition, indicating a key role of this protease in ATXN3 aggregation. Aggregate formation was further dependent on functional Na^{+}- and K^{+}- as well as ionotropic and voltage-gated Ca^{2+}-channels and not observed in iPSC, fibroblasts or glia, thereby providing an explanation for the neuron-specific phenotype of this disease. Our data indicate that developmentally early hESC and iPSC-derived neurons provide a unique window of opportunity to study pathogenic driver mechanisms underlying late-onset neurodegenerative diseases in a pre-symptomatic phase preceding neuronal degeneration.

M6:

THE PATHOLOGY OF PRE-MRNA SPLICING: MECHANISTIC ASPECTS AND DEVELOPMENT OF NOVEL THERAPEUTIC CORRECTION STRATEGIES

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A significant proportion of disease-causing mutations affect pre-mRNA splicing inducing skipping of the exon from the mature transcript. To study the effect of mutations on splicing and identify a correction strategy for therapy, we considered three genes relevant in human pathology, the coagulation *F9*, the Cystic Fibrosis Transmembrane Regulator (*CFTR*) and the *SMN2* (Survival of Motoneuron 2), whose mutations are associated to coagulation factor IX (Haemophilia B), Cystic Fibrosis and Spinal Muscular Atrophy, respectively. In these genes substitutions that affect different splicing regulatory elements (at the donor splice sites, the polypyrimidine tract and exonic sequences) cause the disease inducing aberrant skipping of the exon from the mature transcripts. To provide a novel strategy to correct these different types of natural splicing mutations we developed for each system an Exon Specific U1 small nuclear RNAs (ExSpeU1) that binding by complementarity to intronic sequences downstream of the exons improves its definition. These molecules rescued different types of splicing defects associated to exon skipping and were active on several 5'ss and 3'ss mutations and on exonic substitutions. In cellular models of Haemophilia B and SMA, these molecules induced splicing correction of different types of mutations, thus resulting in several cases of a rescue of protein biosynthesis and functional activity. These results identify a novel therapeutic strategy based on ExSpeU1s to correct, in several human disorders, different types of splicing mutations associated with defective exon definition.

M7:

MECHANISMS OF STOCHASTIC GENE EXPRESSION

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Gene transcription is a stochastic process because most of the proteins required to regulate this process exist in small amounts. One of the best characterized examples of stochastic transcriptional activation is the virus infection- induced expression of the human IFN-b gene, playing a key role in mammalian antiviral response. Activation of the IFN-b gene is a biphasic process requiring three distinct sets of transcription factors bound to the enhancer in response to virus infection. During the early phase of virus infection, the limiting transcription factor NF-kB is captured by 3 defined genetic elements termed NRCs (NF-kB Reception Centers) in a small percentage of infected cells and subsequently it is delivered via interchromosomal interactions to a single IFN-b allele only, thus triggering enhanceosome assembly and monoallelic gene expression in this allele. The produced IFN-b protein amplifies the infection signal by stimulating expression of the IFN-b gene further from both alleles and in a larger fraction of cells. To identify additional genes activated by NRCs we used DNA microarray technologies and have identified 41 genes affected by NRCs. We performed DNA FISH experiments using probes for NRCs and these genes and showed that NRCs associate with all genes, and this association correlates with stochastic monoallelic expression. Remarkably, we found that each expressing cell organizes 2-4 NRC conglomerates in which many virus induced genes are recruited to receive NF-kB and initiate monoallelic gene expression. Single cell PCR analysis verified these data by showing that all NRC-regulated genes are expressed simultaneously in the same cell and in a stochastic manner. Taken together,

these experiments strongly suggest that stochastic patterns of gene expression are due to interchromosomal interactions in a small proportion of cells.

M8:

NUCLEAR RECEPTORS LINK LIPID METABOLISM TO GENOME EXPRESSION

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Introduction: Multicellular organisms have developed intracellular sensors in order to respond to the changing extra and intracellular lipid environment. Some of these proteins belong to the nuclear receptor superfamily. Typically, these receptors regulate the expression of hundreds of genes upon lipid ligand binding and via this change the cell's phenotype. We have been interested in identifying the genomic activity and regulatory role of some of these receptors in cells of the immune system.

Objectives: Using systematic gene expression, genome-wide localization and molecular biological methods we have mapped the activity of the Peroxisome-proliferator Activated Receptor gamma (PPARg) in macrophages and dendritic cells.

Materials & methods: We have used microarray analysis to identify regulated transcription, chromatin immunoprecipitation to identify genomic binding sites and functional assays to characterize cellular phenotypes.

Results: We have identified molecular pathways regulated by PPARg in response to fatty ligands which connect lipid metabolism and immune function in macrophages and dendritic cells. Moreover we have identified transcriptional interactions between nuclear receptors and cytokine mediated transcriptional processes which contribute to cell type specification and tissue specific gene expression.

Conclusion: It appears that nuclear receptors provide immune cells with a coordinated and interrelated network of transcriptional regulators for interpreting the lipid milieu and the metabolic changes to bring about gene expression changes leadingto subtype and functional specification. We will also show that these networks are implicated in various immune diseases and are amenable to therapeutic exploitation.

M9:

MOLECULAR AND CELLULAR MECHANISMS OF MEMORY ALLOCATION IN NEURONAL NETWORKS

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Although memory allocation is a subject of active research in computer science, little is known about how the brain allocates information within neural circuits. Until recently, however, the mechanisms that determine how specific cells and synapses (and not their neighbors) within a neural circuit are recruited during learning have received little attention. Recent findings from our laboratory suggest that memory allocation is not random, but rather specific mechanisms regulate where information is stored within a neural circuit. Our laboratory used a range of single cell manipulation and recording techniques to demonstrate that CREB activity regulates neuronal excitability and consequently the allocation of fear memory to specific cells in lateral amygdala. Our studies suggest that some of the mechanisms involved in the consolidation of one memory (e.g., CREB activation) affect the allocation of the next memory.

M10:

HISTONE ACETYLTRANSFERASES AFFECT TRANSCRIPTION THROUGH DIVERSE MECHANISMS

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Acetylation as a possible posttranslational modification form of histones was recognized and suggested to play a role in RNA biogenesis in the 1960s. In the 1990s the first transcriptional regulator proteins with histone acetyltransferase (HAT) catalytic activity have been discovered, and shortly followed that description of various HAT complexes. By today, thanks to the high throughput chromatin immunoprecipitation and sequencing techniques, detailed descriptions are available on the genome wide distribution of specifically acetylated forms of nucleosomal histones. Nonetheless, a clear cause and effect relationship between specific forms of histone acetylation marks and transcriptional responses has been established only in very few cases. The difficulties are primarily in the robustness and the dynamics of transcription regulation: one form of modification might be functionally substituted by a combination of other forms, and the detection of specific modification forms at different gene regions represents only still frames of the moving picture of transcription. During the last decade our laboratory studied the results of histone acetylation changes both in insect and mammalian models. Our results exemplify clearly that histone acetylation changes can cause changes both in global chromatin structure, or the contrary, can affect the transcription only a small number of selected genes. It is also evident from our data that at some cases related genes respond differently to similar changes of acetylation, and that cells in specific states can cope with abnormal alterations in nucleosomal histone acetylation levels.

M11:

FUZZY COMPLEXES: AMBIGUITY IN PROTEIN INTERACTIONS

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For long, protein complexes were thought to be defined by unambiguous, static contacts. Recent results, however, indicate that residues far outside the binding region can critically influence selectivity or binding affinity via transient, dynamic interactions, yet do not adopt a well-defined structure in the complex. This phenomenon is termed as fuzziness. The dynamic segments can modulate conformational preferences or flexibility of the interface, vary the spacing of the binding motif(s) or serve as a competitive partner. Post-translational modifications, additional interactions or alternative splicing of such structurally heterogeneous regions provide further means to regulate the activity of the complex and expand the functional repertoire of the proteins involved.

Using various recently characterized examples, I'll describe how fuzzy complexes form and the benefits of structural ambiguity in both protein-protein and protein-DNA interactions. I'll also review regulatory pathways within the transcription machinery involving fuzzy complexes. Finally some allostery-related questions will be addressed.

M12:

THE IDENTITY, PLASTICITY AND THERAPEUTIC POTENTIAL OF MESENCHYMAL STEM CELLS

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Postnatal mesenchymal stem or stromal cells (MSC) initially isolated from the bone marrow are also present in the stromal fraction of many other tissues and organs. They have attracted the attention of scientist and clinicians due to their availability, relatively simple recruitment for in vitro expansion, regenerative potential and immunomodulatory properties. Whereas the broad developmental plasticity of MSCs was originally thought to contribute to their demonstrated efficacy in wide varieties of experimental animal models of disease as well as in human clinical trials, new findings suggest that the ability of MSCs to induce tissue regeneration is mainly based on the secretion of a wide array of paracrine trophic- and anti-inflammatory mediators. It seems that there are bidirectional interactions between MSCs and immun/inflammatory cells that determine the outcome of MSC-mediated tissue repair processes. MSCs can interact with cells of both the innate and adaptive immune systems, i.e. with macrophages, dendritic cells, T- and B-lymphocytes, by inhibiting the release of pro-inflammatory cytokines and promoting the survival of damaged cells. Moreover, this immunosuppressive property has also made MSCs attractive candidates in the treatment of autoimmune disorders. Nevertheless, their origin and identity in vivo, possible heterogeneity, and functional roles in adult tissue homeostasis and in the regulation of immune responses in situ have remained enigmatic and are only just starting to be uncovered. So, clinical application of MSCs should be considered with caution at present.

M13:

ADVANCES IN MOLECULAR AND CELL THERAPY OF DUCHENNE MUSCULAR DYSTROPHY

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Duchenne muscular dystrophy (DMD) is the most common childhood genetic disease, affecting one in 3,500 newborn boys, causing progressive muscle weakness, heart and respiratory failure and premature death. This disease is caused by the mutations of the *DMD* gene, and exon skipping by antisense oligonucleotides (AOs) is a novel method to restore the reading frame of the mutated *DMD* gene, and rescue dystrophin expression. We recently reported that systemic delivery of AOs targeting exon 6 and 8 of the canine *DMD* gene to $CXMD_J$, a dystrophin-deficient animal model, efficiently restored functional dystrophin proteins at the sarcolemma of these dogs, and improved performance of affected dogs without serious side effects (Ann Neurol. 2009;65:667-76). To optimize therapeutic AOs for more frequent mutations of the *DMD* gene, we designed AOs targeting exon 51 of the mouse *DMD* gene, and intravenously injected them into mdx52 mice, where exon 52 has been deleted by gene targeting. We found expression of dystrophin at the sarcolemma throughout the body and amelioration of dystrophic phenotypes, including pathology and contractile force. (Mol Ther. 2010;18:1995-2005). This study provides a proof of concept for exon 51 skipping in the DMD animal model and that can be applicable up to 15% of DMD deletion patients. Indeed, clinical trials of exon 51 skipping for DMD patients have been carried out. There has been also considerable progress in understanding how AOs could be applied to the treatment of other neuromuscular diseases, including familial amyotrophic lateral sclerosis and Fukuyama-type congenital muscular dystrophy (Nature. 2011;478:127-31).

M14:

MEMBRANE TRANSPORTERS AND CALCIUM SIGNALING IN HUMAN PLURIPOTENT STEM CELLS

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Pluripotent stem cells represent a new source of biological material allowing the exploration of signaling phenomena during normal cell development and differentiation. Human embryonic stem cell lines, derived from inner cell mass of blastocyst-stage embryos, or induced pluripotent stem cells, generated from differentiated tissues, provide an unlimited source for these studies or even for large-scale drug screening in human tissues. However, until now membrane transporters and intracellular calcium responses have not been sufficiently explored in these cell types and in their differentiated offspring. This is partly due to the special culturing conditions of these cells, the rapid morphological and functional changes in heterogeneous cell populations during early differentiation, and methodological problems in expression and cellular calcium measurements. Here we present the exploration of the expression pattern for ABC multidrug transporter proteins in stem cells. We also demonstrate calcium signal measurements performed in human embryonic stem cells (hESC) and in their derivates, including mesenchymal stem cells and cardiomyocytes. In these studies we have compared the use of the fluorescent calcium indicator Fluo-4, and a cell integrated, stably expressed, genetically engineered, fluorescent intracellular calcium indicator protein. Our data indicate that in pluripotent stem cells both types of indicators can be efficiently used for ligand-induced calcium signal measurements. In contrast, in stem cell-derived, spontaneously beating human cardiomyocytes the application of a selectively overexpressed, uniformly distributed fluorescent calcium indicator protein has a major advantage, and allows reliable screening applications.

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M15:

GENETIC INFLUENCES ON SOCIAL COMMUNICATION

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Social communication skills are essential for everyday human interaction. When impaired, in a specific neurodevelopmental disorder such as Autism, the consequences for later mental health can be profound. Genetic (and possibly epigenetic) influences on social communication are important contributors to such dysfunction.
The genetic architecture of Autism Spectrum Disorders (ASDs) is highly heterogeneous. Early optimism that a few genes of major effect would be discovered has proved unfounded.
Recent research has shown that social-communication spectrum phenotypes in the general population are associated with the same common genetic variants that influence risk for ASDs. We have also learned that small structural variations in the genome, copy-number variations, are relatively common among those with ASDs but are also found, in equivalent regions, among people with quite different psychiatric disorders such as schizophrenia.
Within the past 12 months, the first evidence is appearing from exome sequencing projects on trios, families containing a child with an ASD. Interpretation of the data from such projects will prove challenging.

One objective is to identify potentially causal genes, from the many identified candidates, which lie on a neurodevelopmental pathway that could plausibly support the acquisition of social-communicative skills. Some progress is being made.
We are also beginning to investigate the use of induced pluripotent stem cells (iPSCs), which can be generated from human somatic cells, to study how in ASDs neuronal tissues and their functions could become dysfunctional. This exciting development complements other approaches, and offers the distant prospect of novel treatments.

M16:

PROFESSIONAL POLICY DEVELOPMENT IN GENETIC HEALTH CARE: THE CHALLENGE OF DISCERNING HOPES FROM HYPES

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Genetics and genomics have developed fast in the last decade, but have not revolutionized medicine, as some had expected. While translation of research findings to public health applications is lagging behind, direct to consumer offers of genetic testing have become available. The European Society of Human Genetics (ESHG) is concerned about the way in which commercial companies are currently introducing genetic tests into the market outside of the scope of the traditional healthcare system. At the same time, there is hope that whole genome sequencing and screening programmes will change the landscape of genetic testing in health care a positive way.
There is a paradox between the few genetic tests with proven clinical utility that tend not to be implemented in health care, on the one hand, and the DTC offer of all kind of tests, with or without clinical utility. Associations between genetic variants and disease risks of clinical relevance have been established, for instance for hereditary breast and ovarian cancer, colon cancer (FAP, HNPCC), cardiovascular disorders (familial hypercholesterolaemia, hypertrophic cardiomyopathy). These examples can be used to reflect on the possibilities in using the new genetics in public health. For the latter group of diseases cascade screening (inviting family members) is a very effective approach. To translate the research findings into appropriate clinical applications, such as genetic testing, assessment is needed, for which frameworks of criteria have been developed. The Public and Professional Policy Committee of ESHG stimulates policy development by generating recommendations involving experts, membership and board.

M17:

THE ACE-ING OF GENE- AND CELL THERAPY

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Introduction: Mammalian artificial chromosomes (MACs) are safe, stable genetic vectors, which do not integrate into the host cell's genom and have an unlimited transgene carrying capacity.
Objectives: The combination of MACs with stem cell-based technologies offers a quite novel strategy in gene and stem-cell-based therapeutic applications.
Materials & methods: Weload therapeutic genes onto our MAC system, which is called ACE (Artificial Chromosome Expression system), in a production cell line. Transgene expression and function is tested

in this cell line. The loadedtherapeutic ACE is then delivered into target cell lines to achieve therapeuticeffect (cell therapy).

Results: Presently, this approach is being applied to several type of devastating disease models, including cancer. The MAC technology is excellent to produce inducedpluripotent stem cells in a safe way and MACs could be used to establishvarious cell types for cell therapy.

Conclusion: Inthe mouse model of Krabbe's disease, we could increase the lifespan of mice upto five times with the combined artificial chromosome stem cell method. Currently, we are developing preclinical protocols to treat X-Scid and SMAdisorders and also to eliminate breast cancer in mice.

M18:

EVALUATION OF A PARTIAL GENOME SCREENING OF TWO ASTHMA SUSCEPTIBILITY REGIONS USING BAYESIAN NETWORK BASED BAYESIAN MULTILEVEL ANALYSIS OF RELEVANCE

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Genetic studies indicate high number of potential factors related to asthma. Based on earlier linkage analyses we selected the 11q13 and 14q22 asthma susceptibility regions, for which we designed a partial genome screening study using 145 SNPs in 1201 individuals (436 asthmatic children and 765 controls). The results were evaluated with traditional frequentist methods and we applied a new statistical method, called Bayesian network based Bayesian multilevel analysis of relevance (BN-BMLA). This method uses Bayesian network representation to provide detailed characterization of the relevance of factors, such as joint significance, the type of dependency, and multi-target aspects.

With frequentist methods one SNP (rs3751464 in the *FRMD6* gene) provided evidence for an association with asthma (OR=1.43(1.2-1.8); $p=3x10^{-4}$). The possible role of the *FRMD6* gene in asthma was also confirmed in an animal model and human asthmatics.

In the BN-BMLA analysis altogether 5 SNPs in 4 genes were found relevant in connection with asthma phenotype: *PRPF19* on chromosome 11, and *FRMD6*, *PTGER2* and *PTGDR* on chromosome 14. In a subsequent step a partial dataset containing rhinitis and further clinical parameters was used, which allowed the analysis of relevance of SNPs for asthma and multiple targets. These analyses suggested that SNPs in the *AHNAK* and *MS4A2* genes were indirectly associated with asthma. This presentation will indicate that BN-BMLA explores the relevant factors more comprehensively than traditional statistical methods and extends the scope of strong relevance based methods to include partial relevance, global characterization of relevance and multi-target relevance.

M19:

THE CANCER EPIGENOME

Peter Jones

USC Norris Comprehensive Cancer Center, Los Angeles, CA, USA

Abstract is not available

M20:

IMPLEMENTING PERSONALIZED CANCER MEDICINE

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Cancer genome projects have led to breakthrough observations in understanding the biology of cancers. However, for many cancer types, translating genomic advances to clinical treatment decisions remains a significant challenge. For example, for many potentially "actionable" gene mutations, candidate drugs are not available or remain under development for years to come.

Our project on acute myeloid leukemia (AML) aims to speed up the implementation of personalized medicine in the clinical setting. First, we acquire serial samples from AML patients at different stages of disease progression to better understand the key driver events and clonal evolution of cancer. Second, we profile the response of the cancer cells *ex-vivo* to a panel of 250 high-impact cancer drugs, including both chemotherapeutic agents, targeted clinical drugs and many emerging inhibitors. Drug response is determined over a 10,000-fold concentration range to derive quantitative IC50 values for each drug. Third, we perform exome and RNA sequencing as well as phosphoproteomic profiling of the samples. Fourth, an integrated database is developed to correlate genomic and signaling changes with *ex-vivo* patient drug response profiles. Fifth, we translate promising observations on approved drugs for individualized therapy optimization in the clinic.

Examples will be shown to illustrate the impact of this systems medicine strategy for individualized selection of therapy for patients with drug-resistant AML. We believe that combining many layers of cancer-omics data with comprehensive *ex-vivo* drug response data will provide a new way to personalize and optimize therapies for cancer patients.

M21:

HUMAN CANCER SYNDROMES: LESSONS LEARNED

Edith Oláh

Department of Molecular Genetics, National Institute of Oncology, Budapest, Hungary

More than 30 different hereditary human cancer syndromes have now been defined and attributed to specific germline mutations in various cancer genes. An individual who carries an inherited mutant allele in any of these cancer susceptibility genes has a variable risk of cancer that is influenced by the particular mutation, other genetic and epigenetic mechanisms, and also by dietary, lifestyle, and environmental factors. Though hereditary cancer syndromes are rare, their study has provided powerful insights into the origin and nature of more common forms of cancer. Hereditary breast/ovarium cancer and hereditary colorectal cancer are frequently associated with germline mutations of different genes in pathways critical to genomic integrity. Thus, BRCA1 and BRCA2 with inherited mutations are the key players in conferring hereditary predisposition to breast cancer. Germline mutations in any of the four mismatch repair genes (MLH1, MSH2, MSH6, and PMS2) and in EPCAM/TACSTD1 gene (Kovacs et al. 2009) are the underlying cause of Lynch syndrome, also known as hereditary non-polyposis colorectal cancer (HNPCC), the most common hereditary colorectal cancer syndrome.

Genetic testing of high-risk cancer susceptibility genes is becoming an increasingly important component of the clinical management of at-risk individuals. Yet, much controversy surrounds the estimation of cancer

risk. No susceptibility factor has yet been identified in about 30–50% of clinically well-defined HNPCC and breast cancer families.
The talk will bridge the information available on cancer predisposing genes/mutations with the potential impact on the efforts to effectively prevent, detect, and treat the hereditary forms of cancer.

M22:

MICROTUBULES, MOTORS, MRNAS: TRANSPORT OF *OSKAR* RNPS WITHIN THE *DROSOPHILA* OOCYTE

Imre Gáspár[1], Yanxun Yu[2], Michael Welte[2], Anne Ephrussi[1]
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Establishment of cellular polarity often depends on the underlying polarity of microfilaments and microtubules (MTs) and its interpretation by processive molecular motors. During *Drosophila* oogenesis, *oskar* mRNA molecules assemble into large non-vesicular ribonucleoprotein (RNP) complexes for their transport to the posterior pole of the oocyte. We have recently developed an *ex vivo* assay to study the microtubule dependent motion of *oskar* RNPs with proper spatiotemporal resolution, allowing one to decipher the core and often subtle events of RNP transport. Combining RNA localization studies, ultrastructural and biochemical analyses, and live cell imaging, we demonstrate that although the majority of RNP runs are kinesin heavy chain (KHC) dependent and directed towards the MT plus ends, cytoplasmic dynein is also present and functional on these RNPs and can oppose the action of KHC. Compromising dynein structure and function decreases *oskar* RNP transport efficiency considerably, by affecting plus end directed runs primarily. This effect appears to result from a reduction of KHC associated with *oskar* RNPs. Our recent findings show that proper regulation of the two motors in *oskar* RNP transport depends on Klarsicht. This protein appears to associate with *oskar* mRNA and regulate the motility of the RNPs, adapting the translocation of the mRNA mass towards the posterior pole to different temperatures. We conclude *oskar* RNP transport is an excellent model system for studying the action, regulation and adaptation of at least two different mechanoezymes associated with non-vesicular cargo in a developing organism.

M23:

SIGNAL TRANSDUCTION AND METABOLIC CONTROL OF CELL FATE

Utpal Banerjee
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Intercellular signaling controls cell fate, proliferation and survival. Our laboratory uses *Drosophila* as a model system to study how such pathways interact with metabolic pathways within the cell, including the role of the mitochondrion in the control of cellular functions. We have found that several signaling pathways have a dual function in development and in stress response. Such pathways often utilize metabolites, insulin, ROS, hypoxia, amino acids, nucleosides and neuromodulators as signaling molecules. How such pathways function during differentiation and stress response of blood progenitors will be discussed. Our laboratory is also interested in how cellular signaling pathways modulate metabolism. We have discovered that different conserved growth-promoting signals can have distinctly different effects on mitochondrial organization and metabolic function. Our goal is to understand how and why these signaling pathways modulate metabolism in normal development and disease states.

M24:

CONSERVED EPIGENETIC MECHANISMS CONTROL GENE SILENCING IN FUNGI, ANIMALS AND PLANTS

Gunter Reuter

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Components of protein complexes controlling chromatin differentiation and epigenetic programming were first identified in Drosophila with the help of position-effect variegation Su(var) and E(var) mutations. In Drosophila the coordinated activity of histone demethylases, deacetylases and methyltransferases controls differentiation of alternate chromatin states at the end of cleavage when nuclei differentiate an apico-basal polarity. The histone H3K4me1/me2 demethylase SU(VAR)3-3 (DmLSD1) plays a pivotal role in heterochromatin differentiation whereas the H3K4me3 demethylase LID is essential for euchromatin differentiation. For both enzymes homologues are found in fungi, plants and mammals. In the model plant Arabidopsis thaliana we developed an assay system for transcriptional gene silencing (TGS) which is based on T-DNA transgenes containing tandem repeats of the Luciferase reporter gene expressed under the control of the CaMV 35S promotor. Genetic, immunocytological and ChIP analysis suggest that silencing of the Luciferase transgene repeats is caused by heterochromatisation. Form 98 EMS induced TGS suppressor mutants isolated the mutation sites of 22 mutants defining 13 different genes were determined. Genetic and molecular analysis of the TGS suppressor mutations revealed a molecular pathway of interdependent reactions controlling heterochromatin formation and gene silencing in Arabidopsis. In additon, the maize pathogene Colletotrichum graminicola represents a useful fungal system for epignetic analysis. By systematic knock-out analysis we identified the epigentic factors controlling heterochromatin formation in this fungus. Similarities and differences in processes controlling heterochromatin differentiation and gene silencing in the animal, plant and fungal systems will be discussed.

M25:

INDUCED PLURIPOTENT STEM CELLS TO CREATE 3D NEURONAL TISSUE MODELS

András Dinnyés

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Induced pluripotent stem cell (iPSC) technology directly reprogramming somatic cells to a pluripotent state allows the generation of patient-derived pluripotent cells for disease modeling, drug screening, toxicology tests and, ultimately, autologous cell-based therapies. Our team has created the first mouse and human iPSC cells in Hungary in 2009/2010. Recently, we have reprogrammed somatic cells to pluripotent stem (iPS) cells by introducing reprogramming factors (Oct4, Sox2, Klf4 and c-Myc) encoded in lentiviral plasmid constructs. We have excised the integrating reprogramming cassettes from the genome using the Cre/loxP system thereby giving rise to transgene-free iPS cells. These pluripotent cells are able to differentiate into neuronal precursor cells (NPC) that can produce 2D and 3D neural tissue. Furthermore, we have generated engineered neuronal tissue (ENT) by first differentiating mouse ES and iPS cells into NPC and then by culturing the cells at an air-liquid interface on a membrane for 3 weeks. Results have shown neural differentiation as judged by BIII-tubulin positive cells, and a few, sparsely located GFAP-immunoreactive astrocytes. Overall, the iPSC based methods are developing rapidly and novel cell based assays and clinical

trials with the derived neuronal, cardiac and other cell types demonstrate the validity of the approach for further research and product development.

This work was supported by Grants from EU FP7 ("PartnErS", PIAP-GA-2008-218205; "InduStem", PIAP-GA-2008-230675; "PluriSys" HEALTH -2007-B-223485; "STEMCAM" PIAP-GA-2009-251186; AniStem, PIAP-GA-2011-286264; EpiHealth, FP7-HEALTH-2011-278418; Resolve, FP7-Health-F4-2008-202047) and BONUS HU_08/2-2009-0008, BONUS HU_08/2-2009-0002.

M26:

CONTROL OF EGF RECEPTOR ACTIVITY AND AXIS ESTABLISHMENT IN DROSOPHILA OOGENESIS

Gertrud Schupbach

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In Drosophila the establishment of the anterior-posterior and dorso-ventral axis of the egg and embryo involves signaling between the oocyte and the surrounding follicle cells. Restricted activation of the EGF receptor in the follicle cells by a spatially localized ligand, Gurken, is crucial for this process. Analyzing mutations that affect anterior-posterior axis formation, we have found that the Notch signaling pathway has to interact with the EGF receptor pathway to pattern the posterior follicle cells. The posterior follicle cells then send a signal back to the oocyte that polarizes the cytoskeleton of the oocyte and establishes both the anterior-posterior and the dorso-ventral polarity of the egg and future embryo. We found that the V-ATPase, a multiprotein complex, that is necessary for the acidification of intracellular vesicles, is necessary for Notch activity. Loss of V-ATPase activity leads to patterning defects that can be rescued by expressing the fully cleaved intracellular form of Notch. V-ATPase activity is also required in a mammalian cell culture model for Notch activity. The anterior posterior patterning defects caused by the mutations in V-ATPase highlight the interplay between EGF receptor signaling and the Notch pathway in anterior-posterior axis formation in the developing Drosophila egg chamber.

M27:

FUNCTIONAL ANALYSIS OF THE DROSOPHILA EMBRYONIC GERM CELL TRANSCRIPTOME BY RNAI

Miklós Erdélyi

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Two basic cell types exist in higher organisms: germ cells and somatic cells. Somatic cells have adhesive properties, build tissues and organs, and are genetically inert while germ cells form an immortal cell line allowing maintenance of the species. One of the most interesting questions of developmental biology is how germ cells differentiate from the somatic cells during embryogenesis. *Drosophila melanogaster* provides a powerful experimental system for the genetic dissection and *in vivo* analysis of the early germ line development. To identify and functionally characterize genes involved in embryonic germ line development we performed an RNAi-based functional genomic screen combined with fluorescent *in vivo* video microscopy. For the systematic analysis of the embryonic germ line transcriptome, more than five hundred germ line-localized transcripts were selected by using information of the gene expression databases. Gene

specific double stranded RNAs were injected into embryos and defects in germ line development were monitored by automated *in vivo* imaging. Altogether ca. 110.000 embryos were injected, 14.000 movies were generated and analyzed. By this method, 55 genes were identified to be involved in the development of embryonic germ cells. Phenotypic categories were established and the identified genes were ordered by hierarchical clustering into five gene clusters of similar complex phenotypes. Silencing effects of 37 out of the 55 genes examined could be confirmed by testing their mutant alleles in a genetically sensitized background. Detailed cell biological analysis of the germ cell specific function of the *pbl*, *feo* and *mei-P26* genes has been performed so far.

M28:

POKING MICROTUBULES BRING ABOUT NUCLEAR WRIGGLING TO POSITION NUCLEI

János Szabad

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As reported for several cultured cell types and some neurons of the zebra fish embryo, nuclei wriggle in the follicle epithelium of the Drosophila pre-vitellogenic egg primordia. Wriggling means the succession of sudden and random minor turnings of the nuclei, about three sudden twists in a minute with roughly 12 per twist. Wriggling is strictly dynamic microtubule-dependent and is brought about by the growing microtubules that, while poke the nuclei, buckle and exert forces. Poking seems to happen at the nuclear pore complexes. While wriggling, the nuclei drift about 5 μm in a day along the apical-basal axis in the growing follicle cells. An over two fold excess of the microtubules nucleated in the apical cell region, as compared to those originating in the basal cell cortex, achieve drifting of the nuclei along the apical-basal axis. Wriggling and drifting of the nuclei cease simultaneously when the nuclei become anchored by the actin cytoskeleton. Disturbed microtubule functions lead to failed nuclear positioning. It appears that the wriggling nuclei reveal a thus far not described nuclear positioning mechanism.

M29:

WONDER DEER, ANTLER, OSTEOPOROSIS

László Orosz

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The mythological Hungarian Wonder Deer, surrounded with amazement, is the red deer, *Cervus elaphus.* This royal game develops record trophies in the Carpathian Basin. The annually grown and shed antlers often reaches 30% of the skeleton in weight and display the fastest tissue growth in the animal kingdom. Its calcification within a short time results temporary physiological osteoporosis in the skeleton of the stag. Upregulated genes (36) were identified that were behind the robust growth of the developing („velvet") antler tip: 5 genes linked to tumor biology clearly outlined (ie. strongly silenced in tumors, highly upregulated in antler), 6 were negative regulators (in *Fgf, Tgfb, Wnt* pathways). One positive regulator (*C21orf70*) was discovered. The rest linked to high metabolic demands. In the ossified part of the„velvet" antlers the very high (10-30 fold or more) upregulations along the *Runx2-(Osx,Oc)* controlled (epi)genetic pathway of calcification were recorded in antler (vs. skeleton). Osteoporosis as a phenotype appeared along the evolutionary lineages of both deer and human (separated ca. 85 my ago): „physiological" in the healthy deer for the reproductive success, pathological in human after the reproductive age in the industrialized countries. Gene (orthologous)

expressions „proposed by the deer" separated perfectly the osteoporotic patients from non-osteoporotics. The high discriminatory power of 5 *Wnt* genes as well as *FKBP2* and *TMSB4X* were disclosed (none this two were known to be linked to bone development). The Wonder Deer supports that comparative genomics may provide new targets for biomedical applications. (see MGG 277, 281, 284).

M30:

A ROLE FOR EUROPE IN GENETIC SERVICE PROVISION

Jörg Schmidtke

Hannover Medical School, Hannover, Germany

Under the European Community Treaty, EU action aims at improving public health, preventing human illness and diseases, and identifying sources of danger to human health. Accordingly, the EU health strategy focuses on strengthening cooperation and coordination, supporting the exchange of evidence-based information and knowledge, and assisting national decision-making. This is also true for genetic services, which have acquired a strong European dimension, in particular in the field of rare diseases, now recognised as a major public health issue. This lecture gives an overview on eight areas of European governance, at the levels of the Council of Europe, the European Union, and professional self-organisation, namely: professionalization processes, professional education, public education, rare disease networks, quality assurance, information platforms, basic and translational research, and outreach to low and middle income countries. It emphasizes the role, in particular, of the European Society of Human Genetics and highlights its special activities towards recognition of Clinical Genetics, Laboratory Genetics and Genetic Counselling as European professional specialties, with regard to public and professional education and training, as well as professional policies in many areas of individual-, family- and community-oriented health care in genetics.

M31:

EUROGENTEST: THE WAY FORWARD TO QUALITY GENETIC SERVICES

Jean-Jacques Cassiman

Center for Human Genetics, KU Leuven, Belgium

Genetic services face an ever-increasing number of requests for support, while widespread susceptibility testing and pharmacogenetic tests are lurking on the horizon.

To provide efficient and high quality services to the European population it will be necessary to increase the investment in research, to structure, harmonize and improve the overall quality of these services, while paying special attention to issues resulting from testing including legal, health and health economic impact, IPR (Intellectual Property Rights), ethical and social questions.

In addition the organization and harmonization of external quality assessment schemes will need to be improved and support will have to be provided to the accreditation of the genetic services. Collaboration between academic centers and the private sector on technology development and the validation of genetic tests should generate more rapid translation and more accurate, economical and better testing technologies. The help of Parent and Patient Support Groups, in this effort will not only be very valuable but essential. These are precisely the issues on which the EuroGentest NoE/Co is trying to contribute.

Since its initial start in 2005 as an NoE, funded under FP6, EuroGentest has become a 'Trade mark' for quality European genetic services and has created an unprecedented movement in Europe towards improved quality

of the services. Many of the activities and achievements contribute to the transfer of research results into diagnostics and create the conditions to make sure this can continue in the future so that citizens would benefit rapidly and efficiently from the progress in genetic research.

M32:

TIME TO TAKE TIMING SERIOUSLY IN HUMAN GENETICS

György Kosztolányi

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In a recent work we recommended to extend the approach to genetic disorders with developmental aspect of diseases which, in contrast to the transgenerational understanding of genetics, focuses on how the genome assembled in the zygote controlls the formation of a new individual and how this becomes gradually manifest during the lifespan. One of the most significant characteristic of this aspect is epigenetic modification, for which timing of the environmental factor is of fundamental importance. There are at least two reasons why timing in genetics should be taken seriosly. First, the time when *disease-causing de novo mutations* occur during development contributes significantly to the phenotype. Timing of these postzygotic genetic events may substantially contribute to the genotype-phenotype correlation. It is also well known that during lifespan enormous number of somatic gene mutations occur in various tissues leading to cancer. Second, knowledge is accumulated suggesting that *gene expression* in living cells changes along time. The temporal patterns of gene expression in mammalian cells is largely unknown, although some recent microarray studies could demonstrate gene expression dynamics concluding that expression timing plays important role in cellular responses to cope with an environmental perturbation. It is suggested that timing of genetic events in human organisms, the forth dimension of life, should be incorporated more fully into our understanding of the role of genetics in health and disease. Developmental aspect of genetic diseases has to be taken more seriously not only in explaining genotype phenotype correlation, but also in our scientific studies.

M33:

ORGANIZATION OF CARE FOR GENETIC DISEASES IN A DIVERSE EUROPE

Milan Macek

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EU2 member states policies and actions in the field of rare disease (RD), which comprise majority of genetic diseases, are rapidly evolving. Currently several countries have taken action to adapt their health care system to meet the needs of the RD patient community, or plan to do so. With regard to centres of expertise (CE), there are three categories of countries: 1) those which have a policy regarding RD and have CE within this framework (DK, FR, IT, NO and SE); 2) those which have established CE, though not specifically for RD (BE, HR, CR, SF, GR, IRL, PT, UK) and 3) those which have no CE with this denomination, although they have centres with all the characteristics of a CE. Genetic tests are offered internationally, through both public and private sector genetic testing services. Currently, 956 laboratories offering tests for 1,559 genes are registered with Orpha.net in Europe at large. The test offer differs greatly from one large country to another: Germany (1,141 genes), France (874 genes), Italy (625 genes), Spain (582 genes), the UK (414 genes). The test offer in medium and small-sized countries ranges from 1 to 233 genes. According to available data, only testing for cystic fibrosis is provided by every country. This situation explains the large cross-border flow of specimens,

highlighting the need to provide access to services in other countries when necessary, especially for very rare diseases. Legal and financial issues concerning cross-border testing are not yet fully addressed.

M34:

RARE INHERITED DISEASES AND NEWBORN SCREENING IN SZEGED, HUNGARY

Sándor Túri

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In 2009, the EU Council Recommendation on Rare Diseases identified rare diseases (i.e.: a life-threatening or chronically debilitating condition affecting not more than 5 in 10.000 persons in the Community) It is estimated that between 5 000 and 8 000 distinct rare diseases exist today, affecting between 6-8% of the population in total. In other words, between 27 and 36 million people in the European Union are affected by a rare disease. Twenty-five percent of patients reported waiting between 5 and 30 years from the time of first symptoms to a confirmatory diagnosis of their disease. Forty percent of patients were initially misdiagnosed leading to severe consequences such as inappropriate medical interventions.

Forty years ago, the Hungarian PKU screening began in our Pediatric University Department. Over the years additional disorders were added, such as galactosemia, biotinidase deficiency, congenital hypothyreoidism altogether 26 rare but treatable diseases. The method of screening is mass spectrometry on the first 3 days of life in every newborn of the country. The goal of the Treatment and Research Centre for Rare Diseases is to provide optimal treatment for patients with rare diseases, to investigate rare diseases in national and international networks and to promote cooperation across the professions. There is probably no other area in health where the collaboration between the 27 different national approaches can be as efficient and effective as rare diseases.

M35:

GENETICS OF ROMANY PEOPLE

Béla Melegh

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The Roma (Gipsy) people represent a unique and colorful population of the word: they do not belong to one single nation state; they have numerous different languages, belong to various social, cultural, and religious groups. They have dispersed around Europe, and are often permanently migrating populations, but having no written history or genealogy, their origin and migration during the nomadic periods of their history remain unknown. Their population size is estimated to be in the 10-15 million range, mainly in Central and predominantly in Eastern-Europe; their population's growth rate is higher than that of the surrounding majorities. The geographically dispersed Roma populations, often referred to as the "invisible minority", have been socially marginalized and historically often persecuted. They differ genetically from the major EU populations in several aspects. They have unique diseases with specific founder mutations, including specific neuromuscular phenotypes. Comparing to other populations, we found unique signatures in pharmacogenetically relevant SNPs and haplotypes in VKORC, MDR1, and various CYP metabolizing systems in Roma population samples. We analyzed data from six Roma groups that were genotyped at hundreds of thousands of SNPs, and confirmed that the Roma have shared ancestry with Europeans and South Asians. We estimate that the Roma harbor ~84% European ancestry with an average estimate of about 27 generations or 800 years for an admixture date of the ancestral groups, consistent with the arrival of the Roma in Europe from India.

M36:

GERMLINE AND SOMATIC MUTATIONS IN MELANOMA PATHOGENESIS

Márta Széll

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Melanoma is considered the most lethal form of skin cancers because it can rapidly spread to the lymphatic system and to the internal organs, therefore the early recognition of the lesions is pivotal in its management. This is why it is very important to identify, screen and follow the risk population. Approximately 10% of melanoma cases are considered as familial melanoma and the major locus for melanoma predisposition is the cell-cycle regulatory CDKN2A gene located on chromosome 9q21. The lecture will present the functional characterization of a novel CDKN2A intronic mutation, while the review of another case will demostrate that the detection of the melanoma-predisposing CDKN2A mutation may not be sufficient for the unambiguous identification of the patients at risk. Beside melanoma-predisposing germline mutations, somatic mutations of melanoma lesions gain great importance nowadays, since their identification is pivotal for the novel personalized management of the disease. In the second part of the talk this breakthrouh in melanoma care will be discussed.

O-M1:

GENETIC TESTING OF ADULT-TYPE HYPOLACTASIA IN PRESENT-DAY AND ARCHAIC SAMPLES

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Introduction: The prevalence of adult-type hypolactasia varies ethnically and geographically among populations. A C/T–13910 single nucleotide polymorphism (SNP) upstream of the lactase gene is known to be associated with lactase non-persistence in Europeans.

Objectives: We determined the prevalence of lactase persistent and non-persistent genotypes in current Hungarian-speaking populations and in ancient bone samples of classical conquerors and commoners from the 10th–11th centuries from the Carpathian basin.

Materials & methods: 181 present-day Hungarian, 65 present-day Sekler, and 23 ancient samples were successfully genotyped for the C/T_{-13910} SNP by the dCAPS PCR-RFLP method. Additional mitochondrial DNA testing was also carried out.

Results: In ancient samples the prevalence of hypolactasia was 87%, while 39% in present-day Hungarian and 29% in present-day Sekler samples. The distribution of C/T_{-13910} genotypes among Hungarian conquerors differed significantly from that of present-day Hungarians ($p=0.0002$) and Seklers ($p<0.0001$), respectively. In ancient Hungarians, the T_{-13910} allele was present exclusively in commoners of European mitochondrial haplogroups who may have been of pre-Hungarian indigenous ancestry. To interpret our results correctly, we favoured a multidisciplinary approach, including data on genetic testing of mtDNA, Hungarian history, and direct and indirect evidence from archaeology and ethnography.

Conclusion: The relative absence of lactase persistent genotype may be explained by the Hungarian use of fermented milk products, their greater consumption of ruminant meat than milk, cultural differences, or by their having other lactase-regulating genetic polymorphisms than C/T_{-13910}. This provides additional information on the Asian origin of ancient Hungarians, while present-day Hungarians have been assimilated with the surrounding European populations.

O-M2:

EFFECTS OF PACAP ON CHONDROGENESIS IN HIGH DENSITY MESENCHYMAL CELL CULTURES

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Introduction: Pituitary adenylate cyclase activating polypeptide (PACAP) was originally extracted from ovine hypophysis and later it was also detected in endocrine glands, gastrointestinal tract and gonads. We do not have any evidence of its function in skeletal elements such as hyaline cartilage.

Objectives: We attempted to clarify the possible role of PACAP in cartilage formation and its function during oxidative stress of chondrifying cells.

Materials & methods: Chondrogenesis was investigated in high density cell cultures established from chondrogenic cells of the limb buds of chicken embryos.

Results: mRNA and protein expressionof PACAP and PAC1 receptors were detected. Administration of PACAP1-38 and 6-38 increased cartilage formation equally. Both forms compensated the chondrogenesis-inhibiting and antiproliferative effects of 4 mM H_2O_2. PACAP proteins increased the mRNA and protein expression of PKA, ERK1/2 and P-ERK1/2 either with or without oxidative stress. We also demonstrated that addition of PACAP proteins increased the mRNA and protein expression of Ca^{2+}-calmodulin dependent Ser/Thr protein phosphatase calcineurin and its downstream target, NFAT4. PACAP proteins prevented the reduction of calcineurin activity during oxidative stress.

Conclusion: In chondrifying experimental model, we demonstrated the presence of PACAP and its receptor. Administration of PACAP1-38 elevated cartilage production as do so the PACAP6-38 although it is said to be anantagonist of PAC1 receptor. Our results also give evidence that calcineurin plays important role in the regulation of PACAP signaling pathways.

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O-M3:

PRMT1 AND 8 CONTROL CELL FATE SPECIFICATION OF DIFFERENTIATING EMBRYONIC STEM CELLS VIA SELECTIVELY TUNING RETINOID-INDUCED GENE EXPRESSION

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Introduction: Arginine methylation is a prevalent post-translational modification that is mediated by the protein arginine methyltransferase (PRMT) family. Arginine methylation has been linked to neuronal differentiation and cell type specification. A comparison of the recently identified mammalian PRMT members revealed that PRMT1 and PRMT8 share the highest degree of identity. Moreover, catalytic activity and substrate specificity of PRMT8 is very similar to that of PRMT1. PRMT1 is involved in transcriptional activation of nuclear receptors, including RAR, PXR, FXR, HNF4a.

Objectives: In the recent study we investigated how PRMT1 and 8 can regulate retinoic acid (ATRA) driven gene regulation in the context of stem cell differentiation.
Materials & methods: Retinoic acid (ATRA) induced neural differentiation of mouse embryonic stem (ES) cells was used as a model system.
Results: We demonstrate that PRMT1 is expressed in ES cells and also in the differentiated cells. PRMT1 knockdown cells show increased retinoid responsiveness in a gene selective manner, mainly affecting the Hoxa and Hoxb clusters, suggesting that PRMT1 acts as a gene specific co-repressor. In contrast, PRMT8 is not expressed at the early stage of differentiation, but appears after ATRA treatment, and becomes highly expressed in differentiated neurons. Surprisingly, loss of PRMT8 results decreased retinoid responsiveness, implicating it as a coactivator.
Conclusion: These results provide evidence for a collaborative fine-tuning mechanism for PRMT1 and 8 in retinoid signaling. We will also show details how PRMT1 and 8 contribute in retinoic acid induced embryonic stem cell differentiation.

O-M4:

HYDROGEN PEROXIDE, A PROOXIDANT IN OXIDATIVE PROTEIN FOLDING

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Introduction: Oxidative protein folding in the luminal compartment of endoplasmic reticulum (ER) is thought to be accompanied by the generation of H_2O_2, as side-product of disulfide bond formation.
Objectives: We aimed to examine the role of H_2O_2 produced in the lumen, which on one hand can lead to redox imbalance and hence can contribute to ER stress; on the other hand, as an excellent electron acceptor, H_2O_2 might serve as an additional prooxidant in physiological oxidative folding.
Materials & methods: In our in vivo model, we overproduced H_2O_2 in the ER by treating mice with gulonolactone, which is converted to ascorbate and H_2O_2 intraluminally. In a cellular model, we have used professional secretory plasmacells infected with ER-targeted H_2O_2 eliminating systems to study the consequences of peroxide elimination produced by folding.
Results: Elevation of hepatic luminal H_2O_2 levels of mice resulted in a decrease in microsomal GSH and protein-thiol contents and in a redox shift of certain luminal oxidoreductases. It was accompanied only by moderate signs of ER stress and reversible dilation of ER cisternae, all effects were prevented by concomitant reducing treatment. Antibody producing cells artificially engineered with powerful luminal H_2O_2 eliminating system showed diminished secretion of mature antibody polymers, while incomplete antibody monomers/dimers were accumulated and/or secreted.
Conclusion: The results indicate that local H_2O_2 production promotes, while quenching of H_2O_2 impairs disulfide formation. The contribution of H_2O_2 to disulfide bond formation previously observed in vitro can be also shown in cellular and in vivo systems.

O-M5:

TARGETING THE BREAKPOINT IN DUCHENNE MUSCULAR DYSTROPHY

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Introduction: The process of DNA targeting within the cell - induced by specifically positioned double stranded cleavage of DNA near the mutated sequence - can be applied for gene therapy of monogenic diseases, such as DMD.

Objectives: For this purpose highly specific artificial nucleases have to be developed. The present zinc-finger nucleases exert a minor cytotoxicity, which must be avoided in therapeutic applications [E.M. Händel, T. Cathomen, Curr. Gene Ther. 11, 28 (2011)]. The HNH motif - a conserved beta-beta-alpha-metal-binding structure [J. Orlowski, J. M. Bujnicki, Nucl. Acids Res., 36, 3552 (2008)] comprises the active centre of numerous nucleases. Since it functions under positive allosteric control, it may serve as a catalytic centre of new type of zinc-finger nucleases.

Materials & methods: PCR, the tools of the recombinant DNA technology and physicochemical methods were applied in the experiments.

Results: We developed a method for approaching the mutation sequence in the largest human gene, responsible for DMD. A zinc finger protein was designed using semiempirical method to target the determined DNA sequence in a DMD patient. It was tested for specific binding in in vitro experiments. Furthermore, the conditions of the function of HNH motif from Colicin E7 were investigated in order to apply it for a possible active centre of a specific artificial nuclease.

Conclusion: The results show the possible direction of the development of the new artificial nucleases for gene therapy of monogenic diseases.

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O-M6:

ANTI-INFLAMMATORY EFFECTS OF MOUSE MESENCHYMAL STEM CELLS ON MICROGLIA

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Introduction: The immunosuppressive and anti-inflammatory properties of mesenchymal stem or stromal cells (MSC) have been demonstrated on a wide range of innate and adaptive immune cells.

Objectives: It is well-known that neuroinflammation plays a crucial role in both pathogenic and regenerative processes of the brain and is largely mediated by microglia and astrocytes, therefore we investigated the effects of mouse bone marrow-derived MSCs on microglia.

Materials & methods: We prepared primary mixed glia cell cultures and then selectively isolated primary microglia cells, co-cultured with MSCs and investigated the phagocytic and antigen presenting capability of microglia and the attendant production of pro-and anti-inflammatory factors in the presence or absence of bacterial endotoxin lipopolysaccharide (LPS).

Results: We found that MSCs in co-cultures and also in transwell cultures inhibited the activation of microglial cells and changed the ratio of the secreted pro-inflammatory tumornecrosis factor-a (TNF-a) and anti-inflammatory IL-10 in the supernatants. In addition the phagocytic activity of microglial cells were significantly increased by MSCs. The MSCs also enhanced the antigen presenting capability of microglia and induced morphological changes in these cells. Based on transwell experiments we hypothesize that the cross-talk between these cells is mainly but not exclusively mediated by soluble factors, including prostaglandin E2 (PGE2).

Conclusion: These findings underline the theory that in the presence of MSCs microglia cells undergo alternative activation and gain M2-like (anti-inflammatory) phenotype similarly to peritoneal macrophages. In summary, the observed beneficial effects of MSCs may have relevance to treatment strategies for inflammatory diseases of the central nervous system, apparently by alternative activation of microglia.

O-M7:

TRANSCRIPTIONAL INTERFERENCE NETWORKS COORDINATE GLOBAL GENE EXPRESSION

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Introduction: The recent discovery of non-coding RNAs has changed our view on the regulation of gene expression. Here we put forward a new concept called the 'Transcriptional Interference Network (TIN) Hypothesis' for explaining the genome-wide occurrence of tandemly-oriented genes and the overall expression antisense transcripts. We propose that genes are organized into Genetic Modules composed of genes overlapping each other in both parallel and convergent fashions. The Waterfall Model may explain the one-way inhibitory effect of upstream genes on the downstream genes within a nested gene cluster through transcriptional interference. On the other hand, convergently-positioned genes exhibit a mutually interdependent way of interaction, which is explained on the basis of the Seesaw Model. This model is based on the confrontation of the transcriptional machineries of convergent genes at the overlapping regions.

Objectives: Our aim is to investigate the inhibitory effects exerted by the transcriptional interference mechanisms. Pseudorabies virus (PRV) is used as a model organism for this research.

Materials & methods: Homologous recombination has been applied for the genetic modification of PRV. Real-time RT-PCR has been used to quantify the amount of viral transcripts.

Results: We have detected a genome-wide expression of antisense transcripts. Furthermore, we have shown an interdependent expression kinetics between convergently overlapping transcription.

Conclusion: Transcriptional interference networks provide a straightforward genetic algorithm for an automatic mechanism, which results in a tightly controlled gene expression profile within a genomic locus composed of functionally-linked genes. These mechanisms are supposed to control the embryogenesis, cell differentiation, cell signaling, metabolic pathways, viral replication, etc.

O-M8:

COLORECTAL CANCER EPIGENETICS: CHARACTERISTIC DNA METHYLATION PATTERN UPSETS ADENOMA-DYSPLASIA-CARCINOMA SEQUENCE AT THE EPIGENETIC LEVEL

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Introduction: DNA methylation plays a crucial role in the carcinogenesis of the proximal colon, however in the distal colon it has scarcely been studied.
Objectives: Our aim was to study DNA methylation in multiple genes during adenoma-dysplasiacarcinoma sequence (ADCS) in the distal colon and to identify a characteristic methylation pattern. Our further aim was to correlate DNA methylation levels with mRNA and protein expression levels.
Patients & methods: For DNA methylation analysis 19 healthy colonic, 5 low-grade dysplasia (LGD), 5 high-grade dysplasia (HGD), 10 colorectal cancer (CRC) and 5 ulcerative colitis (UC) samples were endoscopically removed from the distal colon. DNA methylation percentages of 96 genes were determined using Methyl-Profiler PCR array system. For mRNA expression analysis 49 healthy colonic, 25 LGD, 24 HGD and 24 CRC samples were applied for Affymetrix Whole-Genome Expression Profile Microarray. Immunohistochemistry (IHC) for MLH1, MSH2, MSH6, DNMT 1, 3a, 3b and SFRP1 were performed.
Results: 8 genes were hypermethylated in all samples, additionally 34 genes were hypermethylated in LGD, 50 genes in HGD and 30 genes in CRC. A characteristic panel of 10 hypermethylated genes significantly distinguished LGD, HGD and CRC from UC and normal tissue ($p<0.05$). *SFRP1*, *SLIT2* and *MAL* methylation levels inversely correlated with mRNA expression. DNMT3a correlated with overall methylation, showing strongest expression in LGD and HGD. All samples were microsatellite stable (MSS).
Conclusion: Sporadic, distal, MSS CRC has a characteristic methylation pattern. Precancerous lesions have more epigenetic alterations as compared to CRC and in this sense ADCS might be a non-sequential process at the epigenetic level.

O-M9:

ANTI-TUMOR EFFECTS OF PEPTIDE ANALOGUES TARGETING NEUROPEPTIDE HORMONE RECEPTORS IN RODENT PHEOCHROMOCYTOMA CELLS

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Introduction: Malignant pheochromocytoma is a rare but potentially lethal chromaffin cell tumor with a particularly poor prognosis and currently no effective treatments.
Objectives: The targeted therapeutic approach we are pursuing here is based on our previous microarray and RT-PCR analyses, revealing altered expression of neuropeptide hormone receptors in adrenomedullary tumors and cell lines. Additionally, our work in tumor cell lines of both the adrenal cortex and medulla has shown a significant reduction of cell proliferation and survival as well as an increase in apoptosis and necrosis by employing peptide analogues by employing peptide analogues that specifically bind to their expressed receptors.
Materials & methods: We could demonstrate mRNA and protein expression of sst2, GHRH-R and LHRH-R on the parental mouse pheochromocytoma cell (MPC) line as well as on the malignant mouse tumor tissue-

derived (MTT) cell line. Additionally, employing various agonists and antagonists for respective peptide receptors we could demonstrate significant reductions of cell proliferation and increases in tumor cell apoptosis.

Results: Derivatives of somatostatin AN-162 and AN-238 significantly reduced cell numbers of MPC cellsafter 24-72 h and significantly increased caspase 3/7 activation. Furthermore, we could evidence similar anti-tumor effects for GHRH antagonist MIA-602 and LHRH antagonist AN-152 on MPC cells and on MTT cells. Taking advantage of the same cell lines we are now setting up a mouse model of malignant pheochromocytoma, which will than be treated using peptide analogues, selected from in vitro studies to establish therapeutic efficacy.

Conclusion: This study should help to find the most effective peptide analogues with potential for future targeted treatment of neuroendocrine tumors in humans.

O-M10:

ESTABLISHMENT OF A LIVER TRANSGENIC ZEBRAFISH LINE FOR SCREENING ESTROGENIC COMPOUNDS

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Introduction: There is ever increasing demand for cheap, sensitive, simple test systems enabling the detection of pollutants in waters and for replacing animals with alternatives in research. The presence of estrogenic compounds in waste- and groundwater is of international consideration as they pose potential threat to exposed wildlife and humans.

Objectives: Our aim is to develop a transgenic zebrafish line as an alternative toxicological model in which the expression of the fluorescent protein is inducible by estrogenic compounds enabling the detection of their presence in vivo.

Materials & methods: As a liver-specific, estrogen inducible gene, the zebrafish vitellogenin-1 was chosen. 3,5 kb upstream region (promoter and cis regulatory elements) of the gene and the fluorescent protein (mCherry) coding region were cloned in two steps to the transposon-based multsite Gateway system. The construct was then coinjected with transposase mRNA into 1-2 cell stage wild type zebrafish embryos (AB line). The specificity and inducibility of the construct was tested using 100 ng/l 17--estradiol.

Results: About 20% of the injected embryos showed fluorescent signal in the liver while transient, nonspecific expression was detected in the eye, kidney and in the yolk. In the F1, only inducible liver-specific fluorescent signal was detected as a response to estrogenic exposure.

Conclusion: The newly developed transgenic line would enable the in vivo detection ofestrogenic compounds as well as the monitoring of other effects exerted on themost important organs and tissues for toxicology.

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O-M11:

LOW-MUTATION-RATE, REDUCED-GENOME ESCHERICHIA COLI: AN IMPROVED HOST FOR FAITHFUL MAINTENANCE OF ENGINEERED GENETIC CONSTRUCTS

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Introduction: Molecular mechanisms generating genetic variation provide the basis for evolution and long-term survival of a population in a changing environment. In stable, laboratory conditions, the variation-generating mechanisms are dispensable, as there is limited need for the cell to adapt to adverse conditions. In fact, newly emerging, evolved features might be undesirable when working on highly refined, precise molecular and synthetic biological tasks.

Objectives: The reduction of the evolutionary capacity of MDS42, a reduced-genome *E.coli* strain engineered to lack most genes irrelevant for laboratory/industrial applications.

Materials & methods: Plasmid-based genome engineering methods were used to construct different *E.coli* strains. Protein overexpression experiments were performed using the different strains.

Results: Elimination of diversity-generating, error-prone DNA polymerase enzymes involved in induced mutagenesis achieved a significant stabilization of the genome. The resulting strain, while retaining normal growth, showed a significant decrease in overall mutation rates, most notably under various stress conditions. Moreover, the error-prone polymerase-free host allowed relatively stable maintenance of a toxic methyltransferase-expressing clone. In contrast, the parental strain produced mutant clones, unable to produce functional methyltransferase. The surprisingly large stability-difference observed between the strains was due to the combined effects of high stress-induced mutagenesis in the parental strain, growth inhibition by expression of the toxic protein, and selection/outgrowth of mutants no longer producing an active, toxic enzyme.

Conclusion: By eliminating stress-inducible error-prone DNA-polymerases, the genome of the mobile genetic element-free *E.coli* strain MDS42 was further stabilized. The resulting strain represents an improved host in various synthetic and molecular biological applications, allowing stablized production of growth-inhibiting biomolecules.

O-M12:

VELOPHARYNGEAL INSUFFICIENCY (VPI) IN RELATIVES OF INDIVIDUALS WITH CLEFT LIP/PALATE (CL/CP)

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Introduction: CL/CPare the most common facial birth defect, with birth prevalence of about 1/700 in Caucasian populations. CL/CP shows familial aggregation but the family patterns are not simply Mendelian.

Objectives: The aim is to investigate VPI in the unaffected family members of CL/CP probands compared to controls, and then to perform genetic studies.

Materials & methods: People from 4sites in the USA (Pittsburgh, Pa;, St. Louis, Mo; Denver, Co; Houston, Tx) and 1site in Europe (Budapest, Hungary) were assessed for VPI utilizing perceptual screening. 311 non-CL/CP relatives from USA families were assessed, along with 207 controls, then a genome-wide panel of 5,700 uncorrelated SNPs were genotyped to investigate associations between SNPs and CL/CP and VPI in these

families. Families and controls from Hungary will also be presented.
Results: More relatives had VPI than did controls (7.3% versus 2.0%, p-value=0.006) and more case families had VPI than did control families (p-value = 3E-06). Genetic association analyses of the SNPs identified multiple SNPs that were differed in the association patterns between case and control families (p-values 0.0003-0.002).
Conclusion: These results indicate that VPI should be considered as part of the spectrum of CL/CP defects, and can be included in studies to help identifynon-penetrant individuals in CL/CP families. Further, VPI can help to identify genetic associations in CL/CP families.

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O-M13:

TUBULIN-RELATED CEREBRAL DYSGENESIS, NOVEL PARADIGMS FOR UNDERSTANDING THE TUBULINS' ROLE IN THE BRAIN DEVELOPMENT

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Introduction: The agyria/pachygyria-subcortical band heterotopia spectrum comprises a group of rare malformations of the central nervous system. Our survey revealed a live births prevalence of 1 per 37 000 for these anomalies in South-Eastern Hungary over 1992-2006. Several genes (*LIS1, DCX, ARX, RELN, VLDRL*) encoding important cytoskeletal proteins have been discovered previously in association with these malformations. The role of different α– and β-tubulins in brain development has been clarified recently, and mutations in genes (*TUBA1A, TUBA8, TUBB2B, TUBB3*) encoding these proteins have been found responsible for various forms of cortical dysplasia.
Objectives: Our aim is to present the various clinical features of brain malformations due to mutations in the tubulin encoding genes based on our own experience and review of the literature.
Results: The malformation spectrum caused by mutations in the tubulin encoding genes extends beyond the defects of neuronal migration and the cerebral and cerebellar cortical organization is also involved.
Conclusion: Normal function of the microtubules is essential for normal brain development and mutations in the tubulin encoding genes can result in defects of neuronal migration and cortical organization.

O-M14:

AN IN VIVO, WHOLE-GENOME RNAI SCREEN FOR GENES INVOLVED IN AUTOPHAGY IN DROSOPHILA

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Introduction: Autophagy is a conserved pathway for the degradation of cytoplasmic material in lysosomes for subsequent reuse. This process is involved in various physiological and pathological conditions such as aging, cancer, immunity, neurodegeneration diseases etc, and drugs affecting autophagic activity are already used in clinical trials. The main pathway starts with the assembly of preautophagosomal structures from which autophagosomes form. These double-membrane vesicles deliver sequestered cargo for lysosomal degradation.

Objectives: To gain further insight into the molecular mechanisms of autophagy, we completed an in vivo RNAi screen.

Materials & methods: We silenced individual genes in mosaic fat bodies of starved Drosophila larvae and scored autophagy phenotypes using a transgenic reporter assay. We tested the effect of knocking down 7125 genes (90% of the conserved genome), with an estimated 84% gene discovery rate and 2% false positives. Importantly, we identified most known regulators of autophagy including Atg (autophagy-related) gene homologs in a blind fashion.

Results: Through a series of secondary tests including transgenic reporters, immunostainings and vital dyes, we were able to establish three categories from the hits recovered in the screen. Silencing of 139 genes phenocopied the loss of Atg genes, essentially preventing the formation of autophagosomes. 64 RNAi lines resulted in the accumulation of autophagosomes that failed to fuse with lysosomes, while 126 lines blocked proper lysosomal degradation of autophagic cargo.

Conclusion: We were able to approximately double the number of already known regulators of autophagy, and identified numerous genes involved in various inherited genetic disorders or cancer whose role in autophagy has not been described previously.

O-M15:

PHENOTYPE-GENOTYPE CORRELATION IN PATIENTS WITH NUCLEAR AND MITOCHONDRIAL INTERGENOMIAL COMMUNICATION DISTURBANCES

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Introduction: The depletion and multiple deletions of the mtDNA are the result of the mutations nuclear genes responsible for the intergenomial communication. There is a wide variety of clinical syndromes.

Objectives: The aims of this stuty to identify the nuclear genetic defects in patients with depletion or multiple deletion syndromes and analyse the phenotype-genotype correlation.

Patients & methods: Patients with inherited multisystemic disorders and myopathological changes indicating mitochondrial dysfunctions, and children with mtDNA depletion syndrome were involved in the study. The mtDNA deletion was investigated by long PCR, the depletion was detected by real-time PCR. The POLG1, TWINKLE, ANT1 and RRM2B genes were sequenced.

Results: Among 400 patients multiple deletion in 40 cases, mtDNA depletion in 8 cases were detected. In patients with multiple mtDNA deletion myopathy, progressive ophthalmoplegia externa (PEO), exercise intolerance, psychiatric symptoms, ataxia and hypacusis were the most common symptoms. In 30% of the patients minimum 5 organ system were affected. In the depletion cohort serious multiorgan failures were present. Pathogenic mutation in the RRM2B gene was found in one family with autosomal dominant PEO syndrome. The compound heterozygous mutation of the POLG1 gene resulted mtDNA depletion syndrome. In POLG1 gene further 32 different alterations and 2 substitutions previously described as genetic modifier factors were detected. In the TWINKLE gene 7 different substitutions were found.

Conclusion: In our cohort in 6,4% of the patients the pathogenic genetic defects were found. We assume that the detected modifying factors in the POLG gene have also an important role in the background of the clinical phenotype.

O-M16:

SCREENING FOR SARCOMERIC GENE MUTATIONS IN HUNGARIAN PATIENTS WITH HYPERTROPHIC CARDIOMYOPATHY

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Background: Hypertrophic cardiomyopathy (HCM) is a primary disease of the myocardium due to mutations in genes encoding for mainly sarcomeric proteins. The distribution of the disease genes is not known in Hungarian HCM patients.

Patients & methods: We analysed Hungarian HCM patients for mutations in sarcomere genes. The beta myosin heavy chain gene (*MYH7*, exons 3-23), the myosin binding protein C gene (*MYBPC3*, exons 1-35), the toponin T gene (*TNNT2*, exons 8, 11, 14, 15,16) and the troponin I gene (*TNNI3*, exons 7-8) have been analysed in 93, 45, 99 and 99 HCM patients, respectively. The coding exons of the genes were amplified using the polymerase chain reaction, mutation analysis were done using single strand conformation polymorphism (SSCP) or denaturing high performance liquid chromatography (DHPLC) assays. Amplicons with altered migration patterns were direct sequenced.

Results: In the HCM groups we identified five *MYH7* mutations (5%) [Arg719Gln, (exon19); Arg249Gln, (exon 9); Val606Met, (exon 16); Glu924Lys, (exon 23); del930Glu, (exon 23)], eight *MYBPC3* mutations (18%) [Gln1233ter, (exon 33); IVS7+1G>A, (intron 7); 2919-2920delCT, (exon 27); 1831-1832delGT, (exon 18); 486-487delGT, (exon 4); 3462-3464delACT, (exon 31], and one *TNNT2* mutation (1%) [del165Glu, exon 11]. No mutation in the *TNNI3* gene was observed. The Gln1233ter mutation of the *MYBC3* gene was found in three, apparently unrelated families. All of the mutations were present in a heterozygous form. Three mutations in the *MYBPC3* gene (1831-1832delGT, 486-487delGT, 3462-3464delACT) are novel mutations.

Conclusion: These results indicate that the *MYBPC3* gene is the most frequently affected disease gene in Hungarian HCM patients.

P-M1:

MODULATION OF RAC1-DEPENDENT CELLULAR FUNCTIONS BY THE SH3PX-DOMAIN ADAPTOR HOFI/TKS4/SH3PXD2B

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Introduction: HOFI, a Homologue of the five SH3-domain protein (FISH) is a recently discovered regulator of podosomes and lamellipodia. We have found that HOFI is required for the generation of epidermal growth factor receptor (EGF)-induced membrane ruffles and lamellipodia in various cell types, suggesting its involvement in the regulation of cellular movement.

Objectives: Because sphingosine 1-phosphate (S1P) transactivates the EGF-receptor by a Membrane-type-1 matrix metalloproteinase (MT1-MMP)-dependent manner we examined the role of HOFI in migration of HeLa and human umbilical vein endothelial cells (HUVEC) along an S1P gradient.

Materials & methods: Transwell migration and G-lisa assays.

Results: We found that migration of HUVECs across fibronectin- or matrigel-coated membranes is increased while migration of HeLa was decreased in the absence of HOFI. Cellular invasion is dependent on the release of various metalloproteinases. Suppression of HOFI by siRNA resulted in a dramatic decrease in gelatinase activity of HUVECs, which unexpectedly, is in contrast with their increased migratory capacity. Small GTP-binding proteins e.g. Rac-1 and RhoA are major regulators of actin cytoskeleton reorganization. Here we show that S1P-induced Rac1 activation of HOFI-deficient HUVECs is decreased, while RhoA activity is slightly increased.

Conclusion: Taken together, HOFI appears to be a general regulator of several actin-based organelles indispensible for cell motility not only in tumor cells, but also in HUVECs. To establish the signaling pathway in HUVECs connecting one or more of the three S1P-receptors to MT1-MMP and the EGFR via HOFI requires further experiments. Moreover, it is likely that other endothelial functions based on dynamic changes of the actin cytoskeleton may be regulated by HOFI.

P-M2:

OVEREXPRESSION OF THE ABCG2 PROTEIN IN NON-MELANOMA SKIN CANCER COULD AFFECT PHOTODYNAMIC THERAPY OUTCOME

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Introduction: The adenosine-triphosphate binding cassette transporter ABCG2 has recently been characterized as part of the heme efflux system, playing a pivotal role in protecting cells from the accumulation of excess porphyrins, which are the most widely-used photosensitizers in photodynamic therapy (PDT). The high-level expression of ABCG2 makes the cells capable of eliminating toxic amounts of porphyrins leading to an increased resistance to PDT.

Objectives: We aimed to investigate the expression of ABCG2 in non-melanoma skin cancers. Since these lesions are often treated with PDT, we studied the effect of ABCG2 on porphyrin accumulation in an in vitro model of PDT.

Materials & methods: ABCG2 expression in non-melanoma skin cancer samples was examined by immunohistochemistry and the immortalized HaCaT keratinocyte cell line was used to establish the in vitro PDT model.

Results: We observed a strong ABCG2 immunopositivity in the suprabasal epidermis near the tumor tissue in basalioma and basosquamous carcinoma samples. Tumor cells were negative except for the keratotic parts of the lesion. In epidermal hyperplasia, keratoacanthoma, Bowen-carcinoma and squamous cell carcinoma extensive ABCG2 staining of the cancer cells was detected. In seborrhoeic keratosis, stromal cells showed significant ABCG2 expression. Specific inhibition of ABCG2 function in HaCaT cells using the non-toxic fumitremorgin C analog, Ko-134, resulted in increased porphyrin accumulation. Ko-134 had a dose-dependent effect on the sensitivity of cells to PDT.

Conclusion: These results suggest that ABCG2 may serve as a target molecule via which to improve the photodynamic therapy of skin lesions.

P-M3:

EXPRESSION PATTERN OF PLURIPOTENCY MARKERS IN RABBIT EPIBLAST AND EMBRYONIC STEM CELLS

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Introduction: The aim of our work was to analyse the expression pattern of embryonic stem cell specific markers in rabbit embryos and epiblast cells.
Objectives: There are well-known genes that direct pluripotency in the mouse, such as Oct4, Nanog, but their expression pattern during the rabbit embryonic development is not known yet.
Materials & methods: Using culture medium supplemented with two or four inhibitors [(2i: PD032590-(PD), CHIR99021-(CH); 4i: PD, CH, A-83-01(A),Y-27632(Y)], embryonic stem cell (ESC) lines could be derived efficiently from mouse and rat embryos. We cultured the rabbit embryos from eight-cell-stage in RDH, RDH+2i or RDH+4i culture medium until the blastocyst stage. The expressionlevel of epiblast specific Oct4 and Nanog, trophoblast specific Cdx2 and hypoblast specific Gata4 and Gata6 were examined in blastocysts, attached embryos and epiblast like cells.
Results: The expression level of Oct4 and Nanog was significantlyhigher in blastocysts, cultured in RDH+2i culture medium, but there was no significant difference in CDX2, GATA4, GATA6 expression on mRNA and protein level, compared to embryos cultured in RDH medium. Surprisingly, by culturing the embryos in RDH+4i medium, we found high GATA6 expression in epiblast-derived colonies.
Conclusion: We hope that we can maintain the pluripotency of the epiblast derived stem cells in 2i containing medium during long term cultivation.

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P-M4:

FUNCTIONAL PHOTOLYASE SYNTHESIS IN CULTURED HUMAN KERATINOCYTES INDUCED BY A NOVEL mRNA-BASED GENE THERAPY METHOD

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Introduction: Gene therapy based on pseudouridine modified, in vitro synthesized mRNA has the potential for a therapeutic use in various skin diseases.
Objectives: Here, we present successful transfection of cultured human keratinocytes with photolyase encoding mRNA generated in vitro. The photolyase can protect cells from the deleterious effect of UVB because of its ability to repair rapidly cyclobutane pyrimidine dimers (CPDs) during visible light exposure (photoreactivation). This enzyme is absent in humans.

Materials & methods: For delivery of the photolyase mRNA into normal human epidermal keratinocytes and HaCaT cells we applied lipofectamine LTX transfection reagent.
Results: Translation of photolyase protein followed by nuclear translocation was detectable already one hour after the transfection, and seemed to be maintained for at least 3 days. After delivery of a small amount of mRNA (0.25 μg/well) we found the translated photolyase to be functionally active. Using direct ELISA and immunofluorescent assay, after 20 mJ/cm^2 UVB irradiation we detected at least 50% less CPD in photolyase mRNA transfected cells exposed to photoreactivating light, as compared to the control cells not exposed to visible light after UV.
Conclusion: As a consequence, reduction of the antiproliferative effect of UVB irradiation could also be demonstrated. In conclusion, delivery of photolyase mRNA into keratinocytes is an appropriate way to repair UVB-induced DNA lesions, and a good example for the possible application of the novel mRNA-based gene therapy.

P-M5:

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF POLYKETIDE SYNTHASE GENE CLUSTERS FOUND IN NEWLY SEQUENCED BACTERIAL GENOME

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Introduction: Even though remarkable advances were made in medical research and treatments over the last decade, due to emerging and re-emerging pathogens and increase of multi-resistant strains, infectious diseases remain among the primary causes of death worldwide. In respect of the clinical needs, isolation of new microbial natural products (e.g. antimicrobials) and screening for potential producing microorganisms are being facilitated by several new strategies. As a part of these efforts, massively parallel next-generation sequencing (NGS) technologies provide great impacts to typify microbes, not only on the basis of willingly observable characteristics, but also upon their genetic potential.
Objectives: To provide broad insights into the molecular basis of secondary metabolites biosynthesis, *Saccharomonospora azurea* strain SZMC 14600 was discovered.
Materials & methods: The genome sequencing was performed by combining cycled ligation sequencing on the SOLiD 3Plus system with 454 FLX pyrosequencing.
Results: The annotated draft genome sequence has been deposited at DDBJ/EMBL/GenBank under accession numberAHBX00000000. Nevertheless, identifying biosynthetic loci in silico covering the whole range of secondary metabolite including novel or cryptic pathways remains a challenging task. In the present study we provide a comprehensive overview of polyketide synthase (PKS) gene clusters. Complementary to the structural genomics approach, to get insight into the relationship of PKS cluster structure and function, digital transcriptome profiling (RNA-seq) has also been performed.
Conclusion: These data clearly indicate that rare actinomycetes species such as S. azurea is potentially prolific sources of pharmaceutically important secondary metabolites.

P-M6:

DIFFERENTIATION BETWEEN HUNGARIAN AND BRAZIL HUMAN PAPILLOMAVIRUS TYPES AMONG FEMALE ANOGENITAL HPV INFECTIONS

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Introduction: Within some decades after the discovery of human papillomaviruses (HPVs), adequate laboratory diagnostic methods and vaccination for primary prevention have been introduced.
Objectives: We have experienced divergences in HPV prevalence between the continents, and our aim was therefore to compare data from a European and a South-American country.
Materials & methods: HPV PCR and HPV types were determined by known diagnostic methods. A very effective automated nucleic acid isolation procedure was performed.
Results: 333 samples were examined in Szeged and 301 in Sao Paolo, among 15-24-year old women. >80% of the participants in Sao Paolo were married, or lived with a partner, while 90% of the Hungarian women were single. The proportions of those who had their first sexual intercourse at 15 years old were 43.9% (BR) and 20.9% (HUN). The prevalences of low-risk HPV types were 14.3% (BR) and 2.9% (HUN). Examinations of three age groups (15-18, 19-21 and 22-24 y) revealed that among the Brazilian women, the high-risk positivity decreased in the higher age groups, but increased among the Hungarian women. The distributions of HPV types differed in the two countries.
Conclusion: Analysis of the results from different aspects demonstrated many correlations and opposite results. The high prevalence of cervical carcinoma in Brazil with relatively high HPV prevalence may be explained by the rarity of cervical cytological examinations. The frequency of HPV prevention vaccinations was very low in both countries, which may be due to the high expense of the vaccines.

P-M7:

CALCIUM SIGNALS IN PLURIPOTENT STEM CELLS

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Introduction: Human pluripotent stem cells provide new possibilities in generating in vitro disease models for drug screening and for gene- and cell therapy. Human embryonic stem cells (HuESC) have several limitations in this regard, including ethical and immunological concerns. Therefore, the reprogramming of somatic cells into pluripotent state, resulting induced pluripotent stem cells (iPSCs) became a valuable alternative source. However, the similarity and applicability of these two types of pluripotent stem cells remain elusive in many aspects.
Objectives: Our aims were the generation and characterization of hiPSC and their comparison to HuESC, especially focusing on cellular calcium signals.
Materials & methods: In this work human iPSCs were generated from human foreskin fibroblasts by introducing the reprogramming factors (c-Myc, Klf4, Oct4, Sox2, Lin28) in a single expression cassette, using the Sleeping Beauty transposon system. Determination of pluripotency was achieved by immunostaining of stem cell markers and by using a spontaneous differentiation model. The cellular calcium responses were induced by various ligands and detected by loading the cells with the fluorescent calcium indicator, Fluo-4.

Results: We found that both cell types showed the proper expression of markers of pluripotency and gave similar differentiation patterns. The calcium signals induced by ATP, LPA, trypsin andangiotensin II were comparable in hiPS and HuES cells. There was no calcium signal evoked by thrombin, histamine or GABA in the iPSCs.

Conclusion: Our data indicate that the generated hiPS cells in many respects are similar to the HuES cells and suggest that iPSCs may be used as an alternative model for drug screening studies.

P-M8:

A NEWLY IDENTIFIED MISSENSE MUTATION OF THE HR GENE IS POSSIBLY ASSOCIATED WITH A NOVEL PHENOTYPE OF MARIE UNNA HEREDITARY HYPOTRICHOSIS 1

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Introduction: Marie Unna Hereditary Hypotrichosis 1 (MUHH1; OMIM 146550) is an autosomal dominant condition characterized by the development of sparse, twisted hair or complete hair loss affecting the scalp only (alopecia totalis) or other hair bearing areas (alopecia universalis). Besides hypotrichosis, other symptoms rarely occur. MUHH1 is the consequence of mutations located in the hairless (HR) gene.

Objectives: In this study we aimed to identify the underlying genetic abnormality of a Hungarian female patient affected by MUHH1.

Patients & methods: We have identified a Hungarian family with one family member affected by alopecia universalis and limb deformities of all four extremities. The limb deformities were already present at birth, while the hair loss was developed shortly after birth. Aftergenomic DNA was isolated from peripheral blood of the patient, the codingregions of the HR gene were sequenced.

Results: Direct sequencing of the coding regions and the flanking introns of the HR gene revealed a novel mutation in the third exon of the HR gene (c.974G/A, p.Gly325Asp). The affected family member carried the mutation in a heterozygous form, while the clinically unaffected family members and the unrelated controls carried only the wild type sequence.

Conclusion: Based on our results, we have identified a novel mutation of the HR gene (c.974G/A, p.Gly325Asp) associated with novel phenotype of MUHH1 including both hair and limb deformities. Further studies are needed to unreveal whether the newly identified mutation of the HR gene is responsible for the development of both the hair and limb deformities.

P-M9:

PRINS, THE PSORIASIS SUSCEPTIBILITY RELATED NON-CODING RNA REGULATES THE UV-B-INDUCED INTRACELLULAR SHUTTLING OF NUCLEOPHOSMIN

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Introduction: We have previously identified and primarily characterized PRINS, a psoriasis susceptibility related non-coding RNA. PRINS is highest expressed in the psoriatic uninvolved epidermis compared either with normal or psoriatic involved epidermis and it plays a role in cellular stress response.
Objectives: Identification of PRINS-interacting molecules and functional characterization of the identified complex.
Materials & methods: PRINS-interacting proteins were identified with an in vitro RNA binding assay and MALDI-TOF. The functional characterization of the identified PRINS-nucleophosmin (NPM) complex was carried out in an immortalized keratinocyte cell line with an NPM-GFP chimaeric construct.
Results: We identified a chaperon protein, nucleophosmin (NPM) as a direct interacting partner of PRINS. NPM is a multifunctional phosphoprotein primarily expressed in the nucleolus. The nucleolar-nucleoplasmic shuttling of NPM has been previously demonstrated in UV-irradiated fibroblasts and cancer cells. Here we could demonstrate that UV-B irradiation changes the intracellular localization of NPM in human keratinocytes and induces its shuttling from the nucleolus to the nucleoplasm. Gene-specific silencing of the PRINS non-coding RNA in the UV-B irradiated keratinocytes resulted in retention of NPM in the nucleolus.
Conclusion: Our results suggest that PRINSis physically and functionally linked to NPM, thus may plays a role in NPM-mediated cellular stress responses. We suppose that PRINS is part of a ribonucleo complex and its altered expression in psoriatic uninvolved epidermis contributes to the well-established aberrant stress response of psoriatic keratinocytes and as a consequence to psoriasis susceptibility.

P-M10:

RABBIT TRANSGENESIS WITH SLEEPING BEAUTY TRANSPOSON SYSTEM

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Introduction: Since the rabbit physiology and pathology reflects more to human than the rodents, the rabbit is an adequate model to study atherosclerosis, lipoprotein metabolism and cardiovascular diseases. Pronuclear microinjection into fertilized rabbit oocytes is a widely used method to produce transgenic rabbits however its limitation is the low yield of transgenic newborns.
Objectives: As establishing of a transgenic rabbit line is still inefficient thus our goal was to assess the application of Sleeping Beauty (SB) transposon system which is a well-functioning enzyme mediated transgenesis in rodents and other mammalian and vertebrate species.
Materials & methods: A circular plasmid contains YFP reporter gene driven by CAGGS promoter and in vitro synthesized mRNA encoding the hyperactive SB100X transposase was co-injected into the pronucleus of fertilised rabbit zygotes. Injected zygotes were transferred into pseudopregnant females using endoscopy. Newborns were tested for the presence of the transgene at DNA, RNA and protein level.
Results: 15% of newborn rabbits showed high expression level of the transgene in F1 generation. All founders showed low degree of mosaicism but germline transmission was successful in all cases without any transgene silencing.
Conclusion: SB transgenesis is successfully applicable in the rabbit system and is significantly more efficient than the classical approach.

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P-M11:

NMDA-TYPE IONOTROPIC GLUTAMATE RECEPTORS REGULATE COMMITMENT OF CHONDROGENIC CELLS

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Introduction: NMDA-type glutamate receptors (NMDARs) are non-selective cation channels, which primarily are permeable for Ca^{2+} and represent major excitotoxic elements of neuronal synaptic transmission. The functional heterotetrameric channel essentially contains NR1 subunits associated with NR2 and/or NR3 subunits. Growing evidence is on the role of NMDARs in various functions, including differentiation of non-neuronal cells.

Objectives: We aimed to characterise the role of NMDARs in chondrogenesis.

Materials & methods: We applied chondrogenic primary high density cell cultures (HDC) established from limb bud-derived chondrogenic cells of chicken embryos, in which model the majority of chondrocytes differentiates spontaneously on days 2 and 3 and significant amount of cartilage matrix is detectable at the end of culturing (day 6).

Results: We observed the presence of NR1, NR2B, NR3A and NR3B subunits of NMDARs in the plasma membrane of differentiating chondrocytes and cells of HDC produced glutamate. The receptors were proved to function as inward cationic channels with whole cell patch clamp recordings. Out of various pharmacological modulations of NMDARs, only application of the NR1 inhibitor DCKA had significant effect:it increased cartilage formation. In the contrary, siRNA-based transient down-regulation of NR1 blocked this process, reduced both the proliferation rate and cytosolic Ca^{2+}oscillations in HDC. As our most striking result, we observed an almost full recovery of chondrogenesis along the reexpression of NR1 protein.

Conclusion: Delayed chondrogenesis in the absence of NMDARs suggests an essential role of this receptor complex during transition of chondrogenic cells to matrix producing chondroblasts.

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P-M12:

MTOR PATHWAY-DEPENDENT AUTOPHAGY DUE TO NADPH/NADP+ IMBALANCE IN ENDOPLASMIC RETICULUM

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Introduction: The surviving of living organisms are mainly dependent on the ability of cells responding to external signals to make accurate decisions, and to take the proper actions, such as cell growth and division or cell death. Autophagy has an essential role in promoting cellular-survival by "self-eating" of parts of the cytoplasm and intracellular organelles. A classical pathway controlling autophagy during starvation involves the mTOR kinase. It has been recently shown that luminal redox imbalance of pyridine nucleotides in the endoplasmic reticulum (ER) together with oxidative stress results in autophagy.

Objectives: Our goal is to reveal the regulatory mechanism of autophagy induction caused by NADPH depletion at the ER, with a special emphasis to explore its connection with mTOR signalling pathway.
Materials & methods: Luminal NADPH reduction will be accomplished by silencing the NADPH generating enzymes or by addition of pharmacological agent metyrapone in HepG2 cells. The inhibition of mTOR is tested by following the phosphorylation state of the main components of the pathway (such as FRAP, p70S6K, 4E-BP1). Autophagy was monitored by the widely used marker, LC3.
Results: Due to the depletion of luminal NADPH content FRAP the centre molecule of mTOR pathway gets inactivated by dephosphorylation. Interestingly silencing the NADPH generating enzymes or adding metyrapone has different effects on downstream substrates of FRAP.
Conclusion: We show that ER srtess has a significant connection with mTOR pathway in mammalian cells. Our data suggest that redox imbalance caused ER stress is not as drastic inhibiting mTOR pathway as rapamycin addition or starvation.

P-M13:

TNFSF15 SINGLE NUCLEOTIDE POLYMORPHISMS AND HAPLOTYPES IN PSORIASIS AND PSORIATIC ARTHRITIS

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Introduction: Single nucleotide polymorphisms (SNP) and haplotypes of the tumor necrosis factor ligand superfamily member 15 (TNFSF15) gene were reported to contribute to the susceptibility of inflammatory bowel diseases (IBDs) in Japanese and Caucasian populations.
Objectives: We decided to examine these SNPs and haplotypes in psoriasis and psoriatic arthritis having a similar pathogenesis to IBDs.
Patients & methods: Five TNFSF15 SNPs were genotyped in 319 patients with psoriasis, 105 of them had psoriatic arthritis and in 200 healthy individuals. Genotyping was carried out with the PCR-based Assay-by-Design method. Three haplotypes (A, B, C) composed by these five SNPs were also analyzed.
Results: Our findings suggest that the rs6478109 SNP may be a genetic risk factor in psoriasis (p=0.0072), while haplotype C may be a protective genetic factor (p=0,0143) of the disease.
Conclusion: Our results suggest that certain variants of the TNFSF15 gene contribute to psoriasis susceptibility.

P-M14:

DISTRIBUTION OF TRANSFECTED FIBRES ALONG THE REGENERATING SOLEUS MUSCLE

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Introduction: A single intramuscular plasmid injection is capable of making regenerating muscles transgenic. The efficiency of transfection is of special interest when the introduced molecules are expressed only in a few fibres. Determination of efficiency is usually done by analysis of cross sections from the most successful transfected area (i.e. from the central part of the muscle).
Objectives: Our aim was to analyze transfection efficiency on cross sections taken systematically along the whole muscle. It was possible to do this in young rats where muscle fibers run parallel with the longitudinal

axis of the soleus muscle. We also wanted to check if the change of fibre length in regeneration could limit the spatial expression of the transgene.

Materials & methods: Soleus muscles regenerating from notexin induced necrosis were transfected with plasmids coding for green or red fluorescent proteins. After one week, the muscles were dissected, frozen and divided into 6 equal segments in length. Frozen cross sections of each segment were screened for transfected fibres. Fibre length was investigated indirectly using the distribution of neuromuscular junctions (NMJs).

Results: We found declining transfection efficiency from the central to peripheral segments. The distribution of NMJs was similar to that of normal muscles.

Conclusion: Transfection efficiency along the whole regenerating soleus is much lower than it can be calculated from the most transfected parts. This is probably not due to the decline of fibre length during regeneration.

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P-M15:

PHENOTYPIC AND FUNCTIONAL SWITCH OF PERITONEAL MACROPHAGES INDUCED BY MESENCHYMAL STEM CELLS

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Introduction: In recent years it has become clear that the therapeutic potential of mesenchymal stem or stromal cells (MSC) is related not only to their multilineage differentiation capacity but also to their ability to suppress inflammatory and immune responses.

Objectives: Here we studied the influence of mouse bone marrow- (BM) and adipose tissue-derived (Ad) MSCs on peritoneal macrophage (M) polarization in the presence or absence of bacterial endotoxin lipopolysaccharide (LPS).

Materials & methods: We isolated and co-cultured peritoneal macrophages and mesenchymal stem cells from C57Bl/6 mice. The amount of TNFa, IL-10 and PGE2 in culture supernates were measured using ELISA assays.

Results: We found that Ms co-cultured with MSCs consistently showed a higher level of phagocytic activity, increased expression of mannose receptor (CD206), and markedly elevated IL-10, but reduced TNFa levels in the culture supernatants compared to the controls. Even in the presence of high amounts of LPS, stromal cells were able to attenuate classical (M1) polarization of Ms. On the other hand, the MSC induced (M2-like) M polarization appears to correlate with their enhanced ability to induce proliferation of in vivo antigen-primed T cells. Transwell co-culture system revealed that the cross-talk between MSCs and Ms was primarily but not exclusively mediated by soluble factors that include prostaglandin E2.

Conclusion: These results demonstrate that MSCs switch Ms into regulatory cells characterized by low pro-inflammatory and high anti-inflammatory cytokine production, high ability to ingest pathogens and apoptotic cells, and a marked increase in their antigen-presenting potential probably aiming to control hyper-inflammation and tissue regeneration.

P-M16:

IDENTIFICATION AND CHARACTERIZATION THE FUNCTIONAL NLS OF SHADOO PROTEIN

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Introduction: Shadoo protein (Sho) is the newest member of the Prion protein family, which is known from its central role in transmissible spongiform encephalopathies (TSE). Sho shares structural and functional analogies with the Prion protein (PrP), however the physiological function of Shadoo and its role in TSE diseases is not known yet.

Objectives: As, in various experimental set ups, Sho was observed in the nucleus, we aim to identify Shadoo's functional nuclear localization signal (NLS), the crucial amino acids and potential posttranslational modification sites in this region.

Materials & methods: Fluorescent fusion constructs were generated by molecular cloning, transfected to mammalian cell lines, and the fluorescent signal was analyzed by confocal microscopy.

Results: We examined the following potential NLSs in Sho: i) a predicted GR-type NLS (pNLS, aa. 28-37); ii) the RNA-binding RGG-box identified by Corely et al. (aa. 28-41); iii) the $(RXXX)_8$ motif described by our group (aa. 25-61). By deleting the pNLS or the RGG-box motif the almost exclusive nuclear accumulation of the fluorescent fusion protein was abolished. However, when the pNLS sequence or the RGG-box motif of Sho were attached to GFP, they failed to direct the protein to the nucleus. The $(RXXX)_8$ motif was sufficient to accumulate the GFP in the nucleus and its deletion resulted complete elimination of nuclear enhancement.

Conclusion: The pNLS and the RGG-box of Sho is insufficient to direct the fusion protein to the nucleus, although, they are likely part of the complete nuclear localization signal which is contained in the 25-61 segment.

P-M17:

INVESTIGATION OF HIGH-FREQUENCY CA^{2+} OSCILLATIONS IN CHONDRIFYING CELL CULTURES

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Introduction: Cytosolic Ca^{2+} concentration exhibits a characteristic pattern during differentiation of chondrifying primary high density cell cultures (HDC) established from distal limb buds of 4-dayold chicken embryos.

Objectives: During our experiments, we aimed at characterising short-term, high frequency Ca^{2+} oscillations during the differentiation of cells in HDC. We also wanted to identify ion channels that may regulate these oscillatory Ca^{2+} transients.

Materials & methods: In the experimental model applied, spontaneous cartilage matrix formation occurs within a 6-day-long culturing period; chondrifying cells differentiate into chondroblasts on day 3. Rapid Ca^{2+} oscillations were detected in Fluo-4-loaded cells by confocal microscopy on various days of culturing.

Results: Oscillations with the highest amplitude and frequency were identified in cells of 1-day-old cultures. As Ca^{2+} oscillations were completely abolished when free Ca^{2+} ions were eliminated from the extracellular space, we concluded that influx of extracellular ions was required for this phenomenon. Voltage-gated

cation channels were also involved in the regulation of Ca^{2+} oscillations, since administration of K^+, Na^+ and Ca^{2+}channel blockers (tetraethylammonium, tetrodotoxin, $CdCl_2$, nifedipine) considerably interfered with both parameters of these oscillatory Ca^{2+} transients.

Conclusion: We hypothesise that the observed unique Ca^{2+} oscillations in differentiating cells of HDC might play a role in the activation of Ca^{2+} sensitive transcription factors required for the onset of their differentiation programme.

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P-M18:

MANIPULATING THE RNAI RESPONSE BY SUPPRESSING IP_3 SIGNALLING IN CAENORRHABDITIS ELEGANS

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Introduction: RNA interference (RNAi) is a commonly used genetic research tool and a promising therapeutic approach. The inositol-1,4,5-trisphosphate/calcium (IP_3 /Ca^{2+}) pathway is a fundamental intracellular signalling cascade.

Objectives: Our aim was to investigate regulatory pathways of the RNAi mechanism, namely whether the ubiquitous IP_3 /Ca^{2+} signalling interferes with RNAi.

Materials & methods: *Caenorhabditis elegans* was used as a model organism as its highly efficient RNAi machinery makes it uniquely suited to study RNAi.

Results: We show that reducing IP3 signalling in C. elegans significantly enhances the efficiency of RNAi. IP_3 signalling mutants also display enhanced RNAi responses in the nervous system, a tissue normally largely refractory to RNAi. This effect is specific to the disruption of the IP_3-mediated, as opposed to other, Ca^{2+} signalling pathways. We demonstrate that reducing IP_3 signalling enhances the RNAi effect independent of the means of RNAi introduction (ie. exogenous or endogenously transcribed dsRNAs). Tissue-specific rescue experiments demonstrate that IP_3 receptors act non-cell autonomously to enhance RNAi. These results preclude roles for the IP_3 pathway in either cellular siRNA action or in RNAi uptake and thus suggest arole in RNAi export, a largely uncharacterised process of the systemic RNAi mechanism.

Conclusion: Our data provide the first implication of a signal transduction pathway in the RNAi process and imply that RNAi responses may be influenced by an animal's physiology or environment. Understanding these cascades will not only lead to better research tools for manipulating gene function, but also enable us to harness these intrinsic mechanisms for clinical therapy.

P-M19:

THE RS3185480 POLYMORPHISM OF THE ADENOMATOSIS POLYPOSIS COLI DOWN-REGULATED 1 (APCDD1) GENE IS ASSOCIATED WITH ANDROGENIC ALOPECIA

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Introduction: Adenomatosis polyposis coli down-regulated 1 (APCDD1) gene is an inhibitor of the Wnt signaling pathway therefore it may have a role in the development of the skin appendages, moreover a mutation of this gene has been shown to be associated with a rare hair condition, hereditary hypotrichosis simplex.

Objectives: In this study we aimed to investigate whether the single nucleotide polymorphisms (SNP) of APCDD1 gene contribute to the development of a common hair disease, androgenic alopecia.

Patients & methods: The genotypes of 9 SNPs in the coding region ofthe gene have been determined with direct sequencing. 210 patients with androgenic alopecia and 98 controls have been enrolled to the study. The severity of the androgenic alopecia was classified according to Hamilton-Norwood in the case of men and according to Ludwig in the case of women.

Results: We found a significant difference in the distribution of the genotypes of the c.1781C/T, p.L476L SNP(rs3185480) of the APCDD1 gene in exon 5, causing a 3.5 and a 2.8 times increased risk for the development of androgenic alopecia for the homozygote (CI 0.933 –13.125; Nominal Regression p=0.063) and the heterozygote carriers (CI 1.086 –7.217; Nominal Regression p=0.033) of the alleles respectively.

Conclusion: Based on our results we conclude that carrying the c.1781C/T, p.L476L SNP (rs3185480) of the APCDD1 gene either in heterozygous or in homozygous form increases the risk for developing androgenic alopecia.

P-M20:

IDENTIFICATION OF THE R24P MELANOMA- PREDISPOSING CDKN2A MUTATION IN A PATIENT WITH MULTIPLE PRIMARY MALIGNANCIES

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Introduction: A 33 year-old female patient presented at our Department with a slowly progressing pT2b stage malignant melanoma without internal organ involvement. Two years later the patient was diagnosed with multifocal breast cancer which histologically proved to be a Grade III invasive medullar carcinoma. Apart from the mammary tumour PET CT suggested the presence of neoplasm affecting the tail of the pancreas. Explorative laparotomy found a medium differentiated adenocarcinoma of the pancreas with liver and local lymph node involvement. Immunhistochemical examination of the removed tumors proved that they were results of independent primary malignant processes. Family history revealed gastric and laryngeal carcinoma affecting the father of the patient and the father's sister deceased of breast cancer.

Objectives: Occurrence of multiple primary tumours in such a young individual and family history of malignancies suggested genetic predisposition. Therefore our aim was to perform the genetic screening of the patient and her father.

Materials & methods: Direct sequencing of the cellcycle regulatory cyclindependent kinase inhibitor 2A (CDKN2A) and the breast cancer 1 and 2 (BRCA1 and BRCA2) genes.

Results: Our genetic analysis revealed that the patient and her father both carry the relatively common R24P CDKN2A mutation in heterozygote form, however we did not detect any of the 15 most commonly occurring BRCA mutations, characteristic of the European population.
Conclusion: Based on our results we hypothesize that the detected R24P mutation of the CDKN2A gene might cause genetic predisposition to breast cancer independently of other breast cancer predisposing germline mutations.

P-M21:

DIRECTED DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS INTO CARDIOMYOCYTES

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Introduction: Human embryonic stem cell (hESC) lines, derived from inner cell mass of blastocyst-stage embryos can provide an unlimited source of differentiated cells. Among others they have the capacity to differentiate into cardiomyocytes.
Objectives: In spite of the urgent need of pure populations of human cardiomyocytes for large-scale pharmacological and toxicological screening applications, high efficacy methods for cardiomyocyte generation are still lacking. In our present study we examined the potential of directed differentiation methods to enrich cardiomyocytes.
Materials & methods: In order to enrich cardiomyocytes in the differentiation process, methodologies were modified either by the addition of chemical agents, or by a combination of morphogenes and basic fibroblast growth factor (bFGF), followed by selection of cardiac progenitor cells.
Results: The addition of ascorbic acid resulted in an increased number of beating areas (BAs). The combination of ascorbic acid treatment with 5-aza-2'-deoxycytidine even more notably induced the formationof BAs. Induction by the addition of bFGF and morphogenes, required for mesoderm formation, also resulted in an enrichment of cardiomyocytes, indicated by a 10-fold increase in the number of BAs. The percentage of emerging cardiomyocytes could be further increased when a cardiac progenitor-enriched population was sorted out, based on the CAG promoter driven EGFP expression.
Conclusion: As a summary, induction strategies applied in this study resulted in major enrichments of cardiomyocytes, as compared to spontaneous differentiation. Our results suggest that induction followed by purification of cardiac progenitors is a promising strategy to receive pure populations of cardiomyocytes even for large-scale production, supporting drug discovery applications.

P-M22:

COMPREHENSIVE ANALYSIS OF MOUSE MESENCHYMAL STROMAL CELLS DERIVED FROM VARIOUS TISSUES AND ORGANS

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Introduction: Multipotent mesenchymal progenitor cells, termed "mesenchymal stem or stromal cells (MSC)", have been demonstrated to reside in many organs.
Objectives: Here we compared the immunophenotype, differentiation potential and gene expression profile of MSC population derived from adult and juvenile bone marrow, adult adipose tissue, as well as juvenile spleen, thymus and aorta wall.
Materials & methods: Quantitative Real-Time PCR, flow cytometry, immunohistochemistry
Results: Based on the RT-PCR, flow cytometry and immunochemistry measurements, all MSC lines uniformly expressed a large set of genes including well-known mesenchymal markers, such as CD44, CD73, -smooth muscle actin, collagen type I, *Gata6*, Mohawk, and vimentin. In contrast, pluripotency genes and the early mesodermal marker *T*-gene were not expressed. On the other hand, different MSC lines consistently expressed distinct patterns of *Hox* genes determining the positional identity of a given cell population. Moreover, MSCs of different origin expressed a few other transcription factors also reflecting their topological identity and so the body segment or organ to which they normally contributed *in vivo*: 1) thymus-derived cells specifically expressed *Tbx5* and *Pitx2*; 2) spleen-derived MSCs were characterized with *Tlx1* and *Nkx2.5*; 3) *Pitx1* designated femoral bone marrow cells and 4) *En2* appeared in aorta wall-derived MSCs. Thus, MSCs exhibited topographic identity and memory even after long term cultivation *in vitro*.
Conclusion: Based on these results, we suggest that postnatal MSCs isolated from different anatomical sites descend from precursor cells developing in the post-segmentation mesoderm.

P-M23:

DEUTERIUM HAS A KEY ROLE IN TUMOUR DEVELOPMENT – A NEW SUBMOLECULAR REGULATORY SYSTEM

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Introduction: The deuterium/hydrogen (D/H) mass ratio is the largest among stable isotopes of the same element, causing differences in the physical and chemical behaviour between the two hydrogen isotopes. Although the concentration of D is more than 10 mM (150 ppm) in living organisms, the potential role of D was not investigated for six decades.
Objectives: In order to reveal the possible role of naturally occurring D in living organisms, the consequence of the shortage of D was investigated in different biological systems.
Material & methods: To reduce the D-concentration in different biological systems below the natural level we used deuterium depleted water (DDW) in a range of 25 ppm and 135 ppm.
Results: The experiments with DDW revealed that due to D-depletion the cell growth of various cell lines (PC-3, MDA, HT-29, M14,) were inhibited *in vitro*. Deuterium depletion also inhibited the expression of genes (c-myc, H-Ras, Bcl-2, K-Ras, COX-2) having key role in tumor development. During the administration of DDW prostate cancer patients in phase II, double blind clinical trial, the net decrease in the prostate volume was three times higher in the treated group (160.3 cmvs. 54.0 cm, p=0.0019). The one year survival was also significantly higher in patients treated with DDW (p = 0.029).
Conclusion: We suggest that cells are able to regulate D/H ratio and its changes can trigger molecular mechanisms having key role in cell cycle regulation. The decrease in D-concentration can intervene into a hitherto unknown submolecular regulatory system which can serve as new target in anticancer drug development.

P-M24:

FUNCTIONAL ADAPTATION AND ALLOSTERIC REGULATION OF THE DUTPASE SUPERFAMILY

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Introduction: Pyrimidine metabolic pathways and their regulation are interesting – beside their medical relevance – because they reflect the evolution of DNA. The biosynthesis of dTTP is based on uracil metabolism mostly through dUMP. One of the most relevant dUMP producing pathways is built up by members of the dUTPase superfamily, dCTP deaminase (DCD) and dUTPase. dTTP feedback-inhibits DCD by a known allosteric mechanism.

Objectives: We aimed to investigate the relation of enzyme structure, function, regulation and phylogeny of the dUTPase superfamily.

Materials & methods: We performed kinetic analysis on genetically engineered dUTPase pseudoheterotrimers to learn whether allostery observed in the related DCD exist in dUTPase. We also performed a phylogenetic analysis and analyzed literature data.

Results: Our kinetics results show that allosteric communication between active sites does not exist in dUTPase. The absence of an allosteric regulation in this enzyme indicates a simple dUTP elimination task. Our phylogenetic investigation showed that dUTPase is common in every domain of life, while DCD is present only in Prokaryote. Archaea, usually have two genes belonging to the DCD family, one of which has dUTPase function. The existence of such DCD-like dUTPases suggests that the common ancestor of this superfamily was a DCD-like enzyme.

Conclusion: We propose that dUTPase adapted to high specificity and enzymatic efficiency upon the appearance of uracil DNA repair to break down dUTP and avoid uracil incorporation into DNA. Contrarily to DCD, which must have been chiefly responsible for production of dTTP precursors.

P-M25:

AN IN VITRO MODEL FOR THE STUDY OF ENHANCED CYCLIC AMP SIGNALING IN MESENCHYMAL STEM CELLS AND THEIR DIFFERENTIATED DERIVATIVES

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Introduction: Deregulation of cyclic AMP (cAMP) signaling due to early somatic mutations is known to cause diseases such as fibrous dysplasia. However, the effect of increased cAMP levels on mesenchymal stem cells (MSCs) which give rise to differentiated connective tissues is controversial. Also, mature connective tissue cells such as osteocytes or adipocytes are difficult to maintain and study in culture, whereas MSCs can readily be cultured and differentiated toward the desired phenotype.

Objectives: To establish MSC populations with enhanced cAMP production for the investigation of cAMP signaling in MSCs and their differentiated derivatives.

Materials & methods: R201C, a hyperactive mutant of the G protein stimulatory alpha subunit (Galpha-s), was generated by site-directed mutagenesis and introduced into human adipose tissue-derived MSCs by lentiviral transduction. Wild-type G-alpha-s and the hypoactive R231H mutant were used as controls. Transduced cells

were characterized by RT-qPCR, RT-PCR/RFLP, and cAMP assay, and differentiated towards the osteogenic and adipogenic lineages.

Results: Expression of exogenous wild-type and mutant G-alpha-s was verified at the mRNA level. MSCs carrying G-alpha-s-R201C showed elevated cAMP levels following forskolin stimulation, and were successfully differentiated into both osteoblastic and adipocytic directions.

Conclusion: The established MSC-G-alpha-s-R201C cells carry functional hyperactive Gs and differentiate into osteoblasts and adipoblasts. Therefore, MSC-G-alpha-s-R201C can be used as an in vitro disease model of fibrous dysplasia, as well as for the study of altered cAMP signaling in MSCs and their differentiated derivatives.

P-M26:

IMMORTALIZATION OF HUMAN ADIPOSE TISSUE-DERIVED STROMAL CELLS WITH HUMAN TELOMERASE REVERSE TRANSCRIPTASE, BMI-1, AND SV40 LARGE T ANTIGEN

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Introduction: Adipose tissue-derived stromal cells (ASCs) are easy to harvest from human donors and hold great promise for regenerative medicine and pharmaceutical drug testing. However, limited life span of ASC cultures in vitro and large donor-dependent variability present major hurdles to controlled and reproducible experiments.

Objectives: We therefore aimed to establish immortalized ASC cell lines that retain essential features of primary cells but provide steady supply of homogeneous cells for in vitro work.

Materials & methods: Human telomerase reverse transcriptase (hTERT), alone or in combination with murine Bmi-1 or SV40 large T antigen, was introduced by lentiviral transduction into ASCs. The resulting cell lines ASC^{hTERT}, ASC^{Bmi-1}, $ASC^{Bmi-1+hTERT}$and $ASC^{SV40T+hTERT}$ were tested for transgene expression, telomerase activity, surface immunomarkers, proliferation, osteogenic and adipogenic differentiation, karyotype, tumorigenicity, and cellular senescence.

Results: All cell lines have maintained expression of characteristic surface immunomarkers, and none was tumorigenic. However, ASC^{Bmi-1} had limited replicative potential, while the rapidly proliferating $ASC^{SV40T+hTERT}$ acquired chromosomal aberrations, departed from MSC phenotype, and lost differentiation capacity. ASC^{hTERT} and $ASC^{hTERT+Bmi-1}$, on the other hand, preserved all essential MSC features and did not senesce after 100 population doublings. Notably, a subpopulation of ASC^{hTERT} also acquired aberrant karyotype after long-term culture; whether it was a stochastic or deterministic event is as yet unclear.

Conclusion: hTERT alone was sufficient to extend the life span of human ASCs, but ASC^{hTERT} may experience genomic instability during prolonged culturing. The combination of Bmi-1 and hTERT successfully immortalized human ASCs without significantly perturbing their phenotype or biological behavior.

P-M27:

SCREENING AND TESTING COMPOUNDS KILLING SELECTIVELY MULTIDRUG RESISTANT CANCER CELLS

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Introduction: The high number of cancer-related deaths is partly caused by the occurrence of multidrug resistant (MDR) phenotype in tumors. Cancer cells often overexpress P-glycoprotein (Pgp, ABCB1) in their plasma membrane, which mediates resistance against various chemotherapeutic agents. A promising way to overcome MDR is to target resistant cells with MDR-selective compounds that exploit the function of Pgp.
Objective: We expanded our earlier efforts to catalogue the National Cancer Institute's DTP drug repository in search of MDR selective compounds, and to verify the predicted cytotoxic patterns in *in vitro* cell cultures.
Materials & methods: The screening method was based on the correlation between cytotoxicity patterns and Pgp expression levels in the NCI60 database. The candidates with greater estimated toxicity towards MDR cells were obtained from the NCI's DTP drug repository and tested on 3 different parental-MDR cell line pairs. Cytotoxic properties and Pgp-dependency were determined by conventional viability assays.
Results: We identified 63 new MDR-selective candidates, of which 27 were available for testing. Molecules showing *in vitro* MDR-selective property were further investigated. By blocking the function of Pgp with a specific inhibitor, the hypersensitivity of MDR cells could be reversed for all compounds possessing general MDR-selective toxicity.
Conclusion: We established *in silico* and *in vitro* screening platforms to identify compounds with selective, Pgp-dependent toxicity. MDR-selective molecules will serve as a starting point for an ongoing drug development project.

P-M28:

WHOLE TRANSCRIPTOME PROFILING OF MONO- AND CO-CULTURED TWO- AND THREE DIMENSIONAL IN VITRO LIVER MODELS

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Introduction: One of the greatest challenges in the biological science and medicine today is to develop complex functional biological structures such as tissue and/or organs. Encouragement for regenerative medicine strategies are commonly originated from our current understanding of cellular and molecular events of the cells, e.g. proliferation, differentiation, signaling, bimolecular production, and formation of extracellular matrix. Another application of tissue-engineered products is in the toxicological, toxicogenomic research, as alternatives to certain types of animal models. The use of cell culture for toxicity testing and/or drug screening was one of the main expectations, in relation not only to ethical reasons but mainly to the need of reducing times and costs of the safety assessment of increasing number of compounds to be introduced. Toxicogenomic technology is based on the postulate that dugs and/or toxicant exposure always induce a change in gene expression.
Objectives: In this study, we created a scaffold-free in vitro liver model by co-culturing of HepG2 and human fibroblast cells under two and three dimensional condition.
Materials & methods: To elucidate the correspondences and differences between these cell lines, microarray technology and digital transcriptome profiling (RNA-seq) were applied before and after primycin-sulphate exposure.
Results: Here we answer the questions: i) whether there are common variations in genes expression between cell lines; ii) which gene sets response to treatment.
Conclusion: Hence, the results of this study could be used to improve the biological understanding of the underlying mechanisms related to a promising antibiotic.

M29:

NOVEL ROLE OF ASCORBATE IN THE PHOTOSYNTHETIC ELECTRON TRANSPORT. PHYSIOLOGICAL SIGNIFICANCE AND POTENTIAL BIOTECHNOLOGICAL APPLICATION

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Introduction: Life on Earth is driven essentially by photosynthetic solar energy conversion. In eukaryotic photosynthetic organisms, electrons originating from water splitting supply the equivalents to reduce CO2. The water splitting enzyme is a vulnerable component of the electron transport chain and it is particularly sensitive to heat stress. We have shown earlier that when the water splitting enzyme is inactive, ascorbate (Asc) supplies electrons to photosystem II.

Objectives: To study the physiological role of Asc as alternative electron donor.

Materials & methods: For our experiments we used heat-treated *Arabidopsis thaliana* plants, and its Asc-deficient (*vtc2*) and overproducing (*miox4*) mutants. The effect of Asc on biohydrogen production was studied in wild-type *Chlamydomonas reinhardtii* cells. The main techniques used were chlorophyll fluorescence, 820 nm transmission and gas cromatography.

Results: We showed that Asc is capable of donating electrons to PSII at significant rates, with halftimes typically between 20 and 40 ms, depending on the Asc content of the leaves. We could also demonstrate that by donating electrons to PSII, Asc is capable of slowing down donor-side-induced photoinhibition. Further, we show that this mechanism, the supply of electrons without producing oxygen, which inhibits the functioning of the hydrogenase enzyme in green algae, can be utilized for enhancing the photoproduction of hydrogen in *Chlamydomonas reinhardtii.*

Conclusion: We have explored a novel role of Asc in defense against environmental stress conditions, and the phenomenon of alternative electron transport from Asc to PSII was successfully exploited in enhancing biohydrogen production.

P-M30:

IN SILICO ANALYSIS OF THIOTEMPLATE MULTIDOMAIN GENE CLUSTERS IN SACCHAROMONOSPORA AZUREA

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Introduction: A wide range of biologically active products is synthesized by thiotemplate modular systems (TMS) including polyketide synthases (PKS), non-ribosomal peptide synthetases (NRPS) and hybrid PKS-NRPS enzymes. The TMSs are multifunctional proteins that are structurally organized in modules. Each module consists of individual domains for distinctive functions. A variation of domains within the modules accomplishes the structural diversity observed in the resultant products. Furthermore, these metabolites offer wide functional diversity such as antibiotics, immunosuppressive agents and antitumor drug properties. Increasing administration of antibiotics has led to a growing number of antibiotic-resistant pathogens. As the problem of antimicrobial resistance becomes more widespread, the need for new anti-infective agents is more urgent than ever.

Objectives: As part of these efforts, massively parallel next generation sequencing (NGS) technologies provide great impact to typify microbes not only on the basis of readily observable characteristics but also upon their genetic potential.

Materials & methods: To provide broad insights into the molecular basis of secondary metabolites biosynthesis, S. azurea strain SZMC 14600 was discovered. The genome sequencing was performed by combining cycled ligation sequencing on the SOLiD 3Plus system with 454 FLX pyrosequencing.

Results: The annotated draft genome sequence has been deposited at DDBJ/EMBL/GenBank under accession number AHBX00000000. In the present study we provide a comprehensive overview of NRPS and PKS-NRPS hybrid gene clusters. Complementary to the structural genomics, to get insight into the gene expression digital transcriptome profiling (RNA-seq) has also been performed.

Conclusion: Our data will be valuable for further exploring the corresponding non-ribosomally synthesized peptides.

P-M31:

MERKEL CELL CARCINOMA AND MERKEL CELL POLYOMAVIRUS: A HUNGARIAN EXPERIENCE

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Introduction: Merkel cell carcinoma (MCC) is a rare, aggressive tumor that is currently displaying an increasing incidence. The tumor affects mostly elderly individuals of Caucasian origin and occurs more frequently among immunosuppressed patients. A new human polyomavirus, the so-called Merkel cell polyomavirus (MCV), was recently identified in these tumors. The presence of this virus suggests that the tumor has an infectious origin.

Objectives: Our aim was to detect MCV in MCCs and other, randomly selected neoplasms in Hungarian patients.

Patients & methods: Fourteen primary or recurrent MCCs from 12 patients and 32 tumors with other origin (epithelial, melanocytic and other skin tumors) were examined. Viral large T protein (LT1 and LT3), and viral capsid protein (VP1) coding sequences were amplified by PCR and sequenced.

Results: The presence of viral T antigen and/or viral capsid DNA sequences was demonstrated in 11 of the 14 MCC lesions. None of the comparative samples contained MCV DNA.

Conclusion: Our findings strongly support the hypothesis that MCV infection may well be specific for MCC, and MCV may play a role in the pathogenesis of MCC. These results may lead towards more specific diagnostic possibilities and hopefully might result in the development of more effective treatments.

P-M32:

EXAMINATION OF NUCLEIC ACID BINDING OF THE NEWEST PRION PROTEIN, SHADOO, USING AGAROSE GEL SHIFT ASSAY

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Introduction: Transmissible spongiform encephalopathies (TSEs) including Creutzfeldt-Jakob disease, fatal familial insomnia, bovine spongiform encephalopathy and scrapie are fatal, incurable neurodegenerative disorders. TSEs are caused by the infectious isoform of the prion protein (PrPSc) which is formed by a conformational conversion from the cellular prion protein (PrPC). The prion protein family includes two additional members besides PrPC, the Doppel and the newest prion protein, the Shadoo. In 2008 an evolutionary conserved RGG-box motif, which is known to be able to bind RNA, was identified in silico by Gready et al in the amino acid sequence of Shadoo.

Objectives: Here we study the nucleic acid binding features of mouse Shadoo and aim to map its nucleic acid binding region.

Materials & methods: Three deletion mutants of Shadoo were introduced: I deleted the predicted nuclear localization signal (delNLS), the RGG-box (delRGG), as well as the glycine and arginine rich $(RXXX)_8$ motif. Proteins were expressed fused to a mCherry fluorescent tag in *E. coli* and partially purified using Ni-NTA agarose beads. The nucleic acid binding was examined with a newly developed Agarose Gel Shift Assay (AGSA) method, which exploits the advantages of a fluorescent proteinfusion tag.

Results: According to AGSAs, wild type Shadoo is capable of binding both RNA and single or double stranded DNA oligos but not plasmid DNA fragments. The three deletion mutants could not bind nucleic acid.

Conclusion: Mouse Shadoo can bind both RNA and DNA. Even the shortest N-terminal deletion prevents binding. Further experiments aim to finalize mapping the binding region, examine length and sequence specificity of the substrate.

P-M33.

EXAMINATION OF THE EFFECT OF A DOWNSTREAM TRANSLATION INITIATION SITE ON THE LOCALIZATION OF PROTEINS USING THE SECRETORY PATHWAY

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Introduction: Fluorescent proteins are often used for studying protein localization and trafficking. When they are fused to the N-terminus of proteins using the secretory pathway, they must be inserted after the secretion signal. It is a common practice to insert the fluorescent protein in a way which leaves the starting translation initiation (TI) site of the fluorescent protein intact. Our experiments revealed the formation of a truncated form of the fusion protein with an altered localisation.

Objectives: Our aim was to study the effect of the downstream TI-site which was likely responsible for the mislocalisation we detected. We study the possible mechanisms causing this dual localizations on both protein and mRNA level.

Materials & methods: We designed a model for this study, where it is easy to distinguish the full length and truncated proteins by targeting to either the plasma membrane or the nucleus, respectively, and can be visualized by confocal microscopy.

Results: In the presence of both TI-sites we observed nuclear localization in nearly 30% of the transfected cells, which was almost entirely eliminated when the downstream TI site was removed. This made our model

system ideal for the following studies, where we examined whether the effect of the 2nd TI-site occurred at the protein or at the RNA level.

Conclusion: Although such constructs are generally used with double TI-sites it is very important to eliminate the downstream one to avoid the formation of truncated and mislocalised proteins and to decrease the signal: background ratio in fluorescent imaging.

N1:

THE LATERALIZED BRAIN IN PARKINSON'S DISEASE

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Introduction: The onset of PD features starts on either the right or left side.

Objectives: The side of onset appears to determine the prognosis of the disorder and other features. Right side tremor dominant has a better prognosis in contrast to left side dominant bradykinesia-rigidity. In addition, left side onset of motor features is associated with cognitive decline.

Materials & methods: Therefore, an intricate relation appears to exists between the side of disease onset and progression/severity and other non-motor symptoms. Unilateral PD in turn corresponds to neuronal nigrostriatal degeneration in the contralateral hemisphere. Indeed positron emission tomography has demonstrated a positive correlation between symptom asymmetry and brain function (Hoorn et al, 2011), which, corresponds to a unilateral pattern of degeneration.

Results: This phenomenon appears to be exclusive to PD and is not found in other degenerative disorders. Additionally, the variation in motor symptom(s) dominance exhibited in the disorder, conforms with the notion that PD is a spectrum disease with many sub-groups.

Conclusion: Thus, clinical and post mortem studies on "lateralisation" may serve as a vital tool in understanding the mechanism(s) eliciting the characteristic destruction of the SN neurons. Additionally, it may be employed as a predictive indicator for the symptomology and prognosis of the illness thus allowing selective treatment strategies for the pronounced hemispheric degeneration.

N2:

MITOCHONDRIA AND THE PATHOGENESIS OF NEURODEGENERATIVE DISEASES

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Much evidence suggests that mitochondria have a central role in aging-related neurodegenerative diseases. Mitochondria are critical regulators of cell death, a key feature of neurodegeneration. Mutations in mitochondrial DNA and oxidative stress both contribute to aging, which is the greatest risk factor for neurodegenerative diseases. In all of the major neurodegenerative diseases, there is strong evidence that mitochondrial dysfunction occurs early and acts causally in disease pathogenesis. Recent studies have shown that mitochondrial dysfunction occurs early in the course of Alzheimer's Disease and that both beta-amyloid and tau protein oligomers acts synergistically to impair mitochondrial function, contributing to synaptic deficits. In Parkinson's Disease (PD), the genetic causes or early onset PD are linked to mitochondrial dysfunction. The genes parkin and PINK1 are involved in mitophagy, and DJ1 stabilizes Nrf2, a critical genetic pathway involved in modulating oxidative damage. Recent work in Huntington's Disease confirms

that PGC-1alpha, a master regulator of mitochondrial biogenesis, is impaired and contributes to the disease pathogenesis. In ALS, mutant superoxide dismutase binds to mitochondria and impairs the voltage-dependent anion channel and leads to impaired retrograde axonal transport of mitochondria to the nucleus. A number of innovative approaches to treating neurodegenerative diseases are based on modulating transcriptional pathways involved in mitochondrial biogenesis and expression of antioxidant enzymes. These include the PGC-1alpha, and the Nrf2/ARE transcriptional pathways. Other approaches, which are being utilized are the administration of Coenzyme Q10 or creatine. These approaches hold great promise for the treatment of neurodegenerative diseases.

N3:

METABOLIC ENZYME MUTATIONS AND OXIDATIVE STRESS IN MITOCHONDRIAL DISEASES

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Impaired bioenergetics as well as oxidative stress are key factors in the pathogenesis of diseases involving deterioration of neuronal function. Mitochondria play a central role both in the cellular energy production and generation of oxidative stress. Among the mitochondrial enzymes, α-ketoglutarate dehydrogenase (α-KGDH), a Krebs cycle enzyme appears to be critical both in the bioenergetic competence and in the oxidative damage of mitochondria. As a regulatory enzyme, it controls the generation of NADH in the Krebs cycle, therefore the ATP production in mitochondria. α-KGDH is sensitive to oxidative stress and oxidative damage of the enzyme compromises the generation of NADH and ATP in mitochondria. α-KGDH is not only a target of oxidative stress, but it can also generate reactive oxygen species (ROS). ROS generation is attributed to the E3 subunit of the enzyme.

There are mutations in the gene encoding the human E3 subunit leading to a disease called E3-deficiency exhibiting a great deal of clinical heterogeneity always with characteristic neuronal symptoms. In our laboratory, out of the 15 pathogenic mutations known so far, 12 mutations of the E3 subunit have been expressed and purified to homogeneity. The enzyme activity was reduced to varying extent in the mutations and in four disease-causing mutations a significantly enhanced ROS generation was detected. The excessive ROS generation by the mutant enzymes did not correlate with the loss of the normal catalytic activity raising the possibility that this could be an important factor in the pathology and clinical presentation of human E3 deficiency.

N4:

THE PHARMACOLOGY OF SELEGILINE; PAST, PRESENT, FUTURE?

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Deprenyl (selegiline) is an original Hungarian drug, discovered by Knoll and his co-workers and during the past three decades it was almost exclusively used to treat Parkinson's disease.

In an oral dose of 10 mg/day selegiline delays the need for levodopa administration. It is effective in monotherapy at the early stage of the disease and its effectiveness lasts till functioning neurons exist in the CNS.

A new MAO-B inhibitor, rasagiline was recently introduced to therapy. The question arises, whether is there a need for two MAO-B inhibitors in therapy. The answer is yes, but not because of their MAO-B

inhibitory effects, but both selegiline and rasagiline in concentrations too low to inhibit the enzyme activity differently modifies the regulation of apoptotic cascade. The metabolism of the inhibitors are different, selegiline possesses an intensive "first pass" metabolism. The microsomal enzymes convert it to amphetamine, methamphetamine, desmethyl-deprenyl, para-hydroxy metabolites and the conjugates of the formers. In addition, flavin-containing mono-oxygenize produces deprenyl-N-oxide (DNO) with a new chiral center and a quaternary group, which modify membrane penetration. N-oxides can be reduced back to the parent compound. Metabolites might play important role in the complex pharmacological effect of selegiline. The metabolites possessing propargyl-group, strengthen the concept that the propargyl moiety is a pre-requisite of antiapoptotic and neuroprotective action. Parenteral administration increases the bioavailability of selegiline and its higher concentration in the brain might lead to antidepressive activity. Selegiline significantly increased cell to cell adhesion of NGF-naïve and NGF-differentiated PC12 cells of neuroectodermal origin.

N5:

NEUROLOGICAL DISORDERS AND KYNURENINES: FUTURE THERAPEUTIC POSSIBILITIES

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Introduction: L-kynurenine is an intermediate of the metabolism of L-tryptophan to nicotinic acid. L-kynurenine is formed in the mammalian brain (40%) and is taken up from the periphery (60%), indicating that it can be transported across the blood brain barrier. L-kynurenine can be converted into two important compounds: the neuroprotective kynurenic acid (KYNA) and the neurotoxic quinolinic acid (QA).

Objectives: The aim of these experiments was to investigate the effects of novel KYNA analogues in different models of neurological disorders.

Materials & methods: The KYNA analogues were synthesisedstarting from KYNAC by Prof. Fülöp and coworkers.

Results: It was shown neuroprotective effects of a novel KYNA analogue in a transgenic mouse model of Huntington's disease. It was prolonged the survival of the transgenic mice, ameliorated their hypolocomotion, prevented the loss of weight and completely prevented the atrophy of the striatal neurons. We found neuroprotection with a new KYNA analog in thefour vessel occlusion model of ischemia. This effect was robust in the event of pretreatment, and also when the drug was administered at the time odreperfusion. This result is beneficial since a putative neuroprotectant proven to be effective as post-treatment is of much greater benefit. Furthermore, KYNA analogues can give rise to antinociceptive effects at the periphery, in the trigeminal nucleus caudalis, and may also act on migraine generators and cortical spreading depression.

Conclusion: These data suggest that kynurenines are potential treatment strategies of neurological disorders.

N6:

GABA-ERGIC DYSFUNCTION IN SCHIZOPHRENIA: FROM POSTMORTEM STUDIES TO ANIMAL MODELS

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Introduction: Glutamic acid decarboxylase 67 kDa (GAD67) is the primary GABA-producing enzyme in the brain. GABAergic interneuron subtypes can be classified by molecular markers such as neuropeptide Y (NPY), cholecystokinin (CCK), somatostatin (SOM), or parvalbumin (PV). These subtypes also have distinct physiological and morphological characteristics that contribute to differential regulation of pyramidal cell output. GAD67 downregulation is one of the most robust and reproduced findings in post-mortem tissue from schizophrenic patients across many brain regions. However, the importance of GAD67 downregulation across the various interneuronal cell types and their potential role in regulating behavior remain unclear.
Objectives: The objective of the current studies was to determine whether GAD67 downregulation is sufficient to induce alterations in the brain and behavior and whether these alterations are dependent on the interneuron subtype(s) affected.
Materials & methods: We have developed a novel method for silencing GAD67 indistinct subpopulations of interneurons in transgenic mice. We created bacterial artificial chromosome (BAC) constructs containing the NPY or CCK promoter-enhancer elements, an eGFP reporter, and a synthetic microRNA (miRNA) targeted to silence GAD67 mRNA specifically in NPY+ or CCK+ interneurons. Male transgenicmice and their wildtype littermates were subjected to a broad behavioral testing battery to assess general neurological function, learning, memory, anxiety, social behavior, sensorimotor gating, and locomotor activity.
Results: NPYBAC and CCKBAC constructs effectively suppressed GAD67 expression in specific celltypes. While eGFP was detected in NPY+ cells or CCK+ cells, GAD67 expression could not be detected in the two targeted interneurnal subpopulations. Behavioral testing of transgenic and control mice revealed cell-type driven changes in locomotor activity, social behavior, anxiety, memory, and response to amphetamine.
Conclusion: We observed that downregulation of GAD67 in the NPY+ and CCK+ interneurons differentially regulate behavior. Importantly we report that the two mouse lines responded oppositely to an amphetamine challenge, suggesting that GABA system dysfunction and dopaminergic dysregulation are interrelated and warrant further examination. Our observations suggest that dysfunction of particular cell types could underlie particular behavioral dysfunction and may be a promising future avenue for therapeutic targeting of behavioral symptoms associated with various neuropsychiatric disorders, including schizophrenia.

N7:

RECENT ADVANCES IN SUICIDE PREVENTION – THE ROLE OF PSYCHOPHARMACOLGY

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Suicide is a complex, multicausal human behaviour with several biological and psychosocial components. As it is very rare in the absence of current major psychiatric disorders, psychiatric-medical suicide risk factors, particularly current major depression with prior suicide attempt, are the clinically most useful predictors. However, since the majority of psychiatric patients never commit suicide, special illness features and psychosocial factors also play a contributory role. In spite of the fact that around two-thirds of suicide victims have current major depressive episode, over 80% of depressed suicides are untreated or inadequately treated. As suicidal behaviour is a complex phenomenon, its prevention should be also complex. Considering the clinically explorable suicide risk factors in mood disorder patients (family and/or personal history of suicide, early onset, severe depressive episode/hopelessness, agitated/mixed depression, bipolar II diagnosis, comorbid Axis I and II disorders, adverse life situations, lack of social and medical support), suicidal behaviour is predictable with good chance. Follow-up clinical studies show that successful acute

and long-term treatment of unipolar (with antidepressants and/or lithium) and bipolar patients (with mood stabilizers and antidepressants/antipsychotics) reduces the risk of attempted and completed suicide by about 80% even in this high-risk population. Restricting lethal suicide methods, community prevention programs (public education, gate keeper-training, appropriate media reporting, etc.) and psychosocial therapies (psychoeducation, regular aftercare, targeted psychotherapies, etc.) are also effective, particularly in combination with pharmacotherapy.

N8:

BIOLOGICAL PSYCHIATRY RESEARCH IN SZEGED

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Introduction: Searching for biological underpinnings in mental disorders has a tradition in Szeged.
Objectives: To provide brief summary of research.
Materials & methods: Overview of scientific achievements.
Results: Neuropathological observations by Miskolczy (1933) revealed alterations in characteristic brain regions in schizophrenia, where Huszák and Durkó (1962) described various pathochemical features. Latzkovits and co-workers (1974) paved the way to analyse membrane transport for ions in nerve/glial cell cultures. Lithium transport was characterised in patients with affective disorder (Szentistványi and Janka 1979), and in brain cell cultures (Janka et al 1980). Molecular biological studies demonstrated membrane fluidity alterations and gene polymorphisms of apolipoprotein E and other putative risk molecules in Hungarian populations with dementia (Kálmán et al 1994, Juhász et al 2005, Fehér et al 2010, Álmos et al 2011). In vitro approaches explored the features of lipid metabolism and amyloid processing in dementia (Pákáski et al 2009). Collaborative experiments provided new insight into the role of gene expression changes in relationship to pathophysiology and clinical presentation of schizophrenia (Horváth and Mirnics, 2009). Neurocognitive and psychophysiological examinations reported markers (Kéri et al 2001, Csifcsák et al 2008), pattern of decision making (Must et al 2007), and cognitive functioning in the light of brain morphometry (Szendi et al 2006). Functional imaging investigations determined binding to the dopamine transporter in depression (Árgyelán et al 2005). Pharmacogenetic studies found an association between antipsychotic response and gene polymorphism of the dopamine system (Szekeres et al 2004).
Conclusion: Recent and ongoing work in Szeged emanate from the conviction that psychiatry is a clinical neuroscience.

N9:

THE ROLE OF INTRINSICALLY DISORDERED PROTEINS (IDPS) IN NEURODEGENERATIVE DISEASES

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Ageing of the brain leads to impairments in cognitive and motor skills, and is the main risk factor for several common neurological disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD). Altered protein handling (proteolysis, repair system, chaperones) forms a basis for a large number of protein conformational disorders. Extra- and intracellular, as well as intranuclear accumulation of

abnormal proteins, in the form of protein inclusions and aggregates, and dysfunction of the quality control mechanisms are common in all these disorders. Alterations in protein homeostasis occur with age, causing molecular changes such as protein misfolding and aggregation. Many biologically active proteins lack stable tertiary and secondary structure, these are called intrinsically disordered proteins (IDPs), some of them (e.g β-amyloid, α-synuclein) are coupled to neurodegenerative disorders. IDPs exist as assemblies of rapidly fluctuating structures undergoing coupled folding and aggregation process. Protein aggregation is characterized by polymorphism, where soluble oligomers, amyloid fibrils and amorphous aggregates are the final products. Soluble oligomers are inevitable formed during the self-association process and might initiate the neurodegenerative cascades of AD, PD and similar diseases. The emerging consensus that protein misfolding (leading to IDPs) is the cause of several neurodegenerative disorders now offers the opportunity to develop a generic therapy. Soluble oligomers with ID regions are potential drug targets. Recently short peptide fragments and small peptidomimetic molecules have been found also in our laboratory. These molecules bind to the ID regions of β-amyloid and are putative drug candidates.

N10:

BRAIN RHYTHMS AND CELL ASSEMBLY SEQUENCES

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The dominant theoretical form of mental structure of the last century was implicitly a neuropsychological model. At the center of this model, necessary for episodic free recall, planning or logical reasoning, is Hebb's phase sequences of neuronal assemblies, i.e., hypothetical self-propagating loops of neuronal coalitions connected by modifiable synapses. These phase sequences can be activated by exogenous or endogenous (internal) sources of stimulation, independent from environmental determinants of behavior. The neurophysiological implication of this conjecture for episodic recall is that hippocampal networks are endowed by an internal mechanism that can generate a perpetually changing neuronal activity even in the absence of environmental inputs. Recall of similar episodes would generate similar cell assembly sequences, and uniquely different sequence patterns would reflect different episodes. Using large-scale recording of neuronal ensembles in the behaving rat, I will show experimental support of self-perpetuating activity neuronal assemblies and demonstrate how oscillations support precise spike timing across neuronal populations. The physiological characteristics of these assemblies are virtually identical to features of hippocampal place cells controlled by environmental and/or idiothetic stimuli. I hypothesize that neuronal mechanisms introduced for navigation in the physical environment in "simpler" animals are identical to those needed for memory recall and/or planning animals with larger brains. The different appearance of representations in the hippocampus of different species and different segments of the hippocampus in the same species may reflect its functional connectivity with the neocortex and other structures.

N11:

THE MAKING, KEEPING AND LOSING OF MEMORIES

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Memory is fundamental. Qualitatively distinct types of memory enable us to change our behaviour in response to experience, to acquire and use a repository of knowledge, to recollect events from the past, and to plan for the future. The use of memory is changing, with much human knowledge now externalised and sought on-demand on the web, but the loss of memory remains greatly feared. A "Grand Challenge" for neuroscience is to understand the neural mechanisms of the capacity to encode, store and retrieve information, and how these mechanisms go wrong.

In this lecture, I shall touch on the 'making' and 'losing' of memory traces, but focus on new research challenging a long held belief that the systems consolidation process responsible for 'keeping' memories is necessarily a slow one. Specifically, when new learning occurs against the background of well-established prior knowledge, relevant new paired-associate information processed in the hippocampus can be rapidly assimilated into a neocortical network and so expand the knowledge base. Further, this hippocampal-dependent learning is associated with a striking upregulation of immediate early genes in the prelimbic region of the medial prefrontal cortex (mPFC) when the new information is in keeping with established knowledge; and pharmacological interventions targeting the mPFC can prevent new consolidation and the effective recall of both remotely and recently consolidated information.

These findings challenge current orthodoxy and specifically the concept of complementary fast (hippocampal) and slow (cortical) learning systems, and they shed new light on the neural mechanisms of memory consolidation.

N12:

DISTRIBUTION AND FUNCTION OF NEURONAL GAP JUNCTIONS IN THE MAMMALIAN BRAIN

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Gap junctions are specialized membrane regions composed of aggregates of transmembrane channels that directly connect the cytoplasm of adjacent cells allowing direct intercellular movement of ions, metabolites and second messengers. Each intercellular channel is formed by the conjunction of two hemichannels, or connexons, formed by the hexameric assembly of subunit proteins, called connexins (Cxs). Cxs are encoded by a large multigene family formed by 23 different members in mammals. Among identified connexins, 10 (Cx26, Cx30, Cx32, Cx36, Cx37, Cx40, Cx43, Cx45, Cx47, Cx50) are expressed in the brain or retina, and the majority of them have been localized in glial cells. Neuronal gap junctions are the main structural substrates of 'electrical synapses' and the identification of connexins expressed in specific neuronal cell types represents a crucial step for understanding their role in neuronal physiology. Neuronal expression of Cxs has been demonstrated with an array of techniques such as immunoelectron-microscopy, *in situ* hybridization, immunohistochemistry and reporter gene expression in transgenic mice. Combining *in situ* hybridization for Cx mRNAs with immunohistochemical detection for neuronal markers, we provided evidence that Cx45 and/or Cx36 mRNAs are localized in subpopulations of specific neuronal cells both in developing and adult rat brain. Detailed distribution of Cx36 or Cx45 expressing neurons has been determined including Cx36 expression in GABAergic interneurons of cerebral cortex, striatum, hippocampus and cerebellar cortex. Inhibitory GABAergic interneurons in the cerebral cortex, hippocampus, striatum and cerebellum are extensively interconnected by electrical synapses, in agreement with the cellular localization of Cx36.

N13:

UNITARY VOLUME TRANSMISSION BY NEUROGLIAFORM CELLS: BROADENING THE FUNCTIONAL SCOPE OF SINGLE NEURONS

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The presentation addresses mechanisms linking the activity of single neurons with network events by defining the function of a cell type in the cerebral cortex. The key hypothesis is that neurogliaform cells achieve their function in the cortex through an extreme form of spatial unspecificity of release. We showed that neurogliaform cells reach GABAA and GABAB receptors on target cells through unitary volume transmission going beyond the classical theory which states that single cortical neurons act in or around synaptic junctions. Moreover, enrichment of the neurosteroid sensitive GABAA delta receptor subunits on neurogliaform cells suggests that these neurons could gate hormonal actions in neocortical circuits. We propose that the spatial unspecificity of neurotransmitter action leads to unprecedented functional capabilities for a single neuron simultaneously acting on neuronal, glial and vascular components of the surrounding area allowing neurogliaform cells to synchronize metabolic demand and supply in microcircuits.

N14:

FUNCTIONAL ROLES OF ENDOCANNABINOID SIGNALING IN THE CEREBRAL CORTEX

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Endogenous cannabinoids are thought to be retrograde mediators in several forms of synaptic plasticity. Depolarization of cortical pyramidal cells, or a rise in intracellular calcium, evokes a CB_1 cannabinoid receptor-mediated short-term depression of GABA release from afferent inhibitory terminals (depolarization-induced suppression of inhibition; DSI). CB_1 receptors present on interneuron axons mediate both endo- and exogenous cannabinoid actions on cortical GABAergic transmission and associated cognitive functions or disfunctions. Most of the CB_1-modulated interneurons belong to the CCK-containing basket cells, which receive abundant subcortical input, and express several receptors – in addition to CB_1 – that are implicated in anxiety. These features enable CCK-positive interneurons to function as a fine-tuning device for cortical fast oscillations, mediating motivational and emotional impacts.

On the other hand, cannabinoids are known to suppress glutamatergic EPSCs as well. Boutons synapsing on pyramidal cell spines express CB_1 receptors, whereas the endocannabinoid 2-arachidonoylglycerol (2-AG) is synthesized in postsynaptic dendritic spines, thus participating in retrograde signaling. Malfunctioning of this negative feed-back system is involved in epileptogenesis, since a downregulation of CB_1 receptors on glutamatergic terminals is among the most profound molecular changes in lobectomy samples of human epileptic patients observed even before degeneration of CA1 pyramidal cells begins.

Thus, in the cerebral cortex, endocannabinoids are involved in the presynaptic regulation of both GABAergic and glutamatergic transmission, and malfunctioning of this control machinery is implicated in several brain disorders. Identification of the molecular architecture of this signaling system may shed light on its functional roles, and point to new drug targets in pharmacotherapy.

N15:

MAKING MAPS OF THE MIND: MOLECULAR MECHANISMS OF NEURONAL MIGRATION

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The identity, synaptic connections and, ultimately, function of neurons is defined by their position. It is particularly evident in the cerebral cortex where constituent neurons are not generated locally and acquire their proper areal, laminar and columnar position during development by migration from multiple sites of origin. Disruption or even slight slowing of the rate of neuronal migration can induce gross abnormalities such as neuronal heterotopias, lissencephaly and polymicrogyria, or to subtle neuronal malpositions that cause abnormal synaptic circuits. Our strategy has been to study in parallel development of the cerebral cortex in rodents, non-human primates and humans using a variety of *in vitro* and *in vivo* assays, including genetic manipulations in animals as well as the parallel RNA-sequencing of a whole-transcriptome profiling of the embryonic ventricular (VZ), subventricular (SVZ) zones and cortical plate (CP) following laser microdissection. We identified and verified position by QRT-PCR on RNA obtained by LMD and in situ hybridization of approximately 400 genes, including many previously uncharacterized transcripts that are differentially expressed in at least one zone. Genes enriched in the VZ and SVZ/IZ were mostly associated with cell cycle regulation and fate determination, whereas the genes highly expressed in the CP were associated with neuronal functions, synaptic transmission and cell adhesion. Importantly, some of these genes are implicated in the pathogenesis of cognitive disorders such as schizophrenia, mental retardation, childhood epilepsy and autism. The specific functions of these genes are being tested in mice to elucidate their role in development, evolution and cortical abnormalities.

N16:

MODULATING HUMAN CORTICAL EXCITABILITY BY TRANSCRANIAL STIMULATION

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Transcranial electric stimulation techniques have been developed as cheap and efficient tools for modifying cortical plasticity in man. Weak transcranial direct current stimulation (tDCS) (Nitsche and Paulus, 2000) induces plastic aftereffects via membrane polarization: cathodal stimulation hyperpolarizes, while anodal stimulation depolarizes the resting membrane potential, whereby the induced after-effects depend on polarity, duration and intensity of the stimulation. Transcranial alternating current (tACS) (Antal et al, 2008) and random noise stimulation (tRNS) intend to interfere with ongoing cortical oscillations (Terney et al., 2008). Using these techniques, we can induce and modify differently neuroplastic changes with different advantages and disadvantages of tDCS, tACS and tRNS. Plastic aftereffects need a minimal stimulation duration time and may reverse with too long stimulation. Whereas in the normal stimulation duration range of about 10 minutes tDCS allows for excitability increase and decrease, tACS and tRNS induce only excitability increases in particular with higher frequencies between 100 and 600 Hz or in the low kHz range. TACS and tRNS induce less skin sensation than tDCS and accordingly can be blinded better. They are also no longer current flow direction sensitive. These effects are strongly modified by neuropharmacological co-application: L-DOPA leads to a focusing effect in analogy to its otherwise found U-shaped dose dependency. Dopamine agonists may reverse anodal excitatory tDCS into inhibition, SSRI provide the opposite effect. In conclusion transcranial electrical stimulation techniques allow for targeted modulation of cortical plasticity in man.

N17:

ROLE OF CGRP AND CGRP RECEPTORS IN MIGRAINE PATHOPHYSIOLOGY

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Calcitonin gene-related peptide (CGRP) is localized in primary spinal afferent C and Að fibers of sensory ganglia and in the CNS, e.g. colliculi and cerebellum. Trigeminal nerve activation results in antidromic release of CGRP that result in vasodilatation via a CGRP receptor complex (calcitonin-like receptor, CLR, and receptor activity modifying protein 1, RAMP1). At central synapses in the trigeminal nucleus caudalis, CGRP acts on second-order neurons to transmit pain signals centrally. CLR and RAMP1 are widely expressed throughout the brain, and in intracranial arteries and the trigeminal system. CGRP does not induce neurogenic inflammation or sensitization at peripheral meningeal sites but relays nociceptive information to the second-order neurons in the brainstem. Recently, developed CGRP receptor antagonists have excellent antimigraine effects and a low side-effect profile. The CGRP receptor antagonists reduce signaling in the trigeminovascular pathway at multiple sites and at central sites, however, the exact site of antimigraine effect is still discussed.

N18:

NONSYNAPTIC INTERACTION BETWEEN NEURONS

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The neurochemical communication in the central nervous system can take two forms, both having various temporal and spatial limitations: 1. the fast digitally coded synaptic transmission that requires specialized synapse, and 2. the slow but almost unrestricted nonsynaptic transmission that operates in analog mode and provides a continuous tonic influence on millions of cells over hundreds of μm from the release sites (Vizi 1980; 1984; Vizi 2000; Vizi et al. 2010).

In both cases the target neurons are equipped with receptors and transporters sensitive to transmitters released from synaptic (spillover) and nonsynaptic terminals. They are present even in a silent form, and they are of high affinity. In contrast, low affinity receptors are located at the synapse and are exposed to extremely high concentrations of endogenous transmitters (1-5 mM).

The stimulation of extrasynatic NR2B subtype of NMDA receptors is toxic, while activation of synaptic NMDA receptors (mainly NR2A) is neuroprotectiv. Nonsynaptic $GABA_A$ receptors are involved in the effects of neuroactive steroids, ethanol, several anesthetic, hypnotic agents, and some anticonvulsant drugs.

The critical role of Glu transporter in prevention of cells from neurotoxic effect of excessive concentration of Glu is validated by findings that in ischemia in which due to energy failure the transporters operates in a reverse direction.

Thus, extrasynaptic receptors of high affinity may be candidates for therapeutic treatment in a range of disorders in the CNS. Indeed, it was suggested that "nonsynaptic receptors and transporters are involved in brain function and targets of drug treatment".

N19:

CO-OPERATIVE CHRONOCIRCUITS IN THE HIPPOCAMPUS

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Nervous systems evolved to enable organisms interact with their environments using stored experience-dependent information. Interactions on various time scales are reflected in the pattern of neuronal activity observed in the discharge of individual neurons, and in the rhythms generated by synchronous activity. The hippocampus is involved in encoding, consolidating and recalling episodic memories. Adaptations in the wonderful forms of neuronal dendrites and axons in the ordered neuronal circuits formed by about fifty neuronal types of the hippocampus serve the delivery of signals at the right time to distinct domains of the information carrying pyramidal cells. Space and time in the network form an indivisible unity of evolutionary design. Pyramidal cells form representations of experiences and memories in dynamically changing cell assemblies structured as temporal sequences. The spike timing and firing rate of pyramidal cells is governed by a large variety of specialised GABAergic interneuron, which innervate different domains of pyramidal cells and each other. The different interneuron species co-operatively divide time by releasing GABA at different phases of robust network oscillations reflecting synchronisation on several time scales. I will explore specific mechanisms along these general principles, illustrating our current efforts towards defining the players, understanding and explaining their interactions in the hope of gaining insights into neuronal events of the brain in health and disease. Capturing the temporal differentiation in the temporal activity of cortical neurons during behaviourally relevant activity patterns gives the essential parameter for defining cell types and their contribution to the network, the brain and the organism.

N20:

ADAPTATION AND PLASTICITY IN THE DEVELOPMENT AND EVOLUTION OF THE BRAIN

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Development of complex neural circuits depends on general rules, specified by genetic instructions, with adaptive mechanisms to fine-tune connectivity. Such flexibililty, essential for development, might have underpinned evolutionary changes in complex brains. In addition, synaptic plasticity enables neurons to change the strength of their connections in response to the pattern of activity passing through them, helping individuals to match perceptual, cognitive and motor skills to the nature of the world around them. Neuronal plasticity, although genetically determined, enabled humans to escape from the informational limits in the blueprint of their genes and propelled them into a different mode of evolution.

N21:

PAIN IN THE BRAIN – NEUROANATOMICAL AND FUNCTIONAL MRI ANALYSIS OF CORTICAL AND SUBCORTICAL AREAS ACTIVATED BY ACUTE PAIN

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Cross-check on information obtained from functional neuroanatomy, experimental tract-tracing studies and analysis of functional magnetic resonance images goes a long way to have a better knowledge about the topography and functional relevance of brain areas targeted by acute pain. According to the types and characters, pain may elicit rapid changes in the blood flow in several cortical and subcortical brain areas in extreme individual

variations. These pain-related areas may appear on MR images up to 27 locations. They could be classified into two large groups: 1) cortical areas where the pain is localized, characterized and recognized (3 subgroups), and 2) cortical areas and subcortical nuclei which give direct or indirect responses to pain (5 subgroups). The first group (with 3 subgroups) includes the relevant components of the medial and lateral somatosensory, as well as the viscerosensory systems, while the second group (5 subgroups) comprises cortical areas responsible for pain-evoked affective, emotional and motivational responses, visceromotor (autonom) reactions. This group includes the endogeneous pain-inhibitory areas, and areas involved in pain-evoked fear and vision of pain-related memory. For the functional evaluation of the pain signals, a map („Pain in the Brain") has been worked out. The possible greatest variation, the topography of the 27 pain-related brain areas have been mapped on 130 serial sections on the human brain with separate (eight) colour codes, according to their functional relevance. By using this map, responses to the pain in the brain can be localize even three-dimensionally, recognize and evaluate functionally.

N22:

PEPPERS IN DRUG DISCOVERIES: SERENDIPITY, CONCEPT AND BREAKTHROUGH

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For Albert Szentgyörgyi the sweet pepper of the Hungarian paprika was the serendipitous source of his „hexuronicacid" towin the „battle over vitamin C".

Subsequently the pungent ingredient of the peppers from Szeged called capsaicin led to the unexpected discovery of lasting sensory desensitization which followed the strong stimulation. After the nociceptors were discovered in the late sixties our structure-activity studies combined with ultrastructural, electrophysiological and phsychophysical results led to the concept of postulation the existence of a „capsaicin receptor" on C-polymodal nociceptors for triggering the highly selective stimulatory and lasting antinociceptive effects of the compound. Our idea was not accepted and several alternative hypotheses were raised, but two decades later in 1997 the capsaicin receptor (TRPV1) was cloned. TRPV1 became the first temperature-gated cation channel and in fact it operated as an integrative nocisensor membrane protein. Thus, it became extremely promising for discovery a new chapter of analgesics: the selective nociceptor blocking agents. Unprecedental efforts in major drug industries led to over 1000 patents and clinical studies of TRPV1 antagonist drug candides. Furthermore, the first drug in therapy, the 8% capsaicin patch for treatment of severe, therapy-resistant neuropathic pain is already on the market. Within the frame of this overview novel scopes for further perspectives will be outlined, like the unorthodox dual sensory-efferent functions of the TRPV1-expressing capsaicin-sensitive primary afferents, the role of lipid rafts in TRPV1.

N23:

PAIN

John N Wood

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About 6% of the population suffer from poorly treated pain at any one time and mechanically evoked pain sensations are particularly problematic – for example half the population over 60 have osteoarthritis than can result in mechanically evoked pain. Despite this, the underlying transduction mechanisms that cause

mechanically-evoked pain are little understood. Over the past three years considerable progress has been made in defining the sensory neuron subsets that respond to different tissue damaging stimuli. Recent data on the involvement of TRP channels and Piezos in mechanosensation will be presented, as well as new findings on the role of voltage gated sodium channels in distinct types of pain sensations.

Abrahamsen, B., et al The cell and molecular basis of mechanical, cold and inflammatory pain. Science (2008)321(5889):702-5 2008Coste B, et al. Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels.Science. 2010 Oct 1;330(6000):55-60 2010

N24:

OF SPICES, TOXINS AND PAIN: A PERSONAL PERSPECTIVE

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The discovery of the unique pharmacological actions of the pungent agent of red peppers, capsaicin on "pain fibers" by Nicolas Jancsó at the University of Szeged in the late 1940s has fundamentally contributed to the development of pain research. This lecture will touch upon some important issues of the anatomy and (patho) physiology of pain including aspects of the identification and structural plasticity of chemosensitive primary afferent neurons which express the transient receptor potential vanilloid type 1 (TRPV1) ion channel, a key molecular integrator of a variety of noxious stimuli. Special emphasis will be laid on the molecular changes which occur in nociceptive primary sensory neurons in response to neuronal injury. Finally, evidence will be presented for the involvement of glucosylceramide synthase and neuronal gangliosides in the mediation of the capsaicin-induced activation and regulation of the expression of the TRPV1 channel suggesting a new approach to selective nociceptor analgesia.

N25:

DEZSO MISKOLCZY & THE SPECIATION OF MODERN HOMO SAPIENS

Timothy Crow

SANE-POWIC, University Department of Psychiatry, Warneford Hospital, Oxford, UK

In 1933 Dezso Miskolcsy of the Department of Psychiatry in the University of Szeged formulated the hypothesis that schizophrenia was a disorder specific to those areas of the brain that have evolved in Homo sapiens. Thereby he placed the problem within evolutionary theory and as a challenge to the Darwinian gradualist account. What characteristic(s) of the brain evolved in humans and when did this happen? What was the associated function? How and why was it variable?

Here it will be argued that the characteristic was asymmetry across the A-P axis (the cerebral torque), that the associated function was language, and that the change occurred abruptly approximately 160 thousand years ago as a result of a paracentric inversion of a gene Protocadherin11Y and a subsequent change in the ectodomain of its homologue Protocadherin11X on the X chromosome. This gene-pair was created by a duplication from X to Y at the chimpanzee/hominin separation 6 million years ago, has been subject to accelerated evolution, and is expressed in the brain as two cell surface adhesion molecules. (Crow, 2008, 2012).

The PCDH11XY gene-pair is postulated to account for the sexual dimorphism of cerebral asymmetry, and the association between relative hand skill and verbal and spatial ability. Sapiens-specific variation including that

relating to psychosis arises epigenetically from variable pairing of the sex chromosomes in male and female meiosis (Crow, 2012).

N26:

THE NEURO-ADRENAL STRESS AXIS – ROLE OF VITAMIN C

Stefan R. Bornstein, Monika Ehrhart-Bornstein

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Society faces an epidemic of stress-related disorders. Obesity, diabetes, hypertension and mental disease stem from a toxic environment of inappropriate working conditions, over-nutrition and sedentary lifestyle in an increasingly urbanized world. The key stress organ of the human body involved in this emerging cluster of comorbidities is the adrenal gland.

Response of the endocrine system to stress is characterized by the concomitant release of catecholamines from the adrenal medulla and of glucocorticoids from the adrenal cortex. Therefore, the activity of these two embryological different endocrine organs that are united under one organ capsule has to be synchronized. Thus, adrenomedullary chromaffin cells synthesize and release a wide variety of factors such as neuropeptides, neurotransmitters, opioids, growth factors that can control steroidogenesis and ample evidence exists for an extensive intraadrenal paracrine regulation of adrenocortical steroidogenesis. These interactions seem to be especially relevant for the fine-tuning of the gland and for the adjustment of the gland's function in situations of challenged homeostasis in disease or conditions of stress.

The adrenal gland is among the organs with the highest concentration of vitamin C in the body. Interestingly both the adrenal cortex and the medulla accumulate such high levels of ascorbate. Ascorbic acid is a cofactor required both in catecholamine biosynthesis and in adrenal steroidogenesis. Here we provide an overview on the newly elucidated role of vitamin C in adrenal cortex and medulla derived from *in vitro* and *in vivo* studies and its role for stress-related disorders.

N27:

ASCORBATE COMPARTMENTATION

Gábor Bánhegyi

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Although the presence of ascorbic acid as an important water-soluble antioxidant and enzyme cofactor is required in all subcellular compartments, its synthesis takes place only in one specific organelle in both plants and animals. Little is known about the transporters facilitating interorganelle movements and ascorbate concentration and redox state within the compartments. Our aim was to investigate the transport of ascorbate and dehydroascorbate in the ascorbate-synthesizing plant mitochondria and animal endoplasmic reticulum (ER). The connections between transport and the organelle redox homeostasis were also studied.

We observed specific, saturable dehydroascorbate transport in plant mitochondria and mitoplasts prepared from cultured BY2 tobacco cells. Dehydroascorbate transport was inhibited by glucose and genistein suggesting that dehydroascorbate and glucose are transported in plant mitochondria by the same or closely related transporter(s). Similarly, specific bidirectional and preferential dehydroascorbate transport

was found in ER-derived mammalian microsomal vesicles. Dehydroascorbate uptake exceeded the level of passive equilibrium, it had high affinity and low capacity. Glucose cis inhibited and trans stimulated the uptake. Glucose transport inhibitors were also effective suggesting that dehydroascorbate transport is mediated by a GLUT-type transporter in the ER. The intravesicular reduction of dehydroascorbate leads to the accumulation of ascorbate and contributes to the low intraluminal reduced/oxidized glutathione ratio, promoting oxidative protein folding and luminal ascorbate-dependent enzymatic processes. Recent hypotheses about GLUT10 as a putative ER dehydroascorbate transporter may provide a molecular basis for our findings and for a rare inherited human disease, the arterial tortuosity syndrome.

N28:

MIGRAINE FROM MAN TO MOLECULE

Jes Olesen

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A few decades ago it was unclear how migraine should be defined and there were essentially no hard neuro-scientific data on migraine.

The first breakthrough came with a demonstration of slowly spreading cerebral hypo perfusion resembling a cortical spreading depression (CSD). CSD is now accepted as an animal model of migraine with aura. There was no indication of CD in migraine without aura but cerebral and extra cerebral arteries were slightly dilated.

The next wave of human discoveries came from provocation experiments. Nitroglycerine via liberation of NO, histamine, PDE5 inhibitor, PDE3 inhibitors, PACAP but not VIP, PGE2 but not PGF2- could all provoke a migraine attack. This led to extensive animal experimentation searching for the detailed mechanisms of action of these compounds, particularly NO. Mouse and rat models of migraine have been developed using a nitroglycerine injection or infusion. Activation of intra cellular signaling systems in different parts of the brain have been investigated. Recently, the interest has focused on CGRP. It can induce migraine attacks when infused in migraine patients and CGRP antagonists are effective in the treatment of migraine attacks. The liberation of CGRP from dura and nerves has been evaluated and mechanisms blocking CGRP release have been demonstrated.

Many molecules interact in a complex cascade-like fashion and much more work remains before the molecular mechanisms of migraine are fully elucidated

N29:

MITOCHONDRIAL MECHANISMS IN THE CEREBRAL VASCULATURE IN HEALTH AND DISEASE

David W. Busija

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Mitochondria are double membrane organelles found in cells which generate chemical energy in the form of adenosine triphosphate. Traditionally relegated to an energy producing role, we now appreciate that mitochondria are involved in diverse cellular activities and that mitochondrial status and responsiveness can be affected by pharmacological agents, disease processes, and aging. This presentation will describe the role of mitochondrial activation on the initiation of responses that impact the cerebral circulation.

Specifically, the transient activation of ATP-dependent potassium channels on the inner mitochondrial membrane (mitoK_{ATP} channels) leads to immediate and long term protection (preconditioning) of the cerebral vascular endothelium as well as neurons and astroglia against potentially lethal stimuli whether *in vivo* or *in vitro*. Mechanisms of preconditioning include reduced levels of intracellular calcium as well as reactive oxygen species during stress. Studies of preconditioning have led to the realization that many of the signaling events associated with the induction of mitochondrial-targeted cellular protection may also affect cerebrovascular tone. Thus, activation of mitoK_{ATP} channels in isolated cerebral arteries leads to vasodilator responses with specific contributions from endothelium, vascular smooth muscle, and perivascular nerves. Furthermore, even relatively mild metabolic stress, such as occurs with insulin resistance, as well as more potent insults notably ischemia/reperfusion, can dramatically interfere with mitochondrial related events in the brain and cerebral vasculature. Future studies will be directed at developing therapies to target mitochondrial specific responses in patients to reverse the impairment of mitochondrial function by disease processes.

N30:

NEUROVASCULAR COUPLING IN THE INJURED BRAIN

Ferenc Bari

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The close structural and functional interactions between brain and cerebrovascular cell types provide mechanisms for neurovascular coupling (NC; i.e., temporal and spatial relationship between local neural activity and the hemodynamic response). In the past decades, we intensively studied the ischemia-induced alterations in microvascular responses to putative mediators of NC in various animal models. By studying cortical pial arteriolar responses in the newborn piglet after transient global cerebral ischemia, numerous ischemia-sensitive neurovascular mechanisms have been identified virtually affecting all components of the neurovascular unit (neurons, vascular smooth muscle and endothelial cells). Several lines of evidence suggest that the neurovascular dysfunction is in association with the release of reactive oxygen species in the early reoxygenation period. While a number of antioxidants administered as pre-treatments preserved NC, so far only ventilation with hydrogen-enriched air during the reperfusion period was effective as a post- treatment strategy.

In rat models of cerebral ischemia, we studied the hemodynamic consequences of cortical spreading depolarization (CSD, an example for NC) with an advanced multimodal imaging technique. We observed that the cerebrovascular system was less responsive to experimental CSD elicitation in chronic cerebral hypoperfusion as compared with optimal perfusion. Our recent studies indicate that CSD spontaneously occur during global and focal cerebral ischemia. In focal ischemia, the kinetics of and recovery from CSD differ within the ischemic core and penumbra. Under severe ischemic conditions, we observed inverse NC (CSD without normal hyperemic episodes). We conclude that disturbed NC plays a pivotal role in the neuronal damage following cerebral ischemia.

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O-N1:

NEW TRENDS IN THE CLINICAL NEUROSCIENCES

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In the postgenomic era new molecular techniques and biotechnology have increasing role in identification new genes, new disorders, in complex disorders to find the genetic predisposing factors, the development of new therapeutic investigation, understandings the role of brain and behavior, and the mechanism of the neurodegeneration, neuronal plasticity and repair. Monoclonal antibodies, enzyme replacement therapies are already present in the every day clinical practice. Cell and gene therapy is a real hope; clinical trials are ongoing presently in Duchenne muscular dystrophy, lysosomal storage disorders, Parkinson's and Alzheimer's disease. Recent rapid advances in genomics and molecular biology are beginning to reveal a large number of possible new, genome-related, molecular markers for the presence of disease, susceptibility to disease, or differential response to treatment. Such markers can serve as the basis of new genomics-based diagnostic tests for identifying and/or confirming disease, assessing an individual's risk of disease, identifying patients who will benefit from particular interventions, or tailoring dosing regimens to individual variations in metabolic response. These new diagnostics can also pave the way for development of new therapeutics specifically targeted at the physiological consequences of the genetic defect(s) associated with a patient's disease.

Acknowledgement: The work was supported by the TÁMOP.4.2.1.B-09/1/KMR-2010-0001 grant.

O-N2:

FAST TWO-PHOTON IN VIVO IMAGING WITH THREE-DIMENSIONAL RANDOM-ACCESS SCANNING IN LARGE TISSUE VOLUMES

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Introduction: My presentation is based on our Nature Methods paper has been published recently.

Objectives: The understanding of brain computations requires methods that read out neural activity on different spatial and temporal scales. Following signal propagation and integration across a neuron and recording the concerted activity of hundreds of neurons pose distinct challenges, and the design of imaging systems has been mostly focused on tackling one of the two operations.

Materials & methods: Male or female C57Bl/6J wild-type mice (postnatal age 60–130d) were used. In vivo bulk loading was performed with a patch pipette using Oregon Green BAPTA-1AM and sulforhodamine-101 under two-photon guidance (810 nm).

Results: We developed a two-photon 3D laser-scanning microscope with a millimeter z-dimension scanning range and sub-millisecond temporal resolution. We demonstrated its use for single-neuron imaging in vitro by obtaining 3D optical recordings of action potential backpropagation at sub-millisecond temporal resolution with random-access scanning as well as dendritic Ca^{2+} spike forward propagation in several-hundred-micrometer-long neuronal processes with the continuous 3D trajectory scanning mode. We demonstrated

the use of the microscope for imaging neuronal populations in vivo by 3D random-access scanning of Ca^{2+} transients in hundreds of neurons in the mouse visual cortex at 80 Hz.

Conclusion: We performed 3D calcium imaging of action potential backpropagation and dendritic spike forward propagation at sub-millisecond temporal resolution in mouse brain slices. We also performed volumetric random-access scanning calcium imaging of spontaneous and visual stimulation–evoked activity in hundreds of neurons of the mouse visual cortex in vivo. These experiments demonstrate the subcellular and network-scale imaging capabilities of our system.

O-N3:

THE PEROMYSCUS AUDIOGENIC SEIZURE MODEL

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Introduction: Epilepsy is a debilitating disease that can arise from either acquired brain lesions or from an inherited susceptibility to cortical hyperexcitability. At least 40-50% of epilepsies have a presumed genetic basis. Although few human epilepsy syndromes are inherited in a simple Mendelian manner, single gene animal models offer valuable opportunities to isolate gene mutations, to identify underlying molecular mechanisms and to explore strategies for therapy. In 1935 a spontaneous recessive mutation appeared among laboratory stocks of Peromyscus maniculatus artemisiae, which has been maintained as a separate stock since.

Objectives: Charazterize the genetic locus or loci controlling the seizures.

Materials & methods: Homozygousity mapping analyzing a panel of 250 F2 and 100 N2 animals was used.

Results: We have identified 4 candidate markers. A comparative cytogenetic map between Peromyscus maniculatus and Mus musculus reveal that the M. musculus homologs of the candidate markers on P.m. chromosome 1 and chromosome 23 would be on M.m. chromosomes 7 and 5, respectively. The two mapped mouse audiogenic seizure susceptibility genes, mass1 and jams1 are localized on M. m. chromosomes 13 and 10, respectively.

Conclusion: Taken together, our preliminary data suggests that the genetic mutation underlying seizure sensitivity in deer mice resides in a novel gene and is not a homolog of a previously identified susceptibility locus.

O-N4:

TRACTOGRAPHY GUIDED TARGET IDENTIFICATION FOR THALAMOTOMY

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Background: Stereotactic targets for thalamotomy are usually derived from population-based coordinates. Individual anatomy is used only to scale the coordinates based on the location of some internal guide points.

While on conventional MR imaging the thalamic nuclei are indistinguishable, recently it has become possible to identify individual thalamic nuclei using different connectivity profiles, as defined by MR diffusion tractography.

Methodology and Results: Here we investigated the inter-individual variation of the location of target nuclei for thalamotomy: the putative ventralis oralis posterior (Vop) and the ventral intermedius (Vim) nucleus as defined by probabilistic tractography. We showed that there is a substantial variability of peak Vop and Vim locations in normal controls. The mean overlap between individual Vop nuclei was 40.2% and it was 31.8% for Vim nuclei. As a proof of concept, we also present a patient who underwent Vop thalamotomy for untreatable tremor caused by traumatic brain injury and another patient who underwent Vim thalamotomy for essential tremor. The probabilistic tractography indicated that the successful tremor control was achieved with lesions in the Vop and Vim respectively.

Conclusions: Our data call attention to the need for a better appreciation of the individual anatomy when planning stereotactic functional neurosurgery.

O-N5:

STEM / PROGENITOR CELLS FROM ADULT ADRENAL MEDULLA

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Introduction: Transplantation of cells from the sympathoadrenal lineage has been suggested in the treatment of neurodegenerative diseases and pain. Currently, this approach is not practical due to a short age of organ donors, lack of tissue homogeneity and expandable cells, and disappointing survival rates of grafted cells.

Objectives: The capacity to differentiate into neuron-like cells suggests that isolated chromaffin progenitors may have therapeutical potential in the treatment of neurodegenerative diseases. We therefore aim to establish protocols for the enrichment of chromaffin progenitor cells and their differentiation to dopaminergic neurons.

Materials & methods: A method has been established for the isolation of sympathoadrenal progenitor cells from bovine, rodent and human adrenals in sphere cultures (chromospheres). Furthermore, we describe their in situ characterization in transgenic mice with lineage specific labelling of neural progenitor cells.

Results: Our data for the first time proves the existence of proliferation-competent sympathoadrenal progenitor cells within the adult adrenal medulla. These cells express progenitor markers including neural crest markers Sox1 and Sox10, markers of the neural lineage such as musashi1, mash1, NGFR and of the Notch pathway (Notch2, Hes1 and Hes5), in vitro self-renew and form clonal spheres; furthermore, these progenitor cells are capable of differentiation into chromaffin and neural lineages. Electrophysiological analyses of neural cells derived from chromospheres revealed functional properties of mature nerve cells.

Conclusion: Our studies provide evidence that proliferation and differentiation competent chromaffin progenitor cells can be isolated form adult adrenal medulla that might harbor the potential for the treatment of neurodegenerative diseases.

O-N6:

STRESS SCREWING NEURONAL CYTOSKELETON TO ALZHEIMER'S

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Stress is a relatively new and emerging risk factor for Alzheimer's disease (AD). Our group is focusing how severe stress can alter brain characteristics such as neuronal plasticity, due to changes in the metabolism of cytoskeletal proteins. In our recent studies, male Wistar rats were exposed to different type of stress (restraint, foot shock, forced swimming and social) (RS) for different time periods. At the end of the exposure periods, the amounts of b-actin, cofilin, amyloid precursor protein (APP) and mitogen-activated protein kinase 1 (MAPK-1) RNAs and proteins were investigated. The mRNA expressions of b-actin, cofilin and MAPK-1 followed U-shaped time course. Acute (3 days) and chronic (21 days) RS caused a fourfold and tenfold increases, respectively, in hippocampal b-actin mRNA expression. In the case of cofilin mRNA expression, ele-vations were detected in the hippocampus on days 3, 7 and 21. The APP mRNA level was increased on day 21. On protein level, chronic stress elevated the levels of b-actin, cofilin and APP in the hippocampus. The hippocampal cytoskeletal expression profile was stress type dependent, the most robust changes were observed in restrain stress and the least ones in forced swimming. These results suggest that certain stress causes the induction of some genes and proteins that are also elevated in AD selectively in the hippocampal region of the rat brain.

This study was supported by grants from OTKA (83667), ETT (052-07/2/2009) and the Hungarian Ministry of Education and Culture (TÁMOP 4.2.2-08/1-2008-0002, TÁMOP 4.2.1./B-09/1/KONV-2010-0005-3).

O-N7:

CYTOKINE SIGNALLING BY GRAFTED NEUROECTODERMAL STEM CELLS RESCUES MOTONEURONS OTHERWISE DESTINED TO DIE

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Introduction: Following an injury to their axons close to the cell body, adult motoneurons generally die. This type of injury, typically caused by avulsion of the spinal ventral root, initiates the activation of astrocytes and microglial cells and the extracellular space becomes loaded with excessive amounts of glutamate, known to be excitotoxic to motoneurons.

Objectives: The aim of this study was to investigate whether stem cells are able to rescue injured motoneurons.

Materials & methods: Clonal neuroectodermal stem cells (NE-GFP-4C) were grafted into the L4 spinal segment where motoneurons were severely injured by root avulsion. Various techniques were applied to determine the factors that prevent motoneuron loss.

Results: We have provided evidence that, following ventral root avulsion and reimplantation, NEGFP-4C stem cells grafted into the rat spinal cord rescue the vast majority of the motoneurons that would otherwise be destined to die, and enable them to reinnervate peripheral targets. Stem cell grafts produced the modulatory cytokines IL-1-alpha, IL-6, IL-10, TNF-alpha and MIP-1-alpha. The neurons and astrocytes in the ventral horn of grafted animals also produced IL-6 and MIP-1-alpha. The infusion of function-blocking antibodies against all cytokines

into the grafted cords completely abolished their motoneuron-rescuing effect, while neutralization of only IL-10 suggested its strong effectivity on motoneuron survival and a milder effect on reinnervation.

Conclusion: It is suggested that, apart from the anti-inflammatory function of IL-10, the cytokines produced by grafted stem cells, host neurons and glia, otherwise known to act as pro-inflammatory agents, exert a strong modulatory function in the CNS, thus preventing motoneuron death.

O-N8:

THE EFFECTS OF TESMILIFENE, A CHEMOPOTENTIATING AGENT, ON BRAIN ENDOTHELIAL CELLS

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Introduction: Tesmilifene, a tamoxifen-related compound, has chemopotentiating properties in experimental and in clinical cancer studies. Treatment with tesmilifene caused temporary, acute central nervous system (CNS) side-effects in patients indicating the opening of the blood-brain barrier (BBB).

Objectives: The aim was to test the direct effects of tesmilifene on BBB functions by an in vitro cell culture-based model.

Materials & methods: Cell culture: co-culture of primary rat brain endothelial cells with primary rat astroglia and pericyte cells. Barrier integrity: transendothelial electrical resistance; fluorescein and albumin permeability. Efflux pump activity: P-glycoprotein; multidrug resistance associated protein-1(MRP-1). Nitric oxide (NO) release: DAF-FM diacetate as fluorescent indicator. Viability assays: real-time cell electronic sensing (RT-CES); cyclic adenosine monophosphate assay. Gene expression assay: custom Taqman array.

Results: Short-term tesmilifene treatment decreased the resistance of endothelial monolayers, and increased the permeability for albumin and fluorescein. Tesmilifene inhibitied the MRP-1 pump and the production of NO in endothelial cells. Long-term tesmilifene treatment reduced dose-dependently the viability of brain endothelial cells. Using RT-CES we could demonstrate that tesmilifene rapidly decreased the resistance of the endothelial cells with a peak at 1 hour, but this decrease was reversible and a 75% recovery occurred at 18 hours. Tesmilifene also altered the mRNA expression of several tight junction proteins.

Conclusion: Our data support the results from previous clinical studies and animal experiments, and clearly indicate that tesmilifene increases the permeability of the BBB by directly acting on brain endothelial cells. Tesmilifene might be potentially exploited to increase the transport of drugs through the BBB and to treat more effectively CNS diseases.

O-N9:

MECHANISMS OF THE INTERACTION BETWEEN MELANOMA CELLS AND CEREBRAL ENDOTHELIAL CELLS

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Introduction: Malignant melanoma is a tumor which metastasizes to the brain with one of the highest frequencies: brain metastases are diagnosed in 40-50% of the cases. Since the central nervous system lacks a lymphatic system, the only possibility for melanoma cells to reach the brain parenchyma is via the blood stream and the blood-brain barrier (BBB).

Objectives: Our objective was to get an insight into the molecular mechanisms of transmigration of melanoma cells through the BBB.

Materials & methods: For our investigations we have used an in vitro BBB model system based on the culture of cerebral endothelial cells and A2058 or B16/F10 melanoma cells, respectively.

Results: Immunofluorescence studies have shown that the TJ proteins occludin, ZO-1 and claudin-5 disappeared from the membrane of endothelial cells which were in contact with melanoma cells indicating that melanoma cells can transmigrate through the BBB using the paracellular route. Transmigration was accompanied by an increased proteolytic activity, and we have shown that the serine protease seprase plays a role in this process. Furthermore, we have demonstrated that transmigration is significantly enhanced by the Rho-kinase inhibitor Y27632 suggesting, that melanoma cells preferentially use the mesenchymal-type of movement during their transmigration through the BBB. Atomic force microscopic measurements showed that individual binding forces between melanoma and endothelial cells is in the range of 100 –1000 pN and is signigicantly increased in the presence of the Rho-kinase inhibitor.

Conclusion: We conclude that interendothelial junctions, proteolytic activity and Rho-kinases play an imoprtant role in the transmigration of melanoma cells through the BBB.

O-N10:

PROTECTION AGAINST METHYLGLYOXAL-INDUCED TOXICITY IN HUMAN BRAIN ENDOTHELIAL CELLS

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Introduction: Diabetes mellitus results in microvascular complications, such as diabetic neuropathy, retinopathy, and stroke. Diabetes is associated with changes in both barrier and transport functions of the brain endothelium which contributes to the neurological complications including higher prevalence of dementia and Alzheimer's disease in diabetic patients. Methylglyoxal (MG) is a reactive metabolite of glucose and serves as an intermediate between glucose and advanced glycation end products, irreversibly modified macromolecules. Since the plasma concentration of MG and advanced glycation end products are increased in diabetic patients, MG is implicated in diabetes-associated brain endothelial cell injury.

Objectives: Our aim was to test the effects of MG on human brain endothelial cells (hCMEC/D3) and search for protective molecules.

Materials & methods: Cell viability was examined by MTT reduction and real-time cell electronic sensing (xCelligence, Roche). The mRNA expression was determined by costume Taqman gene expression assays.

Results: MG exerted a time- and dose-dependent toxicity on human brain endothelial cells. MG at a dose of 1 mM resulted in about 50% toxicity in a reproducible way. It also decreased significantly the mRNA expression of key blood-brain barrier-related genes, like tight junction proteins occludin and JAM, glucose transporter-1, efflux pumps P-glycoprotein (ABCB1) and BCRP (ABCG2). MG inducedtoxicity in human brain endothelial cells could be prevented by antioxidant molecules and agents inhibiting protein glycation, such as aminoguanidine, vitamin C, -tocopherol and all-transretinal. U0126, a MEK/ERK inhibitor also proved to be an effective protective molecule.

Conclusion: Our data may contribute to the development of compounds for the therapy of brain endothelial dysfunction in diabetes.

O-N11:

THE IMPACT OF AGING ON FOCAL CEREBRAL ISCHEMIA-INDUCED PERI-INFARCT DEPOLARIZATION WITH MULTIMODAL IMAGING IN THE RAT BRAIN.

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Introduction: Spreading depolarization (SD) is a slowly propagating wave of transient neuronal and glial depolarization, coupled with cerebral blood flow (CBF) changes. It occurs in the brain during migraine or after injury. During ischemia, repetitive SD-like peri-infarct depolarizations (PIDs) can impair tissue by worsening the disparity between blood flow and metabolism. Aging may influence the features of depolarization waves in the ischemic brain.

Objectives: The purpose of this study was to identify and characterize PID-related changes in young, middle aged and old animals exposed to mild stroke, using multi-modal imaging of the rat cerebral cortex through a closed cranial window.

Materials & methods: A closed cranial window was placed over the parietal bone in male Wistar rats. The window was loaded with voltage sensitive (VS) dye (RH-1838). Focal ischemia was induced by 30 minutes of transient middle cerebral artery occlusion with a microaneurysm clip. Using two cameras and selected cortical illuminations, multiple image sequences were captured. This multi-modal strategy allows for the study of synchronous changes in: membrane potential (VS dye); cerebral blood volume (CBV); hemoglobin (Hb) deoxygenation, and CBF.

Results: Aged animals (2 years) experienced fewer PIDs, usually without membrane repolarization, and CBF responses associated with PID most often displayed inverse neurovascular coupling.

Conclusion: Age significantly altered the number, shape and flow coupling of PIDs during mild stroke. Following PID, aged animals showed lower CBF in the ischemic area which often did not return to normal, even after repefusion.

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O-N12:

SEVERE SUBACUTE NEUROVASCULAR DYSFUNCTION IS ALLEVIATED BY HYDROGEN IN ASPHYXIATED NEWBORN PIGS

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Introduction: Neurovascular dysfunction (NVD) importantly contributes to the pathomechanism of hypoxic-ischemic (H/I) encephalopathy (HIE) in term neonates. Numerous studies demonstrated severe, transient NVD 1-4h after H/I stress in the newborn piglet -an accepted large animal model of the human term neonate. However, there are virtually no data from the 8-48 h reoxygenation period, although secondary energy failure of neurons often takes place during this time in the course of HIE.

Objectives: The purpose of the present study was twofold: 1) to assess NVD 24 hours after asphyxia in piglets, and 2) to determine if hydrogen gas (H_2) – an antioxidant previously found to alleviate acute NVD after asphyxia– would also affect cerebrovascular reactivity (CR) in this period.

Materials & methods: Anesthetized, ventilated newborn piglets (n=27) assigned to three experimental groups: time control, asphyxia/reventilation with room air (A/RA), and A/RA supplemented with 2.1% H_2 for 4h (A/RA+H_2, n=9-9). Asphyxia (8 min) was induced suspending the ventilation. CR was assessed with closed cranial window/intravital microscopy at 24h reventilation.
Results: We found that vasodilation to graded hypercapnia and NMDA were severely attenuated in the A/RA group compared to time controls, however, vasoconstriction to norepinephrine or vasodilation to nitroprusside-Na were similar. H_2 ventilation fully or partially prevented the attenuation of hypercapnia-or NMDA-induced vasodilation, respectively.
Conclusion: Severe stimulus-selective NVD develops in the subacute phase of HIE. The efficacy of early H_2 treatment to alleviate this NVD suggests the involvement of reactive oxygen species in the mechanism of this late neurovascular dysfunction.

Supported by OTKA K100851.

P-N1:

ABETA1-42 IMPAIRS THETA COUPLED FIRING OF CA1 CELLS IN VIVO

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Introduction: The memory deficits seen in Alzheimer's disease (AD) are results of hippocampal network dysfunction. The function of hippocampal network is largely coordinated by theta oscillation, by controlling the spike timing necessary for coding of neuronal ensembles involved in various higher cognitive processes. Certain memory traces have been hypothesized to be encoded by the firing of CA1 principal cells, which shows phase-preference and theta-dependent firing patterns. A misfolded peptide, Abeta1-42 is widely believed to be the cause of AD. It is synaptotoxic and cause memory impairment in a variety of cognitive tasks.
Objectives: Our aim was to investigate the effect of Abeta1-42 on theta-coupled firing.
Materials & methods: We recorded single-unit and local field potential activities from urethane anaesthetized rats, and applied Abeta1-42 by using microiontophoresis. Theta activity was evoked by a brief tail-pinch.
Results: We show that Abeta1-42 impairs theta-coupled firing activity of CA1 cells, but does not affect theta power. In contrast to vehicle administration which did not alter phase-locked firing, Abeta1-42 severely damaged theta-coupled discharges.
Conclusion: We suggest that the memory impairment seen in early AD may be the result of damaged timing of spikes and neuronal network function.

P-N2:

THE CONSEQUENCE OF PRPC OR SHADOO OVEREXPRESSION ON THE CYTOTOXIC EFFECT OF THE PRPC DCR MUTANT PHENOTYPE

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Introduction: Prion diseases or transmissible spongiform encephalopathies are spongiform degenerations of the brain with variable degree of amyloidal plaque formation, caused by the accumulation of an abnormal isoform of the cellular Prion protein, referred as PrPSc. The d105–125 deletion mutant of the Prion protein,

which causes a neonatal lethal phenotype when expressed in transgenic mice, offers a fresh view in connection with the physiological function of PrPC and how PrPC can be degraded to produce neurotoxic effects. Expressing the PrPCd105–125, referred as dCR mutant, in mammalian neural cells was shown previously to cause hypersensitivity to the toxic effects of certain antibiotics. This hypersensitive phenotype can be rescued by co-expression of wild type PrPC.

Objectives: Our aim is to reproduce these experiments in neuronal SHSY5Y cells and examine whether Shadoo can also rescue this phenotype.

Materials & methods: The Sleeping Beauty transposon system was applied to create stable dCR expressing neural cell lines, while the overexpression of PrP^{C} or Shadoo is carried out using lentiviral expression system. For showing drug hypersensitivity we measured the cell viability with MTT assay with or without Zeocin treatment.

Results: We established a lentiviral expression system in order to investigate these processes as well as to rescue the dCR mutant phenotype by the Shadoo protein, which is - in several respects -seen as a functional analog of the Prion protein.

Conclusion: We created the necessary stable cell lines and optimized the lentiviral system and the MTT assays for the overexpression and cell viability studies, respectively.

P-N3:

INJURY-INDUCED GLIAL CELL REACTIONS IN HISTAMINE DEFICIENT (HDC-KO) MICE

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Introduction: Histamine is a major proinflammatory mediator in the periphery. As a neurotransmitter it can influence several neuronal functions. However, the role of histamine in central nervous system injuries is not fully understood.

Objectives: The aim of the study was to investigate glial and inflammatory reactions related to neuronal injury in histamine deficient mice after peripheral axotomy.

Materials & methods: Animals: 3-month old wildtype Balb/c (WT) and histamine deficient (HDC-KO) mice. Surgery: under anesthesia sciatic nerve on the right side was transected. Immunohistochemistry: rat anti-CD11b, mouse anti-GFAP, mouse anti-MCP1. Cell culture: mixed glial cells from 2 day-old WT and HDC-KO animals were prepared and cultured. Cytotoxicity test: MTT conversion assay. Free radicals detection: ROS measurement.

Results: Five days after the surgery microglia activation developed in the right ventral horn of the spinal cord at the lumbo-sacral part in both WT and HDC-KO animals, detected by CD11b immunostaining. Intensity for intermediate filaments glial fibrillary acidic protein (GFAP) and vimentin, indicating reactive astrogliosis, reached a peak two weeks after sciatic nerve cut in the area of injured motoneurons. At the site of activation of glial cells, spinal cord microvessels were visualized by immunostaining of type IV collagen, and endothelial cells forming the blood-spinal cord barrier were stained for claudin-5 and glucose transporter-1.

Conclusion: Unexpectedly, glial reactions were more intense in HDC-KO mice than in WT mice. Immunoreaction for the monocyte chemotactic protein-1 (MCP-1) was more intense in injured motoneurons in HDC-KO animals. These new data indicate a protective or inhibitory role for endogenous histamine in central nervous system injuries.

P-N4:

INTRACEREBROVENTRICULAR ADMINISTRATION OF THE SYNTETHIC AB1-42 TOTHE RAT BRAIN. CONNECTION OF SPATIAL MEMORY AND SPINE DENSITY

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Introduction: Alzheimer's disease (AD) is associated with an early memory loss and is the major cause ofdementia. AD is characterized by presence of senile plaques which play important role of the pathogenesis. The oligomerization and aggregation of amyloid-beta (Aβ) is believed to be central event in the dementia.
Objectives: Validation of a novel method for measurement of the effect of intracerebroventricular (ICV) administered Aβ1-42 into rats. Evaluation of dendritic spine density in hippocampus CA1 region after ICV injection of Aβ1-42.
Materials & methods: In this study we investigated the cognitive deficits associated with the amyloid pathology. The subjects were Wistar rats. Morris Water Maze and Barnes Maze were used to assess the spatial memory. In the 1st experiment the Aβ1-42 (4 days aggregation) was injected bilaterally into the ventricles with Hamilton syringe and the behavioural tests were assessed after a week recovery. The 2nd experiment the Aβ1-42 (5 days aggregation) was injected and the behavioural tests were assessed after 2 weeks recovery.
Results: In 1st ICV experiment the effect of Aβ1-42 was toxic in the Morris water maze and in Barnes maze. Spatial and reference memory deficit was detected. In 2nd ICV experiment the Aβ1-42 impaired only the spatial learning process in Morris Water Maze. Quantitative analysis of hippocampal dendritic spines was performed on rapid Golgi impregnated neurons. There were significantly higher values of spine density in amyloid-treated group compared to control.
Conclusion: These results suggest the administration of the synthetic Aβ1-42 cause cognitive deficit and the ICV injection method is a valid model in AD.

P-N5:

DIFFERENT COPING STRATEGY OF MICE HAVING HIGH- OR- LOW-ANXIETY RELATED BEHAVIOR

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Introduction: Among certain genetic predisposition, the risk factors of anxiety disorders include contribution from environmental factors. Long-lasting behavioral changes, like maladaptive fear learning and passive coping

strategy are likely underlain by stable changes in gene expression pattern, which are regulated by epigenetic mechanisms. Acetylation of core histones, that was shown to enhance the expression of affected genes, is regulated by two opposing class of enzymes, histone deacetylases (HDACs) and histone acetyltransferases (HATs).

Objectives: Investigating on the anxiety of the two different (HAB, LAB) mouse strains.

Materials & methods: For the experiments we have used two inbred mouse strains (HAB, LAB). The animals were tested in the EPM for eight consecutive days. Another cohort of animals was exposed to EPM for 2 days followed by a change in context: context"B"-blue light. We used two different HDAC inhibitor; sodium butyrate (SB) and valproic acid (VPA).

Results: We found clear difference of anxious phenotype between HAB and LAB mice, furthermore we found that upon context change HAB mice exhibited increased anxiety, however LAB mice showed enhanced open-arm exploration. Our data suggest, that the HAB mice develop increased anxiety over time, which was inhibited by inhibitors of HDACs, SB and VPA.

Conclusion: These results suggest that the mouse lines having extremes in anxiety-like behavior use different coping strategy in novel context. Moreover, we suggest that decreased open arm exploration may be the consequence of either increased anxiety (in the case of HAB) or decreased motivation for exploring open arm (LAB). HDAC inhibitors were anxiolytic in HAB mice when they were not challenged by a context-shift.

P-N6:

ANIMAL EXPERIMENTS ON THE FUNCTIONAL NEUROTOXICITY OF METAL NANOPARTICLES

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Introduction: Environmental presence and health effects of nanoparticles (granules with diameter below 100 nm) constitute an emerging field. Nanoparticles (NPs) from industrial processes like welding can contain metals, including neurotoxic ones. Manganese is often found in metal fumes and is responsible for job-related neurological problems.

Objectives: For modelling occupational metal NP exposure, electrophysiological and behavioural methods were used.

Materials & methods: Synthetic NPs of MnO_2 were given to male Wistar rats (8-10 animals/group) by intratracheal instillation for 3-6 weeks; alone, combined with oral Mn administration, or combined with NPs of other industrial welding fume metals (Fe_3O_4,$Cr[OH]_3$). Body weight was monitored as general toxicological parameter during treatment. At the end, the rats' motor activity was tested in open field box, then they were prepared for electrophysiological recording in urethane anaesthesia. Spontaneous cortical activity (electrocorticogram, ECoG) and evoked potentials (EPs) from the somatosensory cortex were recorded.

Results: Decreased body weight gain was seen in the treated rats. Nano-Mn had stronger effect than oral Mn. The effect of Mn+Cr NP combination was also stronger but Fe diminished this action of Mn. ECoG was shifted to higher frequencies by all treatments applied. In the somatosensory EPs, onset latency was the most sensitive to metal application; in this parameter the same interactions between the metals were seen as in body weight gain.In the openfield test, the time spent with ambulation and rearing decreased while immobility increased, and Fe diminished Mn effect also here.

Conclusion: Experimentsof this kind seem suitable for modelling the occupational neurotoxicity of airborne metals and may provide the base of subsequent mechanistic studies.

P-N7:

A FAST METHOD FOR ASSESSING THE TYPE OF SODIUM CHANNEL INHIBITORS

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Introduction: Our group has previously identified several distinct mechanisms of action for sodium channel inhibitors (SCIs) (Lenkey et al., PLoS One (2010) 5:e15568).

Objectives: We believe that the important question in SCI pharmacology is not their affinity (several therapeutically successful SCIs have rather low affinity values), but their "type"; i.e., their mode of action, most importantly their state-selectivity. We intended to develop a fast method for the identification of individual SCI types, independently of their general affinity.

Materials & methods: We used the standard whole-cell patch-clamp method on Nav1.2 expressing HEK-293 cells.

Results: First we assessed the general affinity of individual drugs using a simple 10 Hz train protocol, and calculated IC_{50} and IC_{20} values. In the second step we applied each SCI in their IC_{50} and IC_{20} concentrations, and assessed their relative affinity under a set of different experimental conditions. The affinity profile of specific drugs gives a concentration-independent measure of state selectivity and kinetics of action. The set of different relative affinity values and their mathematical combinations gives a multidimensional vector for each drug, which can be analyzed using multivariate statistical methods. We have investigated if there is a correlation between the position of individual drugs within the multidimensional space of biophysical properties of inhibition, and the position of the same drug molecule within the chemical space defined by chemical descriptors.

Conclusion: Assessing the type of SCIs requires no more resources than conventional screening, but gives much more relevant information for drug development.

P-N8:

MEMORY EFFECTS OF BENZODIAZEPINE-SITE INVERSE AGONISTS: ARE THEY POTENTIAL COGNITION ENHANCERS?

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Introduction: Benzodiazepine (BZ) site ligands are well established as modulators of learning and memory processing. The ability of BZ site inverse agonists to enhance cognition still asks for further investigation.

Objectives: The purpose of present study was to examine the influence of the standard BZ inverse agonist DMCM on learning processing in active avoidance task in rats.

Materials & methods: The active avoidance test was performed with male Wistar rats in automated two-way shuttle-boxes, in the schedule 2x100 trials (two days) and in the 5x50 schedule (five days), as well as the effects of third day treatment changing. The drugs were given intraperitoneally, 30 min before testing.

Results: DMCM at the dose of 0.1 mg/kg significantly facilitated avoidance retrieval, whereas at the dose of 1.0 mg/kg diminished active avoidance trials. DMCM, at the dose facilitating retrieval of avoidance memory (0.1 mg/kg) significantly ($p<0.05$, comparison of regression coefficients by Student's t-test) and progressively increased acquisition rate during 5 days training, compared to the saline group. In the case of third day

changing of treatments, the intersection of regression rate lines was detected. On the contrary, DMCM, at the dose impairing retrieval (1.0 mg/kg), increased acquisition rate during 5 days training, but not statistically significant compared to the saline group; in the case of third day changing of treatments, the regression rate lines stayed parallel.

Conclusion: These results show that BZ inverse agonists possess dose-dependent bidirectional memory effects. The lower doses show retrieval and acquisition enhancing effects, whereas the higher doses show retrieval and acquisition impairing effects.

P-N9:

HOW ABETA$_{1-42}$ DISRUPTS SYNAPTIC PLASTICITY: EFFECTS ON LTP AND SPIKING ACTIVITY IN HIPPOCAMPAL SLICES

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Introduction: A misfolded peptide, Abeta$_{1-42}$ is widely regarded to be the cause of Alzheimer's disease. It has been shown to impair long-term potentiation, a form of neuronal plasticity thought to underlie learning and memory. The precise temporal control of spiking activity is known to be crucial in synaptic plasticity. Pre- and postsynaptic neuronal discharges separated by few milliseconds may induce modification of synaptic strength, a phenomenon referred to as spike-timing dependent plasticity (STDP). The effect of Abeta$_{1-42}$ on LTP is well documented, however, data about Abeta$_{1-42}$ impact on neuronal firing connected to synaptic plasticity is still lacking.

Objectives: Our goal was investigate the exact mechanism of Abeta$_{1-42}$ on acute hippocampal slices by measuring LTP and spiking activity.

Materials & methods: We applied Abeta$_{1-42}$ onto murine hippocampal slices and recorded field excitatory postsynaptic potentials (fEPSPs) and spiking activity. The Schaffer-collateral was stimulated by injecting a biphasic voltage waveform. Following a stable 10 min control sequence LTP was induced by theta-burst stimulation (TBS) and neuronal discharges were recorded 1 h before TBS and 1,5 h and 3 h after TBS.

Results: We have found that firing rates correlate with the level of LTP. Spiking activity significantly increased after TBS in the control slices, but similarly to LTP, it returned to baseline in the Abeta1-42 treated group after 1.5 h. When $_{\text{Abeta1-42}}$ was applied immediately after TBS, there was no LTP impairment; however, spiking rate was enhanced 3 h after TBS compared to slices having received only TBS. Abeta$_{1-42}$ induced a marked and permanent increase in firing rate 1.5 h and 3h after treatment, without increasing the fEPSP amplitude in slices not subjected to TBS. Control slices, which did not receive either Abeta$_{1-42}$ nor TBS showed unchanged fEPSP amplitude, but a slight reduction of spike number after recording for 3 h.

Conclusion: Based on these results we suggest that the LTP impairment by Abeta$_{1-42}$ might be the consequence of altered spiking activity. Moreover, $_{\text{Abeta1-42}}$ increased excitability, which was not reflected in the fEPSP amplitude.

P-N10:

DOCOSAHEXAENOIC ACID REDUCES BETA-AMYLOID INDUCES TOXICITY IN CELLS OF THE NEUROVASCULAR UNIT

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Introduction: Alzheimer's disease (AD) is characterized by the accumulation of beta-amyloid peptides as perivascular deposits and senile plaques in the brain. The intake of the polyunsaturated fatty acid docosahexaenoic acid (DHA) has been associated with decreased amyloid deposition and reduced risk in AD in several epidemiological trials; however the exact underlying molecular mechanism remains to be elucidated.

Objectives: The aim of the study was to test whether DHA can exert a direct protective effect on the elements of the neurovascular unit, such as neurons, glial cells, brain endothelial cells and pericytes, treated with beta-amyloid.

Materials & methods: The effect of oligomeric beta-amyloid 1-42 and DHA was examined in toxicity tests on cultured cells and ex vivo brain samples; in permeability and functional assays on brain endothelial cells.

Results: A dose-dependent high cellular toxicity was found in viability assays in all cell types and on acute hippocampal slices after treatment with beta-amyloid. The cell morphology also changed dramatically. In brain endothelial cells damaged barrier function, increased para- and transcellular permeability, elevation of the production of reactive oxygen radicals, and inhibition of P-glycoprotein efflux pump was observed after peptide treatment. DHA significantly decreased the beta-amyloid induced toxic effects in all cell types measured by viability assays, and protected the barrier integrity and functions of brain endothelial cells.

Conclusion: Present results indicate for the first time that DHA can protect not only neurons but also the other elements of the neurovascular unit from the toxic effects of beta-amyloid and this double effect may be beneficial in AD

T1:

A TUBERCULOSIS: THE INTERSECTION OF ANCIENT EVIDENCE AND CONTEMPORARY STRAIN VARIATION

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Through the prism of ancient tuberculosis in the Americas, this paper will first present a model for the history of this disease via the medium of skeletal evidence for disseminated TB in the Americas prior to the "Era of Exploration". We present the enigma of the apparent American tuberculosis, given models for the development of human tuberculosis as derived from the bovine form at least 8,000 to 9,000 years ago. Until recently, authors of general books or chapters on the history and co-evolution of our species and *Mycobacterium tuberculosis* have largely ignored the American expression. Since the 1990s, however, this information has been supplemented by aDNA evidence and subsequently by evolutionary models based on contemporary *Mycobacterium tuberculosis* strain and *Mycobacterium sp.* molecular variation. In so doing, the antiquity of ancestral forms of both tuberculosis and humans are proposed to have been in contact for perhaps millions rather than thousands of years. The most parsimonious global history for TB places its origin in Africa, then spreading to South and Southeast Asia. Evidence is presented that supports the fact that it is this form that was carried to the New World during human migration events. Subsequent spread to Europe and increased virulence characterized the pathogen carried around the globe in the 15th century, which continues to plague 21st century global health.

T2:

EVOLUTIONARY CHANGES IN THE GENOME OF *MYCOBACTERIUM TUBERCULOSIS* (MTB) AND THE HUMAN GENOME FROM 9000 YEARS BP UNTIL MODERN TIMES

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With the first reported finding of MTB DNA in ancient skeletons (1), research into microbial evolution became possible. A study of mummies in 18th Century Vác, Hungary found over 65% of the mummies were TB+. However, a 95-year-old woman with calcified mediastinal lymph node positive for MTB DNA, posing the question why was she cured from a childhood Ghone lesion?

In 9000 BP in the village of Atlit Yam in Israel a woman and child had human MTB. This is where a naïve bacterium infected a human with a naïve immune system. Over 4000 years later in biblical Jericho a skeleton is positive for TB. Over this period urbanization developed and the bacillus became exposed to the reactions of the human immune system. As the TB killed off susceptible humans the survivors had genes giving them some resistance to disease similarly the bacteria selected characteristics capable of overcoming host resistance. This has continued until modern times creating a Darwinian process of increasing selection of bacteria. Changes important to our understanding of the co-evolution of both genomes.

We have commenced whole genome studies of the bacteria for genome changes over the millennia and at host susceptibility/resistance genes on the NRAMP and Killer Cell Immunoglobulin-like Receptors (KIRs). Preliminary results will be discussed here.

Spigelman M and Lemma E (1993) The use of the polymerase chain reaction to detect *Mycobacterium tuberculosis* in ancient skeletons. Int J Osteoarchaeol 3; 137-143.

Fletcher H et al. (2003) Widespread occurrence of *Mycobacterium tuberculosis* DNA from 18th-19th century Hungarians. AJPA 12: 144-52.

T3:

THE EVOLUTIONARY DEVELOPMENT OF *MYCOBACTERIUM TUBERCULOSIS* BEIJING GENOTYPE STRAINS AND WORLDWIDE EMERGENCE OF RESISTANCE

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Since the early 1990s, DNA fingerprinting of *Mycobacterium tuberculosis* has been used in contact tracing and source case finding and this brought many new insights in the epidemiology of tuberculosis. Apart from this, the phylogeny of the *M. tuberculosis* complex has been studied extensively and several genotype families have been described, under which the Beijing lineage. Latter genotype family has been associated with multidrug resistance in multiple areas, under which Europe. Moreover, the Beijing strains were found associated with low age of patients and BCG vaccination and this suggests active spread and emergence.

On basis of Whole Genome Sequencing of Beijing strains from China, Vietnam and South Africa, it was recently concluded that Typical Beijing strains are genetically highly clonal and have mutations in genes that

regulate the protein expression. Moreover, the Beijing genotype strains showed, in comparison to East African Indian genotype strains, extremely elevated mutation frequencies for rifampicin resistance and mutants were selected from a large rifampicin concentration window.
In most geographic areas with a high prevalence of Beijing strains, resistance plays a major role. There is increasing evidence of an evolutionary development in this genotype that hampers TB control world wide.

T4:

EVOLUTION OF TUBERCULOSIS IN ANCIENT MUMMIES AND SKELETONS

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The molecular identification of infectious diseases in skeletal and mummified human remains allows a unique insight into the presence, frequency and evolution of pathogens in ancient populations. The analysis of ancient DNA, including extraction, amplification and characterization of specific DNA fragments, made it possible to detect a wide range of bacterial, protozoal and viral infections in tissue samples of mummies and skeleton from different time periods and geographic origins. This holds particularly true for the identification of the *M. tuberculosis* complex, which seems to be more robust than other microbes due to its waxy, hydrophobic and lipid-rich cell wall. These observations provided useful information about the occurrence, but also frequency of tuberculosis in former populations. Moreover, these studies present new evolutionary models and indicate the way of transmission between human and animals. In this paper the most important findings of ancient tuberculosis DNA will be presented and the methodological approach for the detection of pathological conditions in human remains will be reviewed. Paleomicrobiology has the capability to significantly contribute to a better understanding of the evolutionary mechanisms of infectious diseases and their impact on past human populations. Nevertheless, major attention has to be drawn to the avoidance of contamination and the authenticity of the results. The data obtained from paleomicrobiological studies represent an important addition to recent scientific work in the fight against infectious diseases. In the future, it can be hoped that the analysis of ancient pathogen DNA can contribute to the prevention, treatment and possibly even eradication of tuberculosis.

T5:

TUBERCULOSIS: MOLECULAR AND EPIDEMIOLOGICAL INSIGHT – WHAT CAN ANCIENT HUMAN REMAINS TELL US?

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TB remains a major health problem: it is the second cause of death from an infectious disease worldwide (WHO report 2011). During most of the *Mycobacterium tuberculosis* complex (MTBC) evolution, the immune response of human host and changes in human demography and socio-cultural environment have been the most important selective pressures. If MTBC evolved as a human pathogen in Africa and has spread outside Africa with the first migrations of modern humans about more than ten-thousand years ago, its life history traits have been shaped by the immune system. In fact, this immune machinery is effective for controlling tuberculosis infections and numerous studies described a variety of human genetic variants linked to TB. There is increasing evidence that MTBC strains have evolved different ways to overcome human adaptive and innate immune

system. However, the results of these studies reveal some inconsistencies even within study populations. Apart from genetic background and HIV co-infection, other factors such as population density, alcoholism, diabetes and malnutrition (and some others) increase susceptibility to TB infection and TB disease. This highlights the complexity of the problem. Paleomicrobiology analysis and paleopathology give the opportunity to genotype ancient TB strains circulating in past populations. Accessing ancient human pathogens allows us to a better understanding of infectious agents over a longer time scale and confrontation with the dynamic of modern TB strains. Some important questions remain to be answered including the part played by the domestication process, the urbanization and the interactions with other pathogenic agents during TB evolution.

T6:

CRISPR GENETIC DIVERSITY STUDIES AS A MEAN TO RECONSTITUTE THE EVOLUTION OF THE *MYCOBACTERIUM TUBERCULOSIS* COMPLEX

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Clustered Regularly Interspersed Palindromic Repeats (CRISPR) loci were discovered at the end of the 80s in *Escherichia coli*, though designated as such in 2002. Their role in adaptative anti-phage immunity in coordination with CRISPR-associated *cas* genes, and to other physiological mechanisms, created a new field in applied and academic research. CRISPR-associated *cas* genes encode proteins with functional domains typical of nucleases, helicases, polymerases and polynucleotide-binding activities. With the advent of prokaryotic RNA interference linked to CRISPR, regulation of gene expression in bacteria and archaea changed paradigm putting a new emphasis on new mechanisms of lateral gene transfer and phage-based gene flows, which are likely to play important functions in species physiology and evolution.

We will rapidly review recent discoveries gathered on CRISPR interference mechanisms in some models and provide much more detailed information on how CRISPR systems were characterized on the *Mycobacterium tuberculosis* complex (MTBC) whether on membranes or on microbead-based arrays for academic and for operational research on tuberculosis evolution history. In particular, we will described how spoligotyping, the original reverse-line blot hybridization method that characterizes the CRISPR locus diversity was a key factor in the discovery of the main phylogeographical lineages of the MTBC and in many spatio-temporal studies of the tuberculosis disease, before VNTR and Whole Genome Sequencing (WGS) took the lead and became new reference tools to reveal tuberculosis evolution history. We will conclude by showing how an improved spoligo-rif-typing technique could provide new clues on tuberculosis evolution and new tools for tuberculosis control.

T7:

A GLIMPSE OF THE EARLY EVOLUTION OF THE TUBERCLE BACILLUS

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Mycobacterium tuberculosis, the agent of human tuberculosis (TB), is characterized by pandemic clonal spread. In contrast, *Mycobacterium canettii*, and other human TB isolates with unusual, smooth colony morphology, named *M. prototuberculosis*, are non-clonal and restricted to East-Africa. We have shown by comparative pathogenomic analyses of five representative smooth TB strains that their genomes feature up to 100 kb more DNA, fewer

molecular scars, distinct origins of the CRISPR region, traces of extensive intra-species DNA recombination, and up to 60 000 single nucleotide polymorphisms (SNP) compared to the MTBC, providing for the first time insights into the putative progenitor gene pool of tubercle bacilli before the divergence and clonal expansion of the MTBC. Importantly, we also showed that the smooth strains all exhibited lower virulence relative to *M. tuberculosis* in mouse infection models, best demonstrated by a marked default in persistence resulting in complete clearance of the phylogenomically most distant strain after 30 weeks. These defaults were consistently correlated with lower histopathological damages and innate and adaptive immune responses. Our results thus connect the recent evolutionary success of the MTBC to its specific ability to persist in the host, and provide a novel experimental platform for the study of the different molecular factors involved in this process.

T8:

THE EVOLUTION OF *MYCOBACTERIUM TUBERCULOSIS* RESEARCH SINCE ROBERT KOCH'S DISCOVERY

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Although sometimes forgotten, the overarching goal of tuberculosis (TB) research is to deliver solutions to a major public health problem in the form of new knowledge to underpin activities in the diagnostic, prophylactic and therapeutic areas. During the past 130 years progress has been driven by two factors: firstly, insightful thinkers and opinion-formers have heavily influenced biomedical approaches; secondly, access to new technologies has led to greater sophistication in experimental design and clinical applications. To illustrate this, the impact on TB research of the successive technological revolutions will be compared and contrasted in the context of their respective endpoints and protagonists.

T9:

REGIONAL ACTION PLAN TO PREVENT AND COMBAT M/XDR-TB

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Drug resistant TB is spreading at an alarming rate in the WHO European Region. Of the 27 countries worldwide with a high burden of multidrug resistant (MDR)-TB, 15 are in the Region. Annually around 80,000 MDR-TB are estimated to occur in the Region. Most countries have notified cases of Extensively Drug Resistant (XDR)-TB. WHO/Europe has developed a Consolidated Action Plan to Prevent and Combat M/XDR-TB 2011-2015.

Our objectives is to analysis the expected achievements of the Regional Action Plan with the targets of diagnosing 85% of estimated MDR-TB cases; and treating at least 75% of them successfully. The official data reported to WHO were used to analyze the epidemiologic trends. A dedicated transmission model was developed to calculate the expected achievements. WHO CHOICE database was used for calculating the costs of implementing the Action Plan.

Implementation of the Regional Action Plan would lead to the prevention of the emergence of 250,000 new MDR-TB patients and 13,000 XDR-TB patients, saving US$ 7 billion and 120,000 lives. Cost analysis has proved the Action Plan as highly cost effective. The Action Plan and its accompanied Resolution are fully endorsed by the 61st WHO Regional Committee.

WHO/Europe will assist Member States in further development and integration of national MDR-TB response plans and provide the necessary technical assistance. Member States need to improve their health system performance and address TB determinants. There is a need for research and development for new medicines and tools and introduction of patient-friendly services.

T10:

TB IN 2012: BURDEN, STRATEGIES, AND RESEARCH NEEDS

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The WHO estimates that 8.8 million new cases of tuberculosis (TB) and 1.45 million TB deaths occurred in 2010 worldwide. Of all cases, 1.1 million occurred among people with HIV/AIDS, of whom 350,000 were fatal. About 650,000 cases were multidrug-resistant (MDR). XDR-TB has been reported officially from 69 countries. The 2015 Millennium Development Goal of halting and reversing TB incidence rate is estimated to have been reached in 2002, and the total number of cases is declining slowly at 1% per year since 2006. Since the establishment of DOTS in 1995 (later enhanced to Stop TB Strategy), 46 million people have been cured and nearly 7 million lives saved compared to the 1995 standards. Challenges still confront global TB control in 2012, including: unsecured funding for most low-income countries; a third of estimated cases is still not diagnosed or reported; the burden of HIV on TB control in Africa; MDR-TB is threatening achievements world-wide; health policies, systems and services are weak in many settings; and there is limited transfer of new technologies to high-burden countries. TB remains a major global health issue. A four-pronged approach is proposed to foster better control: (1) TB control programmes must optimize case management as described in the Stop TB Strategy; (2) bold policies and properly resourced health system services are crucial to allow effective core TB interventions; (3) address socio-economic determinants of TB; (4) increased investments in R+D of new tools that would ensure a fast incidence decline to target elimination by 2050.

T11:

TUBERCULOSIS – A GLOBAL EMERGENCY: TOOLS AND METHODS TO MONITOR UNDERSTAND AND CONTROL THE EPIDEMIC

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Tuberculosis (TB) remains a major cause of illness and death worldwide. In addition to the WHO's strategy to Stop-TB, we will argue in favor of a concerted and coordinated response by monitoring global TB spread, drug-resistance surveillance and populations at risk.

We will focus on the available tools to identify and analyze the various circulating clones of *Mycobacterium tuberculosis* complex (MTC) worldwide. The role of TB molecular typing in correlating genotyping data and epidemiological investigations, to underline emergence of specific TB clones, and to assess the predictive value of molecular clustering will be described.

The most commonly used genotyping methods to characterize circulating MTC clones are PCR-based spoligotyping and VNTR minisatellites. We will review evidence based on available genotyping information on more than 85000 *M. tuberculosis* complex isolates from 160 countries.

Ongoing developments regarding one of the biggest fingerprinting databases housed at Institut Pasteur de Guadeloupe, use of new web tools, and new evidence regarding minisatellite-based classification of MTC genotypic lineages is described. We will also describe a future application based on bayesian networks (TbBayesNet) which is able to capture hidden knowledge in huge international MTC genotyping databases. It constitutes a useful decision support for phylogenetic classification of strains, for correction and completion of typing profiles (missing MIRU and spoligotype patterns), and even allows to speculate on the geographical origin of a strain, and this, on a total of 15 macro-regions around the globe.
These new tools allow detailed geo-localization and worldwide mapping of TB clusters based on multimarker data.

T12:

TB IN HUNGARY IN THE 20TH AND 21ST CENTURIES

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At the beginning of 20th century tuberculosis (TB) was a major public health problem in Hungary. TB mortality was almost the highest in Europe (400/100.000, 25% of total mortality). Organized TB control was proposed and maintained by civil initiative. The first TB sanatorium was opened in 1901 and TB dispanseries were established. TB mortality gradually diminished, since the twenties TB control institutes and activities expanded (1938: 6000 beds, 87 dispanseries; 1944: 134 dispanseries). After 2nd WW, TB control got high priority in the public health program. Number of TB beds gradually increased (between 1960-63 3000 new TB beds, in 1970 11.259 beds), 166 TB dispensaries covered the country. BCG vaccination of newborns started in 1953 (performance over 98%). In 1950 National TB Institute was established with the task to elaborate and to manage a comprehensive TB control program: local registration of cases, annual reporting to the National Institute, regular training of dispensary-doctors to ensure uniform methods in the practice. Potent TB drugs – SM, PAS, INH - became available that time. Drugs are free of charge through TB dispensaries. In 1960 mass X-ray screening program started. In this period 70% of new cases were detected by screening. Since 1950 TB incidence gradually and continously decreased (annually by 10%). In 1996 TB surveillance was introduced (individual registration and follow up treatment according to WHO recommendation). Characteristics of TB epidemic in the 21st century: low incidence (14,4 / 100.000), concentrated to high risk groups. Resistant cases: previously not treated 13%, previously treated 18,9%, MDR 3,9%.

T13:

LEADS SELECTION AND CHARACTERIZATION OF ANTITUBERCULAR COMPOUNDS USING A NESTED CHEMICAL LIBRARY OF KINASE INHIBITORS

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Discovering new drugs to treat tuberculosis more efficiently and to overcome multidrug resistance is a world health priority. To find antimycobacterial scaffolds, we screened our kinase inhibitor library of more than 12,000 compounds using an integrated strategy involving whole cell-based assays with *Corynebacterium glutamicum* and *Mycobacterium tuberculosis*, and a target-based assay with the protein kinase PknA and

PknB and PknG. We have found several very potent hits inhibiting the kinases but their minimal inhibitory concentrations (MIC) in whole cell-based assay against *M. tuberculosis* were not good. So we screened the entire library in the whole cell-based assay. Several potent "hits" were found in this screening approach, which displayed minimal inhibitory concentrations (MIC) against *M. tuberculosis* below 10 uM and were non-mutagenic and non-cytotoxic. Some of these hits were specific for *M. tuberculosis* versus *C. glutamicum*. The most active hits represented 8 scaffolds and we have identified various strategies for lead optimization and a series of novel potent compounds have been synthesized. With some of the best compounds we have started developing a "target fishing" approach which is based on a specific chemical modification and an affinity chromatography based proteomic technology. The analogs will also be characterized and optimized for avoiding the transporters.

T14:

ANCIENT DNA ANALYSIS - AN ESTABLISHED TECHNIQUE IN CHARTING THE EVOLUTION OF TUBERCULOSIS AND LEPROSY

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Tuberculosis and leprosy range from active multibacillary disease to latent or paucibacillary infections with little or no pathology – suggesting a long time-scale for host and pathogen co-existence. Palaeopathology enables recognition of archaeological cases and PCR can detect DNA of the causative organisms. The lipid-rich cell wall of *Mycobacterium tuberculosis* and *Mycobacterium leprae* restricts permeability, believed to aid the persistence of pathogen DNA after death. It also provides confirmatory biomarkers that are more stable than DNA. Extraction of mycobacterial ancient DNA needs to overcome problems of taphonomic damage, covalent cross-links and co-extraction of inhibitors. Specific primers designed for short fragments and linked to fluorescent probes give good results, especially if targeted at multi-copy loci. Ancient DNA studies have confirmed tuberculosis and leprosy, including many cases with non-specific or no visible skeletal pathology. This is consistent with the natural history of these diseases if left untreated. Co-infections have also been detected. *M. tuberculosis* and *M. leprae* are obligate parasites which have co-evolved with their human host, resulting in a clonal distribution. Therefore genotyping and sub-genotyping based on single nucleotide polymorphisms (SNPs) can indicate their origins, spread and phylogeny. Knowledge of extant genetic lineages at particular times in past human populations can be obtained from well-preserved specimens where molecular typing is possible, using deletion analysis, microsatellite analysis and other methods based on repetitive sequences. Such studies have identified non-bovine tuberculosis from a Pleistocene bison from 17,500 years BP, human tuberculosis from 9000 years ago and leprosy from over 2000 years ago.

T15:

OLD WORLD TUBERCULOSIS: EVIDENCE FROM HUMAN REMAINS WITH A REVIEW OF CURRENT RESEARCH AND FUTURE PROSPECTS

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Tuberculosis (TB) has plagued human populations for many years, and continues to do so globally. Some factors in its occurrence are different today (e.g. the association with HIV), but others such as poverty,

contact with infected animals, migration and specific occupations apply to both past and present TB. This paper takes a broad approach to what we know about the presence of Old World TB in human remains from archaeological sites. Starting with the earliest evidence dated to the Neolithic period (Italy, c.6000 BP), it traces how this infectious disease developed as a major challenge to communities living in more densely populated urban environments in the later and post-medieval periods, particularly in Europe. The paper will critically consider the available diagnostic criteria for TB in skeletal and mummified human remains. It will also focus on the use of biomolecular analysis in furthering our understanding of its origin and evolution, including geographic location of strains and gene variants related to susceptibility. Since the sequencing of the *Mycobacterium tuberculosis* complex genome in 1998, and the first ancient DNA analysis in 1993, it has become possible to answer more challenging questions. Furthermore, the relevance of exploring TB in archaeological animal remains is an area of increased interest, despite the work of Brosch *et al*. (2002) overturning original theories of the co-evolution of *M. bovis* and *M. tuberculosis*. The palaeopathology of TB provides a deep time perspective for clinical studies, allowing the rise and decline of an infection to considered over thousands of years.

T16:

THE EARLY MEDIAEVAL MANOR-PLACE GARS/THUNAU (LOWER AUSTRIA): A TERRAIN OF ENDEMIC TUBERCULOSIS?

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Over the last decades, an increasing number of studies aimed to shed light on the origin and spread of tuberculosis in past human populations. To a great deal these studies were based on macro- and microscopic investigations of pathological lesions and included a variety of features, such as the "classical" Pott´s disease and joint tuberculosis. More recently, also other pathological (unspecific) features probably linked with *M. tuberculosis* infection such as rib lesions (newly built bone formations along the pleural side) and endocranial alterations (small "pits" and "thin new bone formation at the cranial base", etc.) were recorded and discussed in regard to their differential diagnostic potential.

Here we present the results of a systematic palaeopathological survey of the Early Mediaeval population of Gars/Thunau (Lower Austria), which – at this stage – includes 373 individuals,recovered at two archaeological sub-sites, a necropolis within a (densely populated) fortified settlement (including a manor house) at the top of a hill and a riverine settlement with numerous burials in the valley area. At the latter, an area which was probably less densely populated, archaeological evidences for large-scale handicraft and agricultural activities were documented. Thus, we hypothesise that the two contemporary populations of Gars/Thunau differ not only in their social affiliation/condition, but also in their type and frequencies of population density related infectious diseases (in particular tuberculosis). Moreover, we investigated the molecular evidence of the causative organism in three immatures exhibiting pathological changes in the endocranial side and discuss these findings in regard to the macroscopic features observed.

T17:

THE IMPACT OF TUBERCULOSIS TO THE 18TH CENTURY VÁC POPULATION, HUNGARY

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The infectious disease caused by *Mycobacterium tuberculosis* has been called by several names: phtisis, tuberculosis, *morbus hungaricus.* It used to be considered as an endemic disease and it seemed that it had been eliminated but it returned not too long ago.

The studies of partially and completely mummified individuals gave opportunity to analyse the occurrence of tuberculosis in the 18th century population, at a time when the disease was reaching epidemic levels just before the industrialisation and urbanization.

After the discovery of the naturally mummified bodies and skeletal remains of the 265 individuals in 1994-95 in the Dominican Church of Vác, a multidisciplinary research was started within the framework of Hungarian and international co-operation.

The coffins with the names and contemporary church archives offered help in identification and made demographic and micro-history examinations possible. Morphological and radiological studies revealed cases of vertebral tuberculosis. Skeletal and naturally mummified tissues were examined for the presence of *Mycobacterium tuberculosis* complex DNA. The research conducted by Helen D Donoghue and Mark Spigelman discovered that overall 67.7% individuals proved to be positive. This does not mean that all of them died of TB. Many carried the disease but exhibited no symptoms.

The ancient human remains provide excellent material for the understanding of the origin and evolution of TB, and give an opportunity for the study of the population living in the 18th century, before the appearance of medical therapy and the resistance to antibiotics.

The research was supported by the OTKA Grant No 61155 (Hungary).

T18:

PALEOPATHOLOGY OF MYCOBACTERIAL INFECTIONS IN HUNGARY: NEW RESULTS

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Infections by *Mycobacterium leprae* and *M. tuberculosis* complex bacteria may produce pathognomic alterations in human bones on the basis of which they are diagnosed in paleopathology. We have osteological evidence of ancient Hungarian leprosy since more than twenty years. TB discoveries have a longer history: a lot of ancient cases have been discovered during the last half-century. The last 15 years represent a remarkably progressive period in the study of the two diseases. First, we have to mention the introduction of a DNA and lipid biomarker methods for the confirmation of ancient Hungarian leprosy and TB cases. Second, we cannot forget the progresses in the osteological diagnostics of mycobacterial diseases, which facilitate a more precise estimation of the infection prevalences in ancient skeletal populations. Actual studies prove the complementarity of the paleopathological and paleomicrobial techniques. As for some concrete news from this field in Hungary from the last 15 years, first we have to mention the Vác mummy collection which furnished extraordinary paleomicrobial data on past TB infection. Paleopathological investigation of skeletal series has been intensified. The use of new diagnostic criteria, in combination with biomolecular techniques enabled to recognize more ancient Hungarian TB cases – the Neolithic occurrence of this disease has recently been proved. Studies of Paleolithic cases are also in progress. Important paleopathological and paleomicrobial data were obtained on TB-leprosy coinfection in ancient skeletons from Hungary – these results can furnish important new information on past evolution of the two diseases.

Research was supported by OTKA Grant 78555 and SROP 4.2.1./B-09-1/KNOV-210-0005.

T19:

ESX/TYPE VII SECRETION SYSTEMS OF MYCOBACTERIA: INSIGHTS INTO EVOLUTION, PATHOGENICITY AND PROTECTION

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Pathogenesis of *Mycobcterium tuberculosis* depends on the secretion of key virulence factors, such as the 6 kD early secreted antigenic target ESAT-6 (EsxA) and its protein partner, the 10 kD culture filtrate protein CFP10 (EsxB) via the ESX-1 secretion system. ESX-1 represents the prototype system of the recently named type VII secretion systems that exist in a range of actinobacteria. The *M. tuberculosis* genome harbors a total of five gene clusters coding for type VII secretion systems, designated ESX-1 - ESX-5, with ESX-4 being the most ancient system from which other ESX systems seem to have evolved by gene duplication and gene insertion events. The different ESX systems show similarity in gene content and gene order but differ in function, e.g. ESX-1 and ESX-5 systems in *M. tuberculosis* are both implicated in virulence, but for different reasons. While ESX-1 was suggested to be implicated in the lysis of the host cell phagosomes, ESX-5 is implicated in secretion of PE and PPE proteins and cell wall stability. Research on type VII secretion systems has thus become a large and competitive research topic that is tightly linked to studies of host-pathogen interactions of pathogenic mycobacteria. Insights into this matter are of importance for redrawing the patho-evolution of *M. tuberculosis*, which might help improving current strategies for prevention, diagnostics and therapy of tuberculosis as well as for a better understanding of the virulence mechanisms employed.

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T20:

HORIZONTAL TRANSFER IN *MYCOBACTERIUM TUBERCULOSIS* COMPLEX

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Mycobacterium tuberculosis complex (MTC) strains are highly clonal. A first analysis of sequence polymorphism showed a very limited diversity in housekeeping genes in comparison with other bacterial species. However diversity was observed at the level of repetitive sequences, either direct repeats or mobile elements. Transposition of *IS6110* provides an additional promoter to genes adjacent to their insertion site, thus modifying virulence phenotypes as described for the MDR TB outbreak with strain B in Spain. Our analysis using tetranucleotide signatures identified the presence of sequences acquired by horizontal transfer from other bacterial species. Several loci exhibit characteristics of pathogenicity island i.e. presence of genes involved in interaction with host cells and that are flanked by repeats, tRNA sequences or residual mobile elements. Our recent anaysis of the whole genome sequence of 24 strains belonging to different lineages and geographical regions provided evidences for intra-species recombination and natural selection in driving the ongoing evolution of MTC genome strains (Namouchi A. *et al.* Genome Research 2012). This provides a new vision about the genome evolution of *M. tuberculosis* that was considered until now as clonal and evolving through genetic drift.

T21:

PATHOBIOLOGICAL VARIABILITY OF MYCOBACTERIUM TUBERCULOSIS COMPLEX STRAINS

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Pathogens of the *Mycobacterium tuberculosis* complex (MTBC; causative agents of tuberculosis [TB]) remain one of the leading infectious killers worldwide. Only recently, fine molecular typing has indicated that the genetic heterogeneity among MTBC isolates, albeit relatively low, influences the transmissibility and virulence of clinical isolates as well as the induced immune response. However, only limited information about the underlying pathogenetic mechanisms are available.

Due to the monomorphic nature of the MTBC, genomic variation among individual patient isolates has been neglected for decades of research and has not been considered as pathobiological important. However, recent studies applying comparative genomics indicate that the MTBC is composed of manifold phylogenetic lineages that show significant levels of functional genome variation. Subsequently, pathobiological importance of MTBC strain diversity has been confirmed by demonstrating enhanced spread of strains of particular lineages, e.g., in the context of multidrug resistance, defining lineage-specific disease characteristics, and confirming host–pathogen co-evolution and specific host–pathogen interactions. Furthermore, we detected phylogenetic lineage specific transcriptome profiles in liquid cultures and upon macrophage infection.

In conclusion, the genetic diversity among MTBC strains appears to be higher than previously assumed and might have functional consequence as already indicated by differences in virulence model systems. Our preliminary analysis already revealed genotype specific expression signatures and confirm that genomic diversity of clinical isolates translates in specific transciptome profiles.

T22:

A TALE OF TWO GENOTYPES: CONTRASTING PHYLOGEOGRAPHY OF *MYCOBACTERIUM TUBERCULOSIS* BEIJING AND URAL FAMILIES

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Here I will discuss population structure and phylogeography of two contrasting genotypes of *Mycobacterium tuberculosis*, Beijing and Ural, in the context of human history.

Data on 11 MIRU-VNTR loci of 2400 Beijing genotype strains from all continents were studied by phylogenetic and statistical methods. Highest 90% rate and diversity (HGI 0.80-0.95) of Beijing genotype in north China suggest it to be area of its origin. Genetic distances between Beijing populations correlated with geography over uninterrupted landmasses. In contrast, large water and long time-frame generate remarkable outliers. Weak and less expected affinities of distant *M. tuberculosis* populations may reflect hidden epidemiological links due to unknown migrations.

The available spoligotyping data on Ural sublineages were extracted from SpolDB4 database and original publications. Rigorous reanalysis reduced true rate of the Ural genotype in the Ural area in Russia from initial 15% to 7%. In contrast, its frequencies peak elsewhere (north/north-east of Black Sea). However, since this name is used since 2005, it seems most parsimonious to continue its use even if misleading. Ural family is moderately widespread in Eurasia beyond the areas of origin. Ural strains are not marked by increased

pathogenic capacities. This reflects its low contagiosity as a consequence of long-term co-adaptation with human host. The north/east Pontic area may be area of origin and primary dispersal of the Ural genotype in Eurasia.

Large-scale SNP or WGS population-based studies targeting strains from indigenous populations and, eventually, analysis of ancient DNA will better test hypotheses about evolutionary history of the bacterial lineages.

T23:

THE CELL ENVELOPE OF TUBERCLE BACILLI

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Mycobacteria are Gram-positive microorganisms that share with Gram-negative bacteria the property of having two permeability barriers in their cell envelope. This structure in mycobacteria is composed of a plasma membrane surrounded by a thick cell wall, and an outer layer (also called capsule). The cell wall contains very long-chain fatty acids (called mycolic acids) that greatly contribute to the structure and function of the outer membrane. The lipid-rich cell wall contains also various exotic molecules that are species- or type-species specific and may play an important role in the virulence of pathogenic bacteria such as *Mycobacterium tuberculosis*. Comparative analysis of the lipid contents of tubercle bacilli have revealed the diversity and established the structures of the latter compounds, notably those of the phthiocerol family whose biosynthetic pathways has recently been deciphered. The accumulated data may open new hypotheses on the evolution of *M. tuberculosis.*

T24:

ANCIENT MYCOBACTERIAL LIPIDS: KEY REFERENCE BIOMARKERS IN CHARTING THE EVOLUTION OF LEPROSY AND TUBERCULOSIS

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The cell envelopes of pathogenic mycobacteria contain a range of unusual components, including characteristic long-chain lipids. Forty-five years ago in Oxford, under the tutelage of an eminent Hungarian chemist Nicolas Polgar, I determined the essential structures of the mycolic acids and phthiocerol dimycocerosate waxes from *Mycobacterium tuberculosis*. Consideration of the details of these structures resulted in an original, but recently well-verified, proposal of a cell envelope with two distinct lipid domains (Minnikin *in* The Biology of the Mycobacteria, eds. Ratledge & Stanford, Academic Press, London, 1982 pp. 95-184). The resulting coherent, extremely hydrophobic cell envelope has clear importance in pathogenicity, but it is also likely that it contributes significantly to the survival of mycobacterial biomarkers, including DNA, in archaeological materials.

The current lipid biomarkers that are proving to be reliable for the diagnosis of ancient disease are indeed the mycolic acids and the phthiocerol dimycocerosate (PDIM) waxes, which can distinguish tuberculosis and leprosy. The PDIMs have 90-100 carbons but they can be broken down to 26-34 carbon mycocerosic acids and 30-36 carbon phthiocerols. Mycocerosates are profiled by negative-ion chemical-ionisation gas

chromatography-mass spectrometry, but fluorescence high-performance liquid chromatography (HPLC) is used for phthiocerol derivatives. The latter HPLC protocols also yield highly-diagnostic profiles of 70-90 carbon mycolates and diagenetic degradation of the different types can be observed. Additional biomarker lipids, such as 27-carbon mycolipenic acid, are increasingly being recognised. Analysis of material, up to 17,000 years BP, is providing evidence that lipid biomarkers can contribute significantly to understanding the evolution of tuberculosis.

T25:

AG 85: A MAJOR SECRETION PROTEIN OF *MYCOBACTERIUM TUBERCULOSIS* CAN BE IDENTIFIED IN ANCIENT BONE

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Tuberculosis is caused by a group of closely related bacterial species termed the *Mycobacterium tuberculosis* (MTB) complex. These bacterial species are *M. tuberculosis, M. bovis, M. cannetti, M. africanum, M. microti, M. caprae, M. pinnepedi* and the Bacillus Calmette-Guérin (BCG). These pathogenic species are able to survive and grow within macrophages which enable them to evade the host immune system. The key antigenic targets from the MTB-complex, recognized by the cells of the host, were identified as Ag 85 and a secreted 6-kD protein ESAT-6. Elimination of *M. tuberculosis* infection mainly depends on the success of the interaction between infected macrophages and T-lymphocytes, $CD4^+$-T-cells play a crucial role. $CD4^+$ T cells exert their protective effect by the production of cytokines, primarily interferon-y (IFN-y) by stimulation with mycobacterial antigens Ag 85 and ESAT-6. The proteins of the antigen 85 complex are Ag 85A; Ag 85B and Ag 85C, molecular weight is between 31kDa and 30 kDa. Sequences of Ag 85A and 85C from *M. tuberculosis* and *M. bovis* are 100% identical and Ag 85B sequences differ only by one amino acid. This mycobacterial antigen Ag 85 can be identified in human ancient bone by special antibodies in individuals which show in their bones the characteristic vestiges of the tuberculosis disease.

T26:

DETECTION AND MOLECULAR CHARACTERIZATION OF 9000-YEAR OLD *MYCOBACTERIUM TUBERCULOSIS* FROM A NEOLITHIC SETTLEMENT IN THE EASTERN MEDITERRANEAN

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Mycobacterium tuberculosis is the principal etiologic agent of human tuberculosis. It has no environmental reservoir and is believed to have co-evolved with its host over millennia. This is supported by skeletal evidence of the disease in early humans, and inferred from *M. tuberculosis* genomic analysis. Direct examination of ancient human remains for *M. tuberculosis* biomarkers should aid our understanding of the nature of prehistoric tuberculosis and the host/pathogen relationship.

We used conventional PCR to examine bone samples with typical tuberculosis lesions from a woman and infant, who were buried together in the now submerged site of Atlit-Yam in the Eastern Mediterranean, dating from 9250-8160 years ago. Rigorous precautions were taken to prevent contamination, and independent centers were used to confirm authenticity of findings. DNA from five *M. tuberculosis* genetic loci was detected and had characteristics consistent with extant genetic lineages. High performance liquid chromatography was

used as an independent method of verification and it directly detected mycolic acid lipid biomarkers, specific for the *M. tuberculosis* complex.
Human tuberculosis was confirmed by morphological and molecular methods in a population living in one of the first villages with evidence of agriculture and animal domestication. The widespread use of animals was not a source of infection but may have supported a denser human population that facilitated transmission of the tubercle bacillus. The similarity of the *M. tuberculosis* genetic signature with those of today gives support to the theory of a long term co-existence of host and pathogen.

T27:

SANATORIA, ARCHIVES AND SKELETONS: AN INTERDISCIPLINARY APPROACH TO THE STUDY OF PALEOTUBERCULOSIS

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Tuberculosis may be an acute or chronic infection of bone and/or soft tissues. However, human skeletons only registered a small percentage of those cases. From pre-historic and historic populations evidences comes mainly from Pott's disease, followed by changes in the hip and knee joints, and, more recently, by the presence of new bone on visceral surfaces of ribs. This puzzling situation has been discussed by many researchers, including at the light of "osteological paradox".
This work aims to explore the potential of documentary data in the study of paleotuberculosis and to synthesize the advantages and disadvantages of archival research in paleopathology.
Archives from hospitals, sanatoria and cemeteries, especially when dating after Koch discovery of the bacillus and before the development of antibiotics, are useful tools to study the paleoepidemiology of tuberculosis and to improve diagnostic tools. Moreover, these documents help to understand what we lose in the study of skeletons, e.g. tuberculosis of soft tissues, types of tuberculosis affecting children, and the acute and healed cases, not present in identified collections but recorded in hospital/sanatoria files. Data obtained from clinical archives and national statistics will be presented and discussed. As expected pulmonary was by far the most common type of tuberculosis.
At the end, a parallel between the global situation of tuberculosis in late 19th-early 20th centuries and at the present time, with multidrug and extensively drug-resistant strains, will be prepared focusing on socio-cultural determinants of tuberculosis related stigma and patient "non-adherence" to treatment.

T28:

IS IT POSSIBLE TO DIAGNOSE TB IN ANCIENT BONE USING MICROSCOPY?

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In paleopathology, light microscopy, particularly the use of polarized transmission light, has a high value for establishing reliable diagnoses. During the last years, there has been a growth in experience with diagnosing pathological conditions at the micro-level using thin-ground sections prepared from archaeological bone. Thus, the question came up, if it might be possible to diagnose tuberculous disease in archaeological bone using microscopy. As a rule, the reliability of a diagnosis established on the base of thin-ground sections

depends frequently on the state of preservation of the selected sample (e.g., pseudopathology). However, sometimes, although the preservation is rather good, a diagnosis cannot easily be established because the characteristic criteria (e.g., mosaic structure, in Paget's disease) are not clearly observable or seem to be ambiguous. In this case, the pathophysiological nature of the morphological structures should be analyzed (e.g., the speed of the growth of pathological newly built bone formations) which might help to differentiate between nonspecific (e.g., hematogenous osteomyelitis) and specific inflammatory bone diseases (e.g., tuberculous bone disease). To verify this assumption, samples were taken from recent bone collection materials with known disease diagnoses and from archaeological specimens which show lesions suspicious of bone tuberculosis.

T29:

PALEOPATHOLOGY AND PALEORADIOLOGY DATA AS SOURCES FOR THE HISTORY OF TUBERCULOSIS: THE EPISTEMIC AND HISTOGRAPHICAL ISSUES?

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Abstract is not available

T30:

CONTRIBUTION OF 3D RECONSTRUCTIONS TO THE PALEOPATHOLOGY OF TUBERCULOSIS

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In the field of physical anthropology, techniques of three-dimensional imaging have become an essential tool. Indeed, high resolution virtual 3D reconstructions of original specimens contribute to their preservation and broaden the ability for research, teaching and exchanges. We illustrate here the interest of these three-dimensional methods in the field of paleopathology. Far from providing beautiful pictures of limited interest for retrospective diagnosis, 3D analyses are actually the only efficient way for reconstructing processes involved in pathological bone changes.

This leads to the concept of „processual paleopathology" that we would like to introduce in the field.

As for tuberculosis, several paleopathological specimens attributed to this infection and concerning different part of the skeleton (spine, bones and joints) have been analysed using a specific 3D digital chain.

These 3D analyses bring additional and original information: they allow to virtually reconstruct the initial location and aspect of the infectious process, its extension on bone as well as its possible diffusion to the surrounding tissues. Three-dimensional reconstructions also allow a virtual follow-up of the disease with their ability to recreate on a given paleopathological specimen, different virtual stages of the progression of tuberculous infection.

Besides this specific interest, the three-dimensional methodology substantially improves training in paleopathology as well as it increases possibilities to spread pathological specimens to the scientific community, and thus contributes to the knowledge of natural history and evolution of ancient human infections such as tuberculosis.

T31:

TUBERCULOSIS AND SURVIVAL IN PAST POPULATIONS: A PALEO-EPIDEMIOLOGICAL APPRAISAL

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Tuberculosis is still relevant and close to us in light of the effects of recent migration flows and antibiotic resistances observed during the last 15 years.

While the historical assessments of morbidity and mortality from tuberculosis during the nineteenth and twentieth centuries appear sometimes to us greatly exaggerated, they always put TB as the leading cause of mortality. For earlier periods, we know next to nothing for lack of other tools than the calculation of frequencies of lesions in archaeological bones. These lesions most often concern only healed individuals or subjects able to have developed immune resistance and controlled the disease extension by forming visible osteo-articular lesions. They tell us little about the real impact of disease on mortality in past populations.

By following the proposition of Wright (2003), we launched a research hypothesis based on paleopathological diagnosis of tuberculosis and individual age determination by tooth cementum annulations counts to measure somehow the impact of the disease in prehistoric and historic populations.

O-T1:

WHOLE GENOME SEQUENCING OF 199 MYCOBACTERIUM TUBERCULOSIS ISOLATES REVEALS AN ABSENCE OF A MOLECULAR CLOCK OVER SHORT TIME SCALES

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Introduction: Whole genome sequencing allows us to investigate genomic evolution and transmission in *M. tuberculosis* at the highest resolution.

Objectives: To investigate the correlation between epidemiological and WGS data. Additionally investigate mutation rate and genomic evolution in *M. tuberculosis* during transmission.

Materials & methods: 199 isolates were sequenced at ~100 fold coverage. These isolates were collected over two decades in the Netherlands, and represent 42 different RFLP clusters. Epidemiological contact tracing and typing provided evidence for transmission between 92 pairs of isolates.

Results: The mean SNP difference between epidemiologically linked pairs was 1.9 with 37 pairs having no detectable difference. An outlier pair had a SNP difference of 149, despite having a strong epidemiological link and an identical RFLP. Inspection of the sequence data revealed large deletions unique to each isolate providing evidence against recent transmission. A 0 SNP difference was also found between 62 pairs of isolates

that were not previously linked. Phylogenetic analysis revealed a lack of correlation between genetic distance and time. Bayesian methods were also used to infer a mutation rate, but a significant result was never reached; suggesting the absence of a molecular clock.

Conclusion: Analysis of the epidemiologically linked pairs demonstrates the power of WGS to refute and identify possible transmission events. The dataset as a whole revealed an absence of a molecular clock. This is likely to be due to a combination of latency and a low mutation rate. The extreme genomic stability found in *M. tuberculosis* could result in an absence of a clock-like signal over short time scales.

O-T2:

TUBERCULOSIS AT THE ONSET OF AGRICULTURE IN CENTRAL GERMANY

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Introduction: Since the advent of ancient DNA studies 25 years ago *M. tuberculosis* was the first and so far most comprehensively studied ancient pathogen in the emerging field of paleomicrobiology. Modern molecular analysis in combination with paleopathology revealed first precious insights into TB evolution and presented *M. tuberculosis* as a longstanding human-associated pathogen. The occurrence and the epidemic spread of the pathogen is highly linked to social and biological factors of the human host. Therefore it is of particular interest to better understand the significance of TB at time periods of social and nutritional change such as the Neolithic.

Objectives: For the first time in paleomicrobiology a large collection of ancient human remains from the Neolithic will be screened for ancient TB DNA. Our multidisciplinary team will combine different analytical approaches to obtain a more comprehensive picture of the disease during this time period.

Materials & methods: In the present study, 88 individuals from three sites in Saxony-Anhalt (Germany) dated to the Linear Pottery Culture (5450-4775 cal BC) were analysed. Paleopathological analysis was complemented by histological analysis and micro-CT scans. A selected subset of the individuals was further molecularly analysed by using a PCR-based assay targeting the MTBC IS6110 region and via Spoligotyping.

Results: Periosteal pulmonary TB-indicative reactions of varying frequency and degree along the neck of the ribs were found in some individuals (6.5% of subadults, 35.1% of adults). Nine out of 21 molecularly analysed samples were tested TB positive.

Conclusion: In summary, our data clearly indicates that people in the Early Neolithic had to cope with an increased risk of infectious diseases like tuberculosis.

O-T3:

STANNINGTON SANATORIUM FOR TB CHILDREN

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Introduction: The Stannington Sanatorium for children with tuberculosis was opened in 1907 in Northumberland, UK, and was the first such Sanatorium in the UK. In the late 1940s and early 1950s, the need for the Sanatorium changed because of the introduction of effective antituberculous drugs. Since 1985, the archives of the Sanatorium have been in the custody of the Northumberland Archive Service.

Objectives: The project aim is to preserve by digitisation, and make available by internet, a complete clinical and radiographic record of infected children from the period 1939-1943, accompanied by oral histories of surviving patients and staff, and by contemporaneous photographs. Thus anonymous data regarding the clinical course and progression of tuberculosis from an era before the introduction of antituberculous antibiotics will be made available for education and research.

Materials & methods: The records consist of patient discharge books of case notes, which will be dis-assembled and digitised. The X radiographs, 16,100 in number, and referenced to individual case notes will also be digitised. This entire archive will be made available on the internet. 20 oral history recordings will be made, and these and the contemporary photographs will be accessible from the Northumberland Archive Office.

Results: The results of the project will be available on internet.

Conclusion: The project makes available for study and research, the progressive clinical case records and associated radiographs of children with pretreatment tuberculosis. The value of this clinical record is enhanced by the social record of oral and photographic history. It is considered that this archive of tuberculosis is unique.

O-T4:

THE CITY OF ROME, ITS EMPIRE, AND THE SPREAD OF TUBERCULOSIS IN EUROPE

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Introduction: The formation of the Roman Empire constituted an unprecedented joining of Mediterranean and European lands and its capital became the setting for an extensive mixing of peoples. It is likely that Roman civilization played an important role in the spread of tuberculosis and its evolution in Europe.

Objectives: 1) To determine to what extent the city of Rome ca. 100 B.C. to 250 A.D. would have provided a favorable environment for tuberculosis and what opportunities existed for its spread beyond the capital. 2) To analyze whether or not Roman expansion into the provinces resulted in the introduction of tuberculosis and/or an increased burden of tubercular disease.

Materials & methods: Using secondary and primary literature, an analysis was conducted of living conditions in the city of Rome (including sanitation and ventilation) and general Roman social conditions (slavery, domestic life, patronage and relations between social classes) in the first two centuries A.D. A literature review of pre-Roman and Roman-era osteological finds (to date) was performed and biomolecular analyses were noted. The development of these sites was assessed in terms of urbanization and Roman influence.

Results: Living and social conditions were favorable to the efficient spread of tuberculosis in Rome. Mobility was high both into and out of the city. In general, the development of a more urban, Roman way of life correlates with increased tuberculosis prevalence, but this may be an artifact resulting from the relatively small numbers of finds.

Conclusion: The growth of Roman civilization likely facilitated the spread of tuberculosis in Europe.

O-T5:

NOVEL LABORATORY DIAGNOSTIC TESTS FOR TUBERCULOSIS AND THEIR POTENTIAL ROLE IN AN INTEGRATED AND TIERED LABORATORY NETWORK

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Introduction: HIV/AIDS, tuberculosis, and malaria remain major threats that undermine the health of global populations and account for approximately 5 million deaths every year. A lack of adequate laboratory capacity in resource-poor, high-burden countries presents a significant barrier in providing appropriate diagnosis, care, and treatment to patients infected with these and other emerging pathogens. Strengthening laboratory services in high burden countries via implementation of novel diagnostic tests is indispensable to successfully battle against tuberculosis and MDR and XDR tuberculosis.

Objective: Novel tuberculosis diagnostic assays have to be accurate and rapid but should also be applicable to field conditions, affordable and accessible to all patients at different levels of the tiered laboratory diagnostic network in resource poor settings.

Methods: The presentation will overview the latest developments of TB laboratory diagnostics such as automated digital smear microscopy, rapid growth detection and susceptibility testing and rapid molecular testing with focus on performance and applicability to diagnostic needs at different levels of the diagnostic network. Practical evidence collected on the field implementation and scaling up of the new tests will also be overviewed.

Conclusion: A functional national laboratory network should be based on such well developed diagnostic platforms that can provide not only timely and adequate diagnosis but are simple to use, implement and sustain.

O-T6:

ANTIMYCOBACTERIAL ACTIVITY OF PYRIDOPYRIMIDINE DERIVATIVES AGAINST MYCOBACTERIUM TUBERCULOSIS IN A SERIES OF IN VITRO AND IN VIVO MODELS

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Introduction: Tuberculosis (TB) is an airborne infectious disease caused by mainly *Mycobacterium tuberculosis* (MTB) and the first cause of mortality due to a single pathogen. As several drugs in combination have to be used against TB and the number of effective drugs is limited, there is an urgent need to develop new antimycobacterial compounds.

Objectives: Considering that MTB can survive in host cells its elimination could be more efficient with host-cell directed delivery of chemotherapeutic agents. The optimization of the cellular uptake by peptide based drug delivery systems can increase the efficacy of the compounds.

Materials & methods: The dUTPase enzyme (EC 3.6.1.23; Rv2697) plays an important role in preventive DNA repair mechanism and thymidilate biosynthesis. New pyridopyrimidine derivatives were defined using a novel HTS *in silico* docking software (Fregatt). Top hits were assayed *in vitro* for antimycobacterial effect on MTB H_{37}Rv and *M. kansasii* cultures. To get specific compounds with the lowest minimal inhibitory concentration effective hit molecules were optimised and in order to enhance the cellular uptake rate peptid-based carriers and nanoparticle type delivery system (polylactide-co-glycolide, PLGA) were applied. As carrier moiety receptor specific palmitic acid derivative of TKPKG (pal-T5) tuftsin peptide was used.
Results: The compounds has relevant *in vitro* intracellular antitubercular activity on MTB infected MonoMac-6 cells. In a quineia pig infection model, the efficacy of PLGA encapsulated compounds after oral treatment was compared with isoniazid, a first-line antitubercular.
Conclusion: In summary, promising *in vitro* and *in vivo* efficacy of tested pyridopyrimidine derivative and its peptide conjugates gave us attractive antitubercular drug candidates.

O-T7:

7000 YEAR-OLD TUBERCULOSIS CASES FROM HUNGARY OSTEOLOGICAL AND BIOMOLECULAR EVIDENCE

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Introduction: This study derives from the macroscopic analyses of a Late Neolithic (Tisza Culture) population from Hungary. Remains were recovered from a tell settlement at Hódmezővásárhely-Gorzsa (4970 to 4594 BC) from graves within the settlement as well as pits, ditches, houses and as stray finds.
Objectives: One of the most important discoveries from the remains of this late Neolithic population was evidence of tuberculosis. The objective of this study was to confirm its presence on the site.
Materials & methods: Pathological analyses of the seventy-one individuals revealed numerous cases of infections and non-specific stress indicators on juveniles and adults, metabolic diseases on juveniles, and evidence of trauma and mechanical changes on adults. Several cases showed potential signs of tuberculosis and further analyses were undertaken, including biomolecular studies.
Results: The five individuals were all very young adults. HGO-53 was a male with a striking case of secondary hypertrophic osteoarthopathy (HOA), revealing rib changes and cavitations in the vertebral bodies. HGO-08 was a female with resorptive lesions on the vertebrae. HGO-10 (male) and HGO-21 (female) both presented evidence of hypervascularisation on the vertebrae and periosteal remodelling on the ribs. Finally, HGO-48 was a female with abnormal blood vessel impressions and a possible lesion on the endocranial surface of the skull. The initial macroscopic diagnosis of these five cases was confirmed by lipid biomarkers analyses, and three of them were corroborated by DNA analyses.
Conclusion: At present, these are among the oldest palaeopathological and palaeomicrobiological tuberculosis cases in Europe.

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O-T8:

TUBERCULOUS SKELETAL LESIONS AMONG PRE-CONTACT LONGHOUSE PEOPLE OF THE NORTH AMERICAN GREAT LAKES

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Introduction: From the onset of the Iroquoian tradition (ca. 1500 BP) the population of maize farmer/hunter/fishers in the lower Great Lakes expanded and their longhouses lengthened, suggesting a successful adaptation. Nevertheless, skeletal indications of tuberculosis are often seen in the remains from their large ossuaries.
Objectives: Prior studies have focused on spinal lesions, but joint and cranial lesions may also be linked to TB. By considering together these different lesions within large samples, our purpose is to explore the response of these past populations to tuberculous infection relative to population, longhouse and village sizes, and inter-group conflict.
Materials & methods: In a survey of skeletal remains from 20 different sites, dating from 1100 to 350 BP (MNI=936), skeletal lesions attributable to TB on spine, bone, joint and endocranium were assessed.
Results: Skeletal lesions attributable to TB are very frequent. As an example, lesions appear on a quarter of the immature skeletons from one large ossuary (MNI=391), within which immature individuals represent 40% of the sample. Skeletal expressions among the adults indicate a high exposure to TB of these pre-contact people.
Conclusion: Housing is a focus because the intense wood smoke affected sinuses, probably compromised respiratory health and contributed to the dissemination of pulmonary pathogens. Temporary combined vitamin deficiencies (C, D_3) caused by long winters may also contribute to this apparent high frequency of tuberculosis. More complete molecular characterization of this infection is an important goal.

O-T9:

MYCOBACTERIAL BIOMARKER DISCOVERY

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Introduction: The Mycobacterium genus, which is hallmarked by mycolic acids (α-branched β-hydroxylated long chain fatty acids), comprises several human pathogens such as *Mycobacterium leprae* and *Mycobacterium tuberculosis*, the causative organisms of leprosy and tuberculosis, respectively. The diagnosis of tuberculosis from archaeological human skeletal remains is not an easy task. Up to this time numerous studies have detected the presence of the MTBC in archaeological remains by gross osteological examination and biomolecular analyses.
Objectives: The presence of mycolic acids as well as mycobacterial proteins was determined from archaeological skeletal remains.
Materials & methods: We analyzed the presence of free MA fraction and their derivatives in clinical samples as well as tuberculosis infected and non-infected archaeological remains by using MALDI TOF/TOF, LC ESI MS, HPTLC and MALDI LTQ Orbitrap Imaging MS.
Results: During our analytical investigations all techniques were successfully cross validated. The mass spectrometric results were deconvoluted by a homemade search engine algorythm. Among the others, ancient

mycobacterial proteins as well as molecular and quasimolecular ions of methoxy-MA (C84), keto-MA (C80, C81), alpha-MA (C89) were identified from the clinical and ancient human samples.
Conclusion: Our results show that the applied complex analytical strategy is suitable for sensitive and accurate determination of ancient mycobacterial infections.

O-T10:

EVOLUTION OF THE MYCOBACTERIUM TUBERCULOSIS COMPLEX PE AND PPE GENES

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Introduction: The members of the *Mycobacterium tuberculosis* complex (MTBC) originated through an evolutionary bottleneck, leading to a high degree of genetic homogeneity (99.9%), despite differences in their phenotypic characteristics and host ranges. A large percentage of the heterogeneity can be explained by two large gene families, termed pe and ppe. The function of the PE/PPE proteins remains enigmatic, but studies suggest that they are secreted or cell surface associated and involved in bacterial virulence. Some are polymorphic, suggesting involvement in antigenic variation. The expansion of the pe/ppe gene families seems to have occurred during the evolution of the pathogenic slow-growing mycobacteria, as there is an absence of the multigene pe_pgrs and ppe-mptr sub-families in the fast-growing species.
Objectives: To investigate variation in the MTBC pe/ppe gene families to gain insight into pe/ppe evolution and function.
Materials & methods: Comparative sequence analysis was performed of all *pe*/*ppe* genes in 18 MTBC genome sequences, supplemented by whole gene sequencing of 14 genes in a cohort of 40 diverse strains covering all the main lineages of the *M. tuberculosis* phylogenetic tree.
Results: nsSNP's in *pe* (excluding *pgrs*) and *ppe* genes are 3.0 and 3.3 times higher than in non-*pe*/*ppe* genes, respectively. Large differences in mutation type and frequency between both individual genes and gene sub-families were detected.
Conclusion: The apparent absence of selection pressure on these genes suggests neutral evolution, which is inconsistent with the positive selection pressure of "classical" antigenic variation. The high variation rates and absence of selective constraints provides valuable insights into *pe/ppe* evolution and function.

O-T11:

LIPID BIOMARKERS PROVIDE EVOLUTIONARY SIGNPOSTS FOR THE OLDEST KNOWN CASES OF TUBERCULOSIS

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Introduction: *Mycobacterium tuberculosis* is characterised by the presence of unusual cell envelope lipid virulence factors. The remarkable stability and discontinuous distribution of these lipids gives them potential as specific biomarkers for tracing the evolution of tuberculosis. For example, excellent profiles of mycolic acids

have been recorded for the oldest known, 9,000 year old, human case of tuberculosis from Atlit-Yam, Israel (Hershkovitz et al. 2008 PLoS ONE 3:e3426).

Objectives: The distribution of mycolic, mycocerosic and mycolipenic acids and phthiocerol and related diols in the oldest known cases of human (Atlit-Yam, 9,000 BP) and animal (Wyoming bison, 17,000 BP) tuberculosis was investigated. The possible presence of intact complex lipids, such as the phthiocerol dimycocerosates, in archaeological bones was also studied.

Materials & methods: Lipids were extracted, derivatised and fractionated by established protocols (Hershkovitz et al. 2008). Mycocerosic/mycolipenic acid pentafluorobenzyl esters were profiled by negative ion-chemical ionisation gas chromatography-mass spectrometry. Mycolates, phthiocerols and other diols were investigated by fluorescence high-performance liquid chromatography of pyrenebutyric acid derivatives.

Results: Published mycolate results for the Atlit-Yam bones were complemented by excellent mycocerosate/mycolipenate/phthiocerol profiles. For the Wyoming bison, mycolates were degraded but pristine mycocerosate/mycolipenate traces were observed; clear phthiocerol profiles were recorded and diols corresponding to components of "phenolic" glycolipids were indicated. Evidence was obtained for the presence of intact phthiocerol dimycocerosates.

Conclusion: The identified lipid biomarkers indicate that the tuberculosis in the Atlit-Yam bones corresponds to the most common modern strains but that in the Wyoming bison could have more affinity with postulated ancestral strains.

O-T12:

CHARACTERIZATION OF *MYCOBACTERIUM TUBERCULOSIS* ISOLATES FROM BRAZIL, MOZAMBIQUE AND RUSSIA

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Introduction: Hardly any data are available about genotypes and phenotypes from Mtb Beijing isolates from Brazil

Objectives: To evaluate genotype variability and nature and virulence and compare with a selection of Beijing isolates from Mozambique and Russia

Materials & methods: We characterized eight isolates from Brazil, 17 from Mozambique and one from Russia, by spoligotyping and 24-MIRU-VNTR typing. We also used the IS6110-based inverse-PCR in NTF region, sequencing of katG and rpoB, evaluation of 7bp pks15/1 insertion and of major RDs. We evaluated growth in 7H9 medium and infected THP-1 macrophages, followed by measuring intracellular growth and induction of necrosis.

Results: A HGDI of 0.997 with 24-MIRU was observed showing high discrimination; 25% of the strains from Brazil and 53% of Mozambique were modern, as for the strain from Russia. All Beijing isolates had the 7bp pks15/1 insertion, all were RD105 and RD181 deleted, all but two RD150 deleted and all RD142 WT. The Russian isolate had the more pronounced virulence; those from Mozambique intermediate and those from Brazil less virulent. In both regions, modern strains seemed more virulent that the ancient, but less than the Russian isolate.

Conclusions: Our data support the hypothesis that ancestral Mtb strains are less virulent that modern ones. However, when constructing a 24-MIRU genotype based MST and linked the IS6110-NFT genotypes, no logic

correlation between both markers was observed, suggesting that IS6110-NFT may not be adequate in our study population. We are now performing additional typing using newly described SNPs for discriminating between both genotypes and will than reanalyze phenotype/genotype associations.

P-T1:

EVIDENCE OF TUBERCULOSIS IN ANCIENT SYRIA DATING FROM PRE AND EARLY DOMESTICATION

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Introduction: The question of pre-neolithic tuberculosis is still open in paleopathological perspective. One of the major interest is to explore what type of infection could have existed prior to the domestication and at its early stages.

Objectives: Description of paleopathological lesions observed on skeletons coming from two PPNB sites representing pre and early domestication phases in Syria, belonging to the geographical cradle of agriculture.

Materials & methods: Paleopathological study of two Neolithic individuals from Syria: 1) adult skeleton dating from the second half of the 11th millenium BP calibred (Early PPNB) of the predomestication neolithic site of Dja'de El-Mugara (Northern Syria), 2) immature skeleton coming from the site Tell Aswad (Southern Syria) dating from 9800-8600 BP calibrated (Middle PPNB). Laser scanning and microCT scan have been applied. Detection of mycolic acids is in progress; aDNA analyses are planned.

Results: Dja'de adult skeleton exhibits lesions that are typical of an infectious spondylodiscitis: the inferior part of the 9th horacic vertebrae is completely destroyed, the upper plate of the 10th thoracic vertebrae shows lytic rounded cavitations expanding to the vertebral body. Tell Aswad immature individual exhibits plurilamellar periosteal reactions of all the long bones, associated with spina ventosa of the right ulna.

Conclusion: This two paleopathological cases strongly suggest the presence of tuberculous infection before domestication as well as in its early stages. Molecular analyses are in progress in order to better explore the past history of human tuberculosis.

P-T2:

IN VITRO ANTIMYCOBACTERIAL ACTIVITY OF SUBSTITUTED SALICYLANILIDES AGAINST MYCOBACTERIUM TUBERCULOSIS H37RV AND MULTIDRUG-RESISTANT A8 CULTURES

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Introduction: The increase of multidrug-resistant tuberculosis (MDR-TB) is alarming and development of effective new drugs is important. Modifications of first- or second-line antituberculotic drugs are widely used approach. A novel design of new agents is mainly oriented toward the synthesis of prodrug forms; combination of two active molecules in order to achieve their possible interaction with new mechanisms of action.

Objectives: Salicylanilides (2-hydroxy-N-phenylbenzamides) are promising candidates for this purpose due to their antimycobacterial activity. Pyrazine-2-carboxylic acid is the active form of pyrazinamide (PZA), a first-line antituberculotic. The antimycobacterial activity of the substituted salicylanilides alone and in combination with 5-chloropyrazine-2-carboxylic acid was determined.

Materials & methods: Substituted salicylanilides, 5-chloropyrazine-2-carboxylic acid and the combination of these in ester form were prepared and chemically charaterised. The *in vitro* activity of the compounds was determined on *Mycobacterium tuberculosis* (MTB) $H_{37}Rv$ and on A8 MDR cultures. *In vitro* cytotoxicity and cytostatic effect were also tested on human cell cultures.

Results: The minimal inhibitory concentration (MIC) values of the new compounds were determined on MTB $H_{37}Rv$ and on A8 MDR cultures. None of the compounds have *in vitro* cytotoxicity or cytostatic effect on human cells.

Conclusion: Our study has demonstrated the inhibitory effect of the compounds against MTB $H_{37}Rv$ and A8 MDR cultures. The newly synthesised esters can be considered as 'double active' molecules that can play the role of a prodrug with a prolonged liberation.

P-T3:

ENDOBRONCHIAL TUBERCULOSIS IN PATIENTS WITH ACTIVE DISEASE

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Introduction: Endobronchial tuberculosis /EBTB/ is defined as tuberculous infection of the tracheobronchial tree with microbial and histopathological evidence.

Objectives: In order to analyze characteristics of EBTB, a retrospectíve study was carried out in patients with active TB.

Patients & methods: Medical documents of 221 patients with TB between 2006 and 2011 were recruited for analysis. In 91 of these cases, diagnostic bronchoscopy was performed and TB was confirmed by bacteriology, histopathology or clinical course in 46 patients.

Results: The classification of 46 patients' endoscopic findings were the following: 9 edematous-hyperemic, 1 granular, 1 ulcerative, 4 fibrostenotic and 6 tumorous lesions. Large amount of bronchial excretum were seen in 12 cases (serosus, mucosus, purulent or hemorrhagic), while negative endobronchial pattern were found in 13 individuals. Tumorous EBTB was characterized by an endobronchial mass with caseous surface material that totally occluded the bronchial lumen. In all cases of EBTB parenchymal involvement was also present.

Conclusion: EBTB is relatively uncommon manifestation of a common disease. The clinical, radiologic and bronchoscopic presentation of EBTB is non-specificic and it can easily be confused with common pulmonary disorders like bronchial cancer, pneumonia or asthma. Early treatment is of utmost importance to prevent its bronchostenotic complications.

P-T4:

CHANGES IN THE DISEASE PROFILE OF TUBERCULOSIS DURING THE INTRODUCTION OF ANTIBIOTICS – A STUDY OF 20TH CENTURY SWISS PATHOLOGICAL SKELETAL SAMPLES

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Introduction: Tuberculosis is a chronic infection caused by bacteria of the *Mycobacterium tuberculosis* complex (MTBC). Only approximately 10% of infected individuals develop active tuberculosis and in those, approximately 3-5% develop bone lesions. Before the emergence of antibiotic resistance, their use eliminated the causative agent and allowed bone lesion healing to occur although limited healing also occurred in the pre-antibiotic age. Treatment for tuberculosis prior to antibiotics focussed on improving the individuals diet, hygiene and providing rest. In some cases this would allow for the immune system to combat the bacterium and force the disease into remission. During this time, healing can occur.

Objectives: In order to see what effect, if any, antibiotics had on bone pathology we studied samples from the Galler collection, a 20th centuary pathological bone collection.

Materials & methods: We have examined 69 previously diagnosed individuals. By concentrating on these pathological specimens dating from 1925 to 1977, alongside the associated medical records and autopsy reports, we have been able to study how the introduction of antibiotics affected the disease progression.

Results: We see that with the use of antibiotics the disease profile changes dramatically; individuals live longer, healing occurs more frequently and co-morbid chest infections become less common.

Conclusion: We see a clear change in disease progression during the introduction of antibiotics. Therefore, we are also interested in any changes in the causative agent. In order to more completely understand the shift in pathogen over this time period, we are attempting to extract and type MTBC DNA from clinical specimens used in this study.

P-T5:

SPINA VENTOSA: TWO CASES OF OSTEO-ARTICULAR TUBERCULOSIS OF CHILDREN FROM KÖNIGSBERG, PRUSSIA

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Introduction: Materials are received in 2007 from the archaeological excavation of Institute of Archeology of RAS of a Prussian cemetery (former Königsberg).

Objectives: There are two cases (2,1%) among subadult remains which can be identified as tuberculosis. One of individuals is a child, and the second one is teenager. In both cases there are noted specific lesions of lower part of humerus and upper part of ulna of right elbow.

Patients & methods: We present results of application of the microfocus x-ray method. The method is useful for detection of thin and low-contrasting details. There are no limits on the distance between the focal spot and the x-ray detector plane, and images have no area of reduced sharpness.

Results: X-rays revealed classical cystic lesions and "spina ventosa". The typical findings are osseous enlargement of the diaphysis of lower part of humerus and upper part of ulna, and multiple round or oval cystic areas with minimum sclerosis; periostitis and sequestration of affected zones also were noted.

Conlcusion: Cystic tuberculosis is a rare form of skeletal tuberculosis. Tuberculosis of the skeletal system is almost always secondary to hematogenous dissemination. Cystic bone lesions are more frequently encountered in children. There is widening and diffuse rarefaction of the diaphysis and a cortex that is thickened externally, and eroded from within internally. This periosteal reaction leads to a fusiform appearance of the tubular bones and the expansion of the involved bone with the cystic lesion as "spina ventosa". The main differential diagnoses include congenital syphilis, pyogenic osteomyelitis, fungal infections and tumors.

P-T6:

POSSIBLE ASSOCIATION BETWEEN LANGERHANS CELL HISTIOCYTOSIS AND TUBERCULOSIS IN A MEDIEVAL CHILD FROM THE ARCHAEOLOGICAL SITE OF LA GRANÈDE (MILLAU, FRANCE)

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Introduction: The Langerhans cell histiocytosis (LCH) is an immunitary system disease, characterized by abnormal proliferation of histiocytes which have an intense phagocytic activity. Associations between tuberculosis (TB) and LCH have been reported in the literature, at least for adults' pulmonary form. However similar cases concerning children are very rare.

Objectives: To precisely describe our sample and propose a differential diagnosis particularly between LCH and TB.

Materials & methods: The case is a child aged from 1 to 2.5 years old, dating from the 10th century AD, from a paleo-Christian church cemetery. The undertaken work is a precise macroscopic and microscopic analysis using medical imaging.

Results: There are many signs leading to the diagnosis of LCH (plurifocal and pluriostotic osteolytic lesions giving a geographic appearance, "button sequestrum", osteolysis initiation inside the diploë, directional extension of the lesions, age), signs in favor of TB (hypervascularisation of the inner table similar to SES lesions, discoloration areas) and some signs compatible with both diseases (costal lesion and punched out cranial lesions).

Conclusion: LCH seems the most obvious diagnosis; however differential diagnosis cannot exclude TB. LCH is a disease with numerous signs reaching all tissue-types. On the other hand cranial tuberculosis is not well described and understood. The association of the two diseases could explain all of the shown signs (e.g. immunitary deficiencies associated with LCH can facilitate infection development such as TB) and is a hypothesis already proposed in palaeopathological literature.

P-T7:

FROM VIRTUALITY TO REALITY: 3D RECONSTRUCTIONS OF TUBERCULOSIS PROCESSES USING VIRCOPAL® CHAIN

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Introduction: In the field of physical anthropology, accurate virtual tri-dimensional reconstruction of original specimens contributes to their preservation while it improves the means for research, teaching and exchanges. In an interdisciplinary perspective, we have developed a specific 3D digital chain (acquisition -reconstruction - printing) dedicated to anthropology, using a dedicated software program (TIVMI: Treatment and Increased Vision for Medical Imaging), characterized by its accuracy and reliability. This chain has been named VIRCOPAL standing for VIRtual COllection of PALeo-specimens and patented in February 2011.

Objectives: The goal of this presentation is to illustrate the interest of this 3D digital chain to the comprehension of tuberculosis processes with "3Ds" purposes: Diagnosis, Didactic and Diffusion.

Materials & methods: Several skeletonized and mummified specimens exhibiting TB lesions have been digitalized, using CT or micro-CT scans; the images were reconstructed by TIVMI and finally printed on a high resolution 3D printer (Objet -Eden 250™). These specimens can be reconstructed and printed on demand for research, trainings, exhibitions.

Results: Cases can be presented in a specific TB training package corresponding to different aspects of skeletal tuberculosis. In addition to the printed versions of paleopathological specimens, including additional digital sections, enlargement or extraction of regions having specific interest for retrospective diagnosis, original digital data are also available on a DVD.

Conclusion: This 3D digital chain allows a better understanding of the expression and evolution of skeletal TB and increases the possibilities for research, training and exhibitions. This methodology will be extended to other different skeletal pathologies besides tuberculosis.

P-T8:

A NEW SPINAL TUBERCULOSIS CASE FROM THE ÁRPÁD PERIOD (11TH-13TH CENTURIES AD) FROM ZALAVÁR, WESTERN HUNGARY

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Introduction: The distribution, antiquity and epidemiology of tuberculosis have previously been studied in the osteoarchaeological material of the eastern part of Hungary, mainly on the Great Plain. The purpose of this study is to increase the number of known skeletal tuberculosis (TB) cases in the western part of Hungary,

Transdanubia, especially in the area of Zalavár, one of the most significant regions during the 9th-13th centuries AD. In this study we present a new case found at the site of Zalavár-Vársziget-Hadrianus templom. Previously three cases of spinal TB were identified from this cemetery, but during the continuous investigation we found an additional case.

Objectives: Description of alterations indicating severe spinal TB.

Materials & methods: Paleopathological analysis was based on morphological observation supported by radiological analyses.

Results: The juvenile female individual has a severe collapse, fusion and ankylosis between the lower (7th and 12th) thoracic vertebrae. The 5th and the 6th thoracic vertebrae are fused, but significant bone resorption occurred on their bodies. Beside this fusion and the ankylosis, vertebral hypervascularisation was observed on the 12th thoracic and on all the lumbar vertebrae. Neither rib, nor endocranial lesions were detected in the analysed case.

Conclusion: In summary, based on the skeletal morphological changes, this case shows classical alterations indicating a healing stage of TB, with advanced gibbus deformity in the lower thoracic region.

P-T9:

GROWTH AND DISEASE PATTERNS IN INFANTS AND CHILDREN FROM THE OTTOMAN DOR, NORTHERN ISRAEL

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Introduction: This research is part of a larger survey on health and disease patterns in past human populations of Israel and focuses on infants and children aged 0-12 years (N=39) from the Ottoman Dor. Previous anthropological studies have shown that this population suffered from numerous stress events in early infancy and childhood that resulted in delayed growth and ill-health.

Objectives: Malnutrition and infections were among several factors suggested to have contributed to poor health status, yet no attempt has been made to determine possible causative agents of these infections.

Materials & methods: We performed anthropological analysis of the skeletal remains and created health status cards for each individual. In parallel, we looked for the traces of pathogenic bacteria at the DNA level.

Results: The most common pathologies recorded were periostitis in the long bones (50%) and *cribra orbitalia* (42%). Other pathologies included porosity of the hard palate and maxilla, porotic hyperostosis of the skull, rounded nasal aperture, periostitis on ribs, circumferential pitting and lytic lesions on vertebral bodies, periodontal disease and dental enamel hypoplasia. These findings suggested the presence of anemia, metabolic and infectious diseases in the population studied. Five cases of infectious diseases have been detected by means of ancient DNA analysis: 4 cases of leprosy and one case of co-infection of leprosy and tuberculosis.

Conclusion: Anthropological and molecular findings were consistent and indicated that tuberculosis and leprosy were present in infants and children aged 0-12 years from the Ottoman Dor. This research is supported by a grant (60/09) from the Israel Science Foundation (ISF).

P-T10:

VARIABLE HOST SUSCEPTIBILITY TO INFECTION WITH MYCOBACTERIUM TUBERCULOSIS LINKED TO THE GENOTYPE OF STRAIN

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Introduction: In the present study, we have determined the phylogenetic position of a particular *Mycobacterium tuberculosis* (Mtb) isolate, Mtb79112, which was originally isolated by professor Denis Mitchison in the 1960s in South India and found as low virulent in guinea pigs (1).

Objectives: We have investigated the strain with the aim to identify potential genotype-phenotype relationships.

Materials & methods: The Mtb isolate was subjected to genome sequencing and animal infection studies.

Results: We have identified this strain as belonging to the "ancestral", TbD1+ Mtb strains that are prevalent in South India and other parts of southern Asia. As expected, the strain was attenuated in the tested guinea pigs using aerosol infection, instead of the previously used intramuscular injection. Strikingly, in C57BL/6 mice that were infected in parallel with the same Mtb79112 containing aerosol as the guinea pigs, the Mtb79112 strain fully retained its virulence and caused lung and spleen lesions that were comparable or even more pronounced than the lesions obtained with the Mtb H37Rv and 79499 control strains (both TbD1-).

Conclusions: Analysis of the genome sequence of Mtb79112 and comparison with the sequence of MtbH37Rv is presently ongoing in order to identify the genetic differences between Mtb strains that might account for the different host-pathogen interaction in the tested animal models. More insight into this matter should also help to elucidate why certain phylogenetic Mtb lineages are associated with disease in particular human populations in restricted geographic locations.

P-T11:

SPOLIGORIFTYPING - A NEW DPO-BASED DIRECT-HYBRIDIZATION ASSAY FOR EFFECTIVE TB CONTROL ON A MULTIANALYTE MICROBEAD-BASED HYBRIDIZATION SYSTEM

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Introduction: Multidrug resistant (MDR-TB) represent an important challenge for global tuberculosis (TB) control. Rapid, reliable and cheap tests are needed in the fight against TB.

Objectives: We wanted to provide a new assay allowing simultaneous spoligotyping (i.e. CRISPR-based genotyping) and rifampin drug-resistance mutations in the *rpoB* gene hot-spot characterization in a single tube and in a few hours.

Materials & methods: This method uses Dual Priming Oligonucleotide (DPO) primers that allow efficient multiplexing PCR amplification and requires a microbead-based analytic system such as Luminex or Magpix. We tested this method on a set of 114 previously phenotypically characterized MDR-TB (n = 101) or drug-susceptible (n = 13) TB DNA extracted from clinical isolates obtained from patients living in Bulgaria, Nigeria and Germany.

Results and discussion: We developed a new high-throughput 53-Plex test called «spoligoriftyping» that allows *M. tuberculosis* complex genotyping and multidrug resistance detection at the same time. We show that our method is 100% correlated to *rpoB* gene hot spot sequencing results for SNP typing and 100% correlated with classical spoligotyping. Compared to classical drug susceptibility testing, spoligoriftyping sensitivity and specificity were 98 and 100%respectively. This type of assay opens the way to the implementation of locally and specifically-adapted genotyping methods. Such methods allow both epidemiological and patient-relevant information to be produced simultaneously and are paradigmatic of a power of new generation cheap multiplexed assays.

Conclusion: We believe that spoligoriftyping implementation in routine could improve tuberculosis control globally and contribute to reach the millennium goals in TB burden reduction.

P-T12:

THE MYCOBACTERIAL DUTPASE: BIOCHEMISTRY, PHYSIOLOGY AND MOLECULAR INTERVENTION

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Introduction: Thymidine biosynthesis is essential as dTTP is one of the nucleotide building blocks of DNA. Three major pathways exist for dTTP synthesis in humans, while in mycobacteria only one of these is present. This one involves the dUTPase reaction. In addition, the constantly produced dUTP needs to be eliminated to prevent DNA uracilation. Therefore, dUTPase is also required to eliminate excess dUTP.

Objectives: We aimed to relate the previously deciphered in vitro reaction mechanism of dUTPase to physiology in the living mycobacterial cell.

Materials & methods: *Mycobacterium smegmatis* was used as a fast growing model for mycobacterial thymidine biosynthesis. Marked allelic replacement, growth assays and *in vitro* enzyme assays were used.

Results: We found that mycobacterial dUTPase genes (*dut)* exhibit over 85% sequence identity and has a genus-specific surface loop absent in the human dUTPase. The *k*nock-out of *dut* resulted in lethality, which could be reverted by complementation with the wild-type *dut*. We assayed complementation with four dUTPase mutants with *in vitro* characterized enzyme activity. Importantly, mutant *dut* lacking the genus-specific loop was enzimatically unaffected, but was unable to complement lethality. However, partially or fully inactive mutants having the genus-specific loop could revert lethality. Growth assays are in progress to reveal the effect of decreased dUTPase activity on various stress conditions.

Conclusion: Our results prove that *dut* is essential in *M. smegmatis* and that essentiality is brought about by the mycobacterium-specific dUTPase motif and not by the enzymatic activity. Therefore, we propose that targeting the mycobacterium-specific motif will potentially yield an efficient, specific antimycobacterial treatment.

P-T13:

SKELETAL TUBERCULOSIS IN A LATE NEOLITHIC SERIE FROM HUNGARY

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Introduction: The aim of this study is to present a unique tuberculosis (TB) case discovered in Hungary. Between 2006 and 2008 a Late Neolithic-Early Copper age settlement and cemetery with more than two thousand graves were excavated at Alsónyék-Bátaszék in the South-Western part of Hungary. Consequently this site is the largest excavated Neolithic cemetery in Europe.

Objectives: The still ongoing multidisciplinary investigation implies among others anthropological and paleopathological analysis, which provide invaluable direct sources of the features, spread and roles of specific infectious diseases.

Materials & methods: During the examination of the skeletal material unambigious traces of spinal TB were found. The analysis was carried out using morphological observation supported by radiological analyses.

Results: In the case of an adult male skeleton, between the lower thoracic and the upper lumbal region of the vertebral column, there is a marked collapse, ankylosis and severe angulation, which indicates long standing TB.

Conclusion: The anthropological and paleopathological investigation of the cemetery is still in progress, therefore we may expect the occurrence of further TB cases, which would have modified our recent results of the prevalence and epidemiology of this specific infectious disease. With regard to that the finding is among the earliest occurence of spinal TB in European prehistory it reveals the significance of this case.

P-T14:

EVALUATION OF INTERFERON-GAMMA RELEASE ASSAY FOR THE DETECTION OF ACTIVE MYCOBACTERIUM TUBERCULOSIS INFECTION

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Introduction: QuantiFERON®-TB Gold In-Tube (QFT-GIT) is an interferon gamma release assay (IGRA) for the diagnosis of *Mycobacterium tuberculosis* infection.

Objectives: The aim of this study was to evaluate the usefulness of QFT-GIT in a population with mandatory BCG vaccination.

Patients & methods: Authors performed a retrospective analysis of 146 patients with Quantiferon test compared to clinical, bacteriological and histopathological findings between September 2009 and October 2011. Patients were selected to active (A, n=32) and non-active (NA, n=114) tuberculosis (TB) groups.

Results: In group A, 3/8 (37,5%) cases with positive bacteriology were false-negative with QFT-GIT, while all (4/4) the histopathologically confirmed cases had positive IGRA. In the clinically proved, active TB subgroup, 17/18 (94,4%) were shown QFT-GIT-positive. In two extrapulmonary cases, Quantiferon tests were false-negative (2/2). In group A, the sensitivity of IGRA was 26/32 (81,3 %). In the NA group, false-positive results

were found in malignancy 4/24 (16%), inflammatory diseases 3/35 (8,5%) and rheumatoid arthritis 5/13 (13,3%). In the post TB infected patients, 6/9 (66%) were IGRA positive. In 9 patients with repeated QFT-GIT tests, 4/9 (44%) had different results.

Conclusion: A single positive Quantiferon result can't confirm active tuberculotic condition. Further prospective trials are required for the assessment of its clinical value.

P-T15:

EVIDENCE OF MYCOBACTERIUM TUBERCULOSIS AT 18TH/19TH CENTURY SLAVES IN ANSE SAINTE-MARGUERITE (GUADELOUPE)

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Introduction: During the American colonization in the 18th and 19th century numerous Africans were captured and shipped to the Americas. Straining living and working conditions led to chronic diseases and high mortality rates. Slaves in the Caribbean were mainly forced to work on sugar cane and other plantations. They were buried in cemeteries like Anse Sainte-Marguerite on the isle of Grande-Terre (Guadeloupe).

Objectives: Our objective was to identify possible diseases of a defined historical group with molecular biology methods.

Materials & methods: The Caribbean cemeteries were examined by archaeologists and physical anthropologists. The morphological study of the osseous remains of 272 individuals revealed signs of bone tuberculosis in 20 cases and a high frequency of periosteal reactions which could indicate initial stages of bone tuberculosis. 11 samples from the cemeteries were examined for ancient DNA (aDNA). The samples were extracted with established procedures and examined for the cytoplasmic multicopy *ß-actin* gene by PCR.

Results: There were two positive results with the size of 202 base pairs (bp). We also obtained an amplification product for the *Mycobacterium tuberculosis* complex DNA (IS6110) with the size of123 bp. The result was confirmed by sequencing.

Conclusion: This is the first aDNA evidence for a *M. tuberculosis* complex infection at slaves on Guadeloupe.

P-T16:

A NOVEL POLYMER-BASED DNA PURIFICATION METHOD SUPPORTS REMOVAL OF CO-PURIFIED PCR-INHIBITORS FROM ANCIENT TISSUE EXTRACTS

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Introduction: Nucleic acids extracted from ancient tissue samples are generally co-purified with variable amounts of heterogeneous PCR-inhibitors which may hinder enzymatic downstream applications (e.g. PCR). Various commercial and custom DNA purification techniques share the common problem of losing considerable amounts of nucleic acids and in co-purifying large quantities of PCR-inhibitors.

Objectives: A novel DNA purification technique is described which supports the removal of co-extracted PCR-inhibitors while limiting the loss of DNA.

Materials & methods: The novel polymer-based DNA purification technique was applied on the DNA extracts of both widely used DNA extraction techniques, the Phenol/Chloroform DNA extraction and Silica-based DNA extraction. In addition the P69 method was compared to two common purification methods with Spermine and PEG8000.

Results: The P69 method displays a unique purifying potential after various extraction techniques applied on different natural and artificial samples. Moreover, in samples with high inhibitor content it is possible to reprecipitate with P69 up to 4x without considerable loss of DNA.

Conclusion: In summary the P69 method purifies the tested tissues better than the conventional methods tested. Beside its high DNA purification potential the method has the following additional advantage:
P69 protocol is fast; approx. 25min • No overnight incubation needed even for precipitation of small DNA fragments • P69 precipitates efficiently DNA fragments bigger than 50bp • Polymer has not to be added again for reprecipitations • Polymer-type and polymer-amount are not inhibitory to PCR • P69 could purify the tested extracts better than other conventional method

P-T17:

BONE TUBERCULOSIS IN THE ROMAN PERIOD PANNONIA (WESTERN PART OF HUNGARY) – CASE REPORT

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Introduction: One major category of pathological processes that leave their mark on bones is infectious diseases. Among them, tuberculosis (TB) is one of the most important.

Objectives: Skeleton of an adult woman excavated in the western part of Hungary and dated to the Roman Period provided opportunity for direct observation of tuberculous spondylitis.

Materials & methods: The skeleton (KE91, site: Gyor-Frigyes laktanya) was subjected to gross observation, mycolic acid and proteomic analyses by MALDI TOF/TOF tandem mass spectrometry.

Results: On the skeleton of the 25-30 years old female alterations indicating spinal tuberculosis can be observed in the remaining portions of the vertebrae. The symptoms include: hypertrophic bone formation on Th2-Th3; porosity and bone reaction on bodies of Th4-Th5; reactive bone formation with large lytic focus on Th3-Th4. None of the latter vertebrae showed any evidence of marked collapse and gibbus, only a slight angulation can be considered.

Conclusion: Despite the fact that we only had few data regarding the occurrence of skeletal TB from earlier periods, this disease was definitely present in the area of Hungary in Roman times. Urbanization, new public utilities, improving life circumstances and increased density of population (not only in Hungary but the whole Roman Empire, too) made the spreading of infectious diseases easier. Our results supported by mycolid acid and proteomic analyses contribute to a better interpretation of TB morphology and widen our knowledge of public health in the Roman Period populations.

P-T18:

LIVING AND DYING WITH TUBERCULOSIS AS REVEALED BY THE ARCHIVES OF THE PORTUGUESE SANATORIUM CARLOS VASCONCELOS PORTO (1918-1991)

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Introduction: Sanatoria archives have the potential to unveil useful information that complements and improves our knowledge regarding the paleoepidemiology, paleopathology and social history of tuberculosis (TB). The sanatorium Carlos Vasconcelos Porto (SCVP), inaugurated in 1918 at São Brás de Alportel (Algarve), was the first private institution devoted to TB treatment in Portugal. Until 1952 it served exclusively for the internment of employees from the railway company – "Companhia dos Caminhos-de-Ferro" (CP). Afterwards it was incorporated in the public health system at the National Institute for Tuberculous Assistance –"Instituto de Assistência Nacional aos Tuberculosos" (IANT).

Objectives: This research presents the study of 315 clinical files from SCVP archives which encompass two groups of male patients: before and after the introduction of antibiotics and BCG vaccination and the epidemiologic transition in Portugal.

Materials & methods: The group 1 consisted of 128 CP patients, interned between 1931-1944; group 2 included 187 IANT patients, interned between 1955-1961.

Results: The study of these archives revealed daily life routines of patients and physicians as well as medical procedures. Moreover, when biographic data from groups 1 and 2 were compared the following results are highlighted: the mean age at admission was 40,4 and 39,0 years old respectively; average duration of internment was respectively 325,0 and 361,9 days; mortality during internment was higher in the first (17%) than the second group (11%), although statistically non-significant. Additionally, the mean weight gain in individuals from group 1 was 7.7 kgs, ranging from -10.5 to 41.5 kgs.

Conclusion: This investigation brings new knowledge to the social history of tuberculosis in Portugal.

P-T19:

INFANTS WITH ATYPICAL SKELETAL TUBERCULOSIS FROM THE 8-9TH CENTURY OF HUNGARY

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Introduction: In spite of its probable higher prevalence, a little paleopathological evidence is available on infant tuberculosis.

Objectives: The aim of this study is to present infant cases of skeletal tuberculosis from the 8-9th century (Late Avar Age) cemetery of Szeged-Kiskundorozsma-Kettőshatár.

Materials & methods: The systematic paleopathological investigation on skeletal remains of 360 specimens was carried out using standard bioarchaeological methods.

Results: The paleopathological study revealed a wide range of different pathological conditions, including infectious diseases, such as tuberculosis. Beside some adult individuals the bone remains of two subadults exhibited skeletal morphological features referring to tuberculous infection. A 7-8 years old infant shows pathological alterations (lytic loci, periostitis) in multiple localizations (ribs, iliac bone, endocranial surface,

lumbar vertebrae). These lesions could be attributed to multifocal skeletal tuberculosis. Cervical and thoracic vertebrae of a 12-13 years old infant reveal severe osteolytic lesions. In the cervical region the end plates of the vertebrae remained intact, but the corpus of thoracic vertebrae are completely destroyed. Assessment of lesion morphology led us to consider more diseases affecting this individual at the same time, but tuberculosis seems to be the most likely diagnostic option.

Conclusion: This study enriches our knowledge of tuberculosis in juveniles of past populations, furthermore sheds light on the health conditions of this Avar Age population.

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P-T20:

TUBERCULOSIS ACROSS EUROPE – AN ANCIENT DNA STUDY

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Introduction: Since the first publications on ancient DNA, a number of studies have reported evidence of *Mycobacterium tuberculosis* complex (MTBC) DNA from human remains displaying pathological alterations suggestive of tuberculosis (TB). Some of these studies also looked for the presence of single nucleotide polymorphisms (SNPs). The latter were shown in clinical research to distinguish between species and strains of the MTBC and ultimately led to hypotheses about possible evolutionary pathways for TB.

Objectives: The study presented here analysed a large number of European skeletal samples for the presence of MTBC DNA and aimed to differentiate between the members of the MTBC.

Materials & methods: More than 200 samples spanning the Roman period up to the 19th century were screened for multi-copy number insertion elements IS6110 and IS1081, using real-time and standard PCRs. Positive samples were further examined for a set of single nucleotide polymorphisms and Insertions/Deletions (InDels) using conventional PCR typing.

Results: To date, samples from about 15 individuals have given unequivocal results for MTBC DNA, most of which are derived from UK archaeological sites. Additionally, five samples also yielded SNP and InDel data, respectively.

Conclusion: Our results support the palaeopathological differential diagnosis of tuberculosis for a number of individuals analysed. They further indicate that ancient strains of the MTBC are diverse throughout different time periods, thereby coinciding with the evolutionary pathways suggested in previous studies. The results for one of the samples suggest possible double infection with two different strains of the MTBC.

P-T21:

PRELIMINARY RESULTS FROM THE PALEOMICROBIOLOGICAL STUDIES OF A HUNGARIAN ANTHROPOLOGICAL SERIES

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Introduction: Paleomicrobial research helps to identify human pathogens in ancient human remains and thus provides considerable information on the onset and development of infectious diseases.
Objectives: The aim of the actual molecular paleomicrobiological analysis was to diagnose bacteria of the *Mycobacterium tuberculosis* complex in the 16-17th centuries AD series of Bácsalmás-Óalmás (Hungary). This study has been conducted in the ancient DNA Laboratory of the Institute for Mummies and the Iceman, EURAC Research, Bolzano, Italy.
Materials & methods: A PCR-based assay targeting the multicopy IS6110 region was performed in a subset of the Bácsalmás samples. DNA was extracted using two different extraction methods, a Phenol/Chloroform-based DNA extraction and a Silica-based DNA extraction.
Results: From the 8 examined cases one individual had a positive PCR result for the insertion sequence IS6110 in both DNA extraction methods. Another mycobacterium positive case was only diagnosed in a Phenol/Chloroform-based DNA extract. However, together with the results of the mtDNA hypervariable region 1 PCR the Silica-based DNA extraction clearly outperformed the Phenol-Chloroform extraction.
Conclusion: This preliminary molecular study indicates the necessity in paleomicrobiology to adapt and test the planned molecular analysis on a subset of the samples before starting the analysis of a whole series. In the next steps of our project, in addition to the methodological studies mentioned above, the paleomicrobiological analysis of the complete series and comparative studies of aDNA is planned to be undertaken.

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P-T22:

TUBERCULOSIS AS PROBABLE ETIOLOGY OF TWO KNEE ANKYLOSES FROM MEDIEVAL HUNGARY. CONTRIBUTION OF MEDICAL IMAGING AND 3D RECONSTRUCTION

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Introduction: Osseous ankylosis of the knee associated with tuberculosis does not count as a common phenomenon in contemporary populations, thanks to modern diagnostic techniques and medical interventions. This expression relating to the natural history of the disease can mainly be evidenced nowadays through osteoarcheological specimens, medical reports or books dating from the pre-antibiotic era.
Objectives: Our aim was to investigate whether special medical imaging techniques together with ancient reports and books can help us diagnose advanced-stage tuberculous alterations in osteoarcheological specimens.

Materials & methods: We report here complete ankyloses of the knee observed on two medieval skeletons, excavated in the Bátmonostor cemetery (Hungary). Macroscopic and radiological analyses were completed. 2D and 3D reconstructions were performed by means of specific software programs, such as Amira® 5.2.2 and TIVMI.
Results: 2D CT slices evidenced cavities involving both metaphyses and epyphyses. The 3D reconstructions revealed volumetric morphology of the circumscribed lytic lesions, as well as clear "image-mirror" lacunar volumes. These lesions penetrated the physes and showed a special extension towards the articular space.
Conclusion: Macroscopic and digital analyses allowed us to propose tuberculous infection as the most probable etiology of these two cases. We consider medical imaging including volumetric reconstructions as a valuable technique for retrospective diagnosis of skeletal tuberculosis besides molecular validation.

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P-T23:

JUVENILE CASES OF SKELETAL TB FROM THE TERRY ANATOMICAL COLLECTION (SMITHSONIAN INSTITUTION, WASHINGTON DC, USA)

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Introduction: TB can cause characteristic skeletal changes, such as tuberculous spondylitis (Pott's disease), arthritis or osteomyelitis. However, the morphology of TB lesions in dry bones is frequently not specific, especially in early stage infections, atypical localisations or in immature skeletons.
Objectives: In order to better define the variability of skeletal expression of tuberculosis, TB related lesions and their association were studied in skeletons of individuals who died from TB during the first half of the 20th century.
Materials & methods: Macro-morphological study of 1728 skeletons from the Terry Anatomical Collection was carried out. Among the numerous tuberculous cases we studied there were three juvenile individuals who died from TB that are of particular interest.
Results: The spine of the first case exhibits lesions related to a multifocal cystic TB spondylitis, associated with rib lesions caused by a pneumo-pleural infection. The second skeleton has frontal and parietal endocranial lesions that can be attributed to TB meningitis. The associated multifocal vertebral and costal abnormalities may represent an early stage in the development of skeletal TB lesions. The skull vault of the third juvenile skeleton is perforated by lytic lesions compatible with a diagnosis of cranial TB. These lesions are associated with bone forming endocranial lesions that suggest TB meningitis. The postcranial skeleton shows mainly osteolytic lesions.
Conclusion: These case-studies provide insight regarding the different manifestations of skeletal TB and provide the basis for identifying this infection in archaeological human remains.

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P-T24:

MYCOBACTERIUM TUBERCULOSIS -MYCOBACTERIUM LEPRAE COINFECTIONS FROM HUNGARY: OSTEOLOGICAL AND BIOMOLECULAR FINDINGS

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Introduction: Today tuberculosis is a frequent cause of death in leprosy and it may have been similar in the past. Paleomicrobiology has recently proved TB-leprosy coinfection in past human material (1). However, the skeletal evidence of concomitant leprosy and tuberculosis is extremely infrequent in paleopathology.
Objectives: The aim of our research is the re-evaluation of three potential paleopathological cases of TB-leprosy coinfection.
Materials & methods: Two previously decribed leprosy cases from Hungary (KD517, 7th century AD; LS79, 11th century AD) and a recently published (2) medieval infectious case (ZV228) have been re-evaluated with morphological, aDNA and lipid biomarker methods.
Results: In LS79 new morphological studies provide evidence of leprosy-TB co-infection, while in KD517 they only suggest this diagnosis. In these two cases both DNA and lipid biomarker analyses testified concomitant leprosy and tuberculosis infection. In ZV228 TB-leprosy coinfection can be suggested on a morphological basis, biomolecular analyses are still in progress.
Conclusion: These results furnish important data for the study of past evolution of the two mycobacterial diseases.

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P-T25:

TUBERCULOSIS INFECTION IN A LATE-MEDIEVAL HUNGARIAN POPULATION

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Introduction: The 16-17th AD century Bácsalmás-Óalmás (Hungary) skeletal series has already been the subject of several paleopathological studies on TB-related bone lesions. Initial macromorphological research has suggested a low tuberculosis infection rate within this population.
Objectives: Due to recent development of macroscopic and molecular diagnostic methods in paleopathology and paleomicrobiology, a 5 year international research program was recently started in order to re-evaluate the TB-related lesions in the complete Bácsalmás-Óalmás series.
Materials & methods: Skeletal material of 205 individuals was chosen for the macromorphological investigation, which was focused both on classical/advanced stage skeletal TB alterations and atypical/early-stage TB lesions. Paleomicrobiological analysis was used to study the presence of *Mycobacterium tuberculosis* DNA both in morphologically positive and negative cases. Samples were examined for the repetitive element IS6110 in the *M. tuberculosis* complex. Paleoproteomical analysis of the samples is still in progress.
Results: From the 205 re-examined skeletons 135 possible TB infection cases were found; among them 12 cases were selected for further biomolecular examinations. 6 cases were positive with hot-start PCR for IS6110.
Conclusion: Compared to the previously described few tuberculous cases in this series, we identified a much higher prevalence of *Mycobacterium tuberculosis* infected skeletons. The atypical/early stage skeletal lesions occur significantly more frequently than the so called 'classical' alterations. The paleomicrobial analysis confirmed the *M. tuberculosis* infection in half of the samples selected for this pilot project. Our preliminary results indicate a better preservation of *M. tuberculosis* DNA in the compact long bones.

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P-T26:

NITROQUINOXALINES: NEW POTENTIAL ANTI-TB COMPOUNDS

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Introduction: Number of TB infections increased dramatically in the last decades. Moreover, new strains emerged resistant against the classical antituberculosis drugs.
Objectives: Vichem Ltd has a compound library containing about fifteen thousand molecules designed for pharmacologically active compounds. To find antimycobacterial compounds, we screened this library against *Mycobacterium tuberculosis* at EPFL. Based on the results a promising lead structure was selected and many analogs have been synthesized in VICHEM's laboratory.
Results: Among some other structures 5-nitroquinoxaline derivatives exhibited good inhibitory concentration (MIC90<4 μM) against the bacteria and were non-mutagenic and non-cytotoxic. Detailed study of structure-activity correlations has been done and the crucial chemical functions on the quinoxaline scaffold were determined. It was proved that nitro function in position 5 is essential as well as bromine or trifluoromethyl groups in position 7. In case of the best compound MIC90 was 1.3 μM. Search for the target enzyme of nitroquinoxaline was successful: DprE1 (*decaprenylphosphoryl-β-D-ribose 2'-epimerase*) was identified. This protein is necessary for the synthesis of the cell wall arabinans because its inhibition leads to cell lysis and bacterial death.

Conclusion: We have found a new, patentable compound family, which is a promising drug candidate. Synthesis of several new analogs is in progress.

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P-T27:

TB LINEAGES: DO WE HAVE THE TOOLS TO IDENTIFY PATHOGENIC SPECIFICITIES?

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Introduction: The relevance of any lineage is proportional to the biological importance of the trait that characterizes it, i.e. to the adaptation this trait conferred. If one trait provides a higher fitness, the individuals belonging to this lineage may have outnumbered their closest relatives. This results in a gap between all distances separating individuals within this lineage and distances separating them from other individuals.

Objectives: One aim in evolutionary biology of TB is to identify whether different TB lineages require different control measures because they have different pathogenic features such as drug resistance, transmissibility, host specificity, etc. The delineation of these lineages is crucial to test for correlations. Until now, TB families have been mainly delineated using spoligotype locus (CRISPR TB locus) that is considered neutral and that can exhibit convergence. Our goal is to check whether these families correspond to biologically relevant lineages.

Materials & methods: We describe here a method called Affinity Propagation that clusters data without any supervision. This method identifies a different number of clusters according to a penalty on how distant the clustered data are allowed to be. When the same number of clusters is identified even if the penalty is changed, these clusters may result from adaptive mutations.

Results and conclusion: We validated the Affinity Propagation method on spoligotype families. We show in addition T2 family is as relevant as S or EAI families. We plan to further use this method on VNTR and SNP data to refine families' delineation in TB.

P-T28:

TUBERCULOSIS: A DEMOGRAPHIC ANALYSIS AND SOCIAL STUDY OF ADMISSIONS TO A CHILDREN'S SANATORIUM (1936-1954) IN STANNINGTON, NORTHUMBERLAND

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Introduction: The concept of sanatoria was developed in Britain in the 19th century. They were institutions that were opened throughout the world where people with TB were admitted for rest, a good diet, fresh air, graduated exercise and treatment.

Objectives: To understand the differential impact of TB on male and female children at a 20th century children's sanatorium in terms of different age groups, the types of TB the children suffered, and the relevance of socioeconomic background to TB occurrence.

Materials & methods: 1987 patient records at the Northumberland Record Office at Morpeth from Stannington, Northumberland, north-east England dating from 1936-1954 were studied.
Results: Showed that more females than males were admitted to Stannington during that period, there were peaks of 6 and 13 years of age for admissions, over 63% (1199) children had pulmonary TB, and 12% (230) had bone or joint involvement. Most children where data existed came from urban backgrounds in the Newcastle-upon-Tyne and Gateshead areas. The implementation of chemotherapy at Stannington in 1946, the end of the 2nd World War in 1945, and the founding of the National Health Service in 1948 did not have any great effect on who was admitted to the sanatorium.
Conclusions: This study contributes to understanding the burden of TB for children in early 20th century north-east England.

P-T29:

NEW INSIGHTS INTO THE COMPLEX FORMATION OF ESAT-6 AND CFP-10 OF M. TUBERCULOSIS

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Introduction: Esx proteins of the WXG family that are secreted by "Type VII secretion systems" (T7SS) are among the most important molecules involved in host-pathogen interaction of *M. tuberculosis.* The 6 kDa early secreted antigenic target (ESAT-6) and the 10 kDa culture filtrate protein (CFP-10) are the most studied representatives.
Objectives: As only partial knowledge is available on the function of the ESAT-6 and CFP-10 proteins, the aim of our study was to determine if the biological activity of ESAT-6 and CFP-10 is linked to the formation of a 1:1 heterodimeric complex
Materials & methods: Using targeted mutagenesis, we constructed recombinant strains of *M. tuberculosis* that have selected amino acids in ESAT-6 and CFP-10 replaced by cysteines in order to potentially express ESAT-6 and CFP-10 that are covalently linked by disulfide bonds and so prevent dissociation of the complex.
Results and conclusions: Secretion analysis of these recombinants strains showed that modified ESAT-6 and CFP-10 were produced and secreted by the recombinant strains. Immunological studies that were carried out to test the potential of the complex to induce T cell response against ESAT-6 and CFP-10 showed that presentation of epitope to MHCII remained intact in the recombinant strains. Similarly, virulence studies in mice indicated that strains with covalently linked ESAT-6 and CFP-10 remained virulent. Furthermore, experiments that are focused on the potential biological activity of ESAT-6 and CFP-10 are ongoing.

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P-T30:

PROBABLE CASES OF SKELETAL TUBERCULOSIS FROM THE NEOLITHIC PERIOD OF HUNGARY – A MORPHOLOGICAL STUDY

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Introduction: The historical change from foraging to settled farming in the Neolithic period – associated with larger, denser populations, a sedentary lifestyle and widespread domestication of animals – might have influenced the prevalence of infectious diseases, such as tuberculosis.

Objectives: The aim of this study is to present new data on the occurrence of tuberculosis in the Neolithic period of Hungary based on paleopathological study of skeletal remains from the Tisza culture tell settlement of Vésztő-Mágor.

Materials & methods: The paleopathological investigation of the 30 individuals was carried out using macromorphological methods.

Results: In spite of the poor state of preservation 4 probable cases of tuberculosis (two subadults, one young and one mature adult individual) were discovered. It should be noted that most of the detected alterations (superficial vertebral changes/hypervascularisation, endocranial alterations, potential stress indicators or infection markers, such as cribra orbitalia and long bone periostitis) could be considered as atypical or early stage TB lesions. In order to confirm the supposed diagnoses, further investigations (e.g. DNA or lipid biomarker analyses) are planned.

Conclusion: These results contribute to improving our knowledge on the occurrence of TB in past populations of Hungary.

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P-T31:

DRUG DEVELOPMENT AGAINST MYCOBACTERIUM TUBERCULOSIS PKNB, PKNG AND PKNA KINASES

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Introduction: *Mycobacterium tuberculosis* genes encode 11 Ser/Thr protein kinases from which PknA, PknB and PknG were found essential for growth.

Objectives: The aim of this study was to identify small molecular weight inhibitors on PknA, PknB and PknG kinases.

Results: Previously we performed the biochemical screening of more than 1000 compounds of Vichem's NCL˜ against the previously mentioned tree kinases where several hit molecules inhibited PknB or PnkG kinases in the nanomolar range but none of them possessed good MIC on *Mycobacterium tuberculosis*. Based on the structures of these hits (benzothiophenes) and the ones coming from the phenotype screen of the library (nitroquinoxalines, benzoquinoxalines) a joint pharmacophore was built. After the structure similarity search in the NCL˜ 9 benzo-thienopyrimidine molecules were selected as joint pharmacophore. Unfortunately none of these compounds had significant inhibitory effect on the PknX targets, but two of them showed to inhibit the growth of *M. tuberculosis* at 5μg/ml MIC. In simultaneously other 1000

compounds were tested in target based screening where a common hit was found with IC50 ≈200 nM for PknB and PknG, moreover it inhibits PknA with IC50 = 6 μM.

Conclusion: We hypothesize that the thick cell wall of the bacteria is the main reason why neither this common hit nor the previous hits inhibited *M. tuberculosis*. To confirm our hypothesis and resolve this problem we are trying to monitor the path of the compounds and improve their permeability.

This work was supported by the grants NM4TB (LHSP-CT-2005-0189230) and MuKiT (NIHANR, OMFB-00132/2010).

P-T32:

TWO POSITIVE TB CASES IN THE LATE NIGROVITS FAMILY, 18TH CENTURY, VÁC, HUNGARY

I. Szikossy[1], I. Pap[1], Á. Kustár[1], G. Pálfi[2], E. Molnár[2], K. Karlinger[3], Cs. Korom[3], B. Kovács[3], M. Spigelman[4,6], H. D. Donoghue[5]

[1]Hungarian Natural History Museum, Department of Anthropology, Budapest, Hungary, [2], Department of Biological Anthropology, University of Szeged, Szeged, Hungary, [3]Semmelweis University, Department of Diagnostic Radiology and Oncotherapy, Budapest, Hungary, [4]Department of Anatomy and Anthropology Sackler Medical School Tel Aviv University Israel, [5]Centre for Clinical Microbiology and Centre for the History of Medicine, University College London, UK, [6]Royal Free & University College Medical School, Centre for Clinical Microbiology, RoyalFree Hospital, Rowland Hill Street, London, UK

Introduction: The small Hungarian town of Vác lies to the north of Budapest on the bank of the Danube. During the renovation of the Dominican Church in 1994-95 there was an extraordinary discovery. Researchers discovered the remains of 265 deceased individuals untouched for 200 years. Many were naturally-mummified and well-preserved. The anthropological material from the crypt is stored in the Department of Anthropology of the Hungarian Natural History Museum, Budapest.

Objectives: Our aim was to analyze the occurrence of tuberculosis in an 18th century family.

Materials & methods: Skeletal and naturally mummified tissues from the remains were examined for the presence of *Mycobacterium tuberculosis* complex (MTB) DNA, using specific nested PCR for the IS6110 locus. Overall, 157 specimens from 232 (67.7%) individuals proved to be positive. Two of them were members of the late Nigrovits family. The father, József Nigrovits (No. 29) died aged 55 years on 11.11.1793. His son, Antal Nigrovits (No. 54) died on 16.07.1803, aged 22 years.

Results: The son had an extremely deformed and asthenic body. The father showed a slightly curved neck region. We tried to determine the cause of their diseases with the help of radiological imaging technique and virtual 3D reconstruction. We reconstructed a virtual 3D model of the deformed back of body 29 using the CT scan data of the vertebral column, in order to investigate the morphology of each affected bone.

Conclusion: This study of tuberculosis illustrates a family history of the disease.

The support of OTKA 61155 and OTKA K 73441 is greatly acknowledged.

P-T33:

THE NUN WITHOUT A HEART -A TB CASE FROM THE 18TH CENTURY, VÁC, HUNGARY

I. Szikossy[1], Á. Kustár[1], G. Pálfi[2], L. A. Kristóf[2], I. Horányi[3], K. Karlinger[4], B. Kovács[4], E. Riedl[5], M. Spigelman[6], H. D. Donoghue[7], O. Dutour[8], H. Coqueugniot[9], I. Pap[1]

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Introduction: A large series of well documented, naturally mummified individuals came to light during reconstruction work at the Dominican Church in Vác, Hungary, in 1994-1995. The anthropological material of the crypt is stored in the Department of Anthropology, Hungarian Natural History Museum, Budapest. The coffins, untouched for nearly 200 years, contained the remains of 265 individuals.
Objectives: Our study is part of a complex multidisciplinary research study of the 18-19th century Vác mummies.
Materials & methods: The late Terézia Sándor, a nun with the Poor Clares from Pozsony (Pressburg), died in September 1783 at the age of 40. Researchers discovered a round, sharp-edged incision in the heart region of her body. CT examination clearly showed that her heart had been removed.
Results: Our aim was to answer the question, "Why was the heart removed?" To determine this, radiological examinations were done. The investigation confirmed that the heart was missing. DNA analysis of the sample taken from the body showed the presence of *Mycobacterium tuberculosis* complex. Besides pulmonary TB, serious vertebral damage (probable TB spondylitis) was found on the dorsal part of the mummy's spine.
Conclusion: Following the closure of the convent in Pozsony (Pressburg) in 1872, it is possible that Terézia Sándor was returning to her home town, when tuberculosis (and the probable Pott's disease) forced her to stay in Vác. She died there in 1783 and was buried in the Dominican crypt, but her heart was apparently taken to her hometown for separate burial.

The support of OTKA 61155 and OTKA K 73441 is greatly acknowledged.

P-T34:

MOLECULAR DETECTION OF MYCOBACTERUM TUBERCULOSIS INFECTION IN PATIENTS TREATED IN UNIVERSITY HOSPITALS OF SZEGED

G. Terhes[1], B. Kele[1], A. Somfay[2], J. Deák[1]

[1]*Institute of Clinical Microbiology, University of Szeged, Szeged, Hungary* [2]*Department of Pulmonology, University of Szeged, Szeged, Hungary*

Introduction: About fifteen years ago, molecular methods were introduced to detect TB in our laboratory. On the basis of applied diagnostic technique, two periods can be distinguished. In the first period, clinical specimens were tested simultaneously by Ziehl-Neelsen staining, culture and molecular methods. Molecular methods were set up in our laboratory, while culture was performed in the regional centre. While during the second period, because of the changes in test request, only molecular method was carried out to detect the presence of *M. tuberculosis* complex, in our laboratory.

Objectives: Our aim was to analyse the results of these two periods.
Materials & methods: Clinical specimens were digested and decontaminated, in the case of the first period, samples were cultivated. During this time, three molecular methods were used, while in the second period *M. tuberculosis* was detected using real-time PCR.
Results: During the first period, 862 samples were analysed and 87 (10.09%) proved to be positive using traditional and molecular methods. After this time, our institute performed PCR only if rapid results were necessary in patients with respiratory infections, and to rule out tuberculosis in severe or unusual clinical pictures in which infection has arisen. PCR was also performed in the case of patients with pulmonary nodule from differential diagnostic point of view. In the second period, 1691 specimens were tested and the presence of nucleic acid of *M. tuberculosis* complex was confirmed in 69 (4.08%) specimens.
Conclusion: The use of molecular methods provides rapid results, but the culture of TB is not indispensable because of drug sensitivity testing.

We have commenced whole genome studies of the bacteria for genome changes over the millennia and at host susceptibility/resistance genes on the NRAMP and Killer Cell Immunoglobulin-like Receptors (KIRs). Preliminary results will be discussed here.

Spigelman M and Lemma E (1993) The use of the polymerase chain reaction to detect *Mycobacterium tuberculosis* in ancient skeletons. Int J Osteoarchaeol 3; 137-143.
Fletcher H et al. (2003) Widespread occurrence of *Mycobacterium tuberculosis* DNA from 18th-19th century Hungarians. AJPA 12: 144-52.

Dr. László Botka
Mayor of Szeged

Dear Professors,
Dear Participants,
Dear Guests,

It is a great pleasure for me to welcome all of you in Szeged, who came to participate in the conference series on the occasion of the 75th anniversary of Albert Szent-Györgyi's Nobel Prize Award. The City of Szeged is greatly honoured to be the host of this international event of high importance. Szeged counts as the second biggest educational, research and cultural centre in the country, after Budapest. The figure of 30,000 university students that study here is almost equal to the population of a medium-sized Hungarian town. The network of research institutions is the largest after the capital. Influential research and development activity has become concentrated in Szeged, assuring an outstanding position in the country in the field of research and development. Szeged can be proud of many achievements. In 2003 the city obtained the Europa Nostra Award for preserving the cultural heritage: the total reconstruction of the walking street, including all houses, statues, covering of streets and squares. In 2004, for modern solutions during the investments Szeged was given the "Climate Star" Award by the international Climate Alliance. In 2006, Szeged, the first city ever in Hungary, was awarded the Europe Prize. The Council of Europe has bestowed this distinction to one European city every year, since 1955. It is given for developments, improvements, foreign relations and distributing European idea. It is also a great achievement for the city that a gigantic laser research centre will be built here in the framework of a European project called Extreme Light Infrastructure (ELI). On the other hand Szeged is a beautiful city with plenty of sunshine, friendly people where visitors can discover a lot. We hope all of you will be enriched by lots of pleasant experience during your stay. On behalf of the City of Szeged I want to express my thanks to you all for your strong co-operation and contribution to the success of this significant conference.

Inrtoducing Szeged

In 1879, the city of Szeged was struck by an immense natural disaster: the river Tisza flooded the whole city, as a result of which 95 percent of the buildings was destroyed. Szeged was totally reconstructed after the great flood, and within 5 years a beautiful downtown with palaces was born.



contemporary pictures of the Great Flood of 1879

Our city is the cultural centre of the Southern Great Plain. Here operates one of the most significant theatres of the country, and the Open Air Festival is also held here every summer (July-August), the traditions of which date back to the 1930's, where globally known stars perform noted operas, ballets and musicals in front of an audience of over 4000 people per show.



National Theatre Open Air Theatre Reök Palace

The Reök Palace, which is a beautiful Art Nouveau building, was originally built in 1907. After being renovated and fully equipped, it was opened as a gallery and art centre of the city in 2007. Not only fine art exhibitions can be seen here but several cultural programmes are held in the building like literary evenings, music concerts and chamber theatre performances as well.

Szeged is famous for the hospitality of its inhabitants, it is one of the most visited destinations in Hungary. Among our specialities there are the well-known Pick salami, the handmade Szeged slippers, the ground red paprika and the fish soup.

The Szeged Youth Days, at the end of every August, with over 70 thousand participants, have become one of the most significant youth festivals of the country. We celebrate the Day of Szeged on 21 May every year. On this occasion the visitors can enjoy the atmosphere of the Wine Festival and the Bridge Fair. There are also numerous other international festivals like the Rose Festival in June, the Fish Festival in September, the Paprika Festival in November.

Bridge Fair

Day of Szeged

Rose Festival

The largest thermal water base of Hungary can be found here as well. Our medicinal springs from the depth of 1000 meters have been the base of development for a wide scale of therapeutical and health-related services continually. Today one of the biggest therapeutical and wellness centre of the country called “Aquapolis” based on thermal water can be found in Szeged, which has a water surface of more than 4400 m^2.

Anna Spa

Anna Well

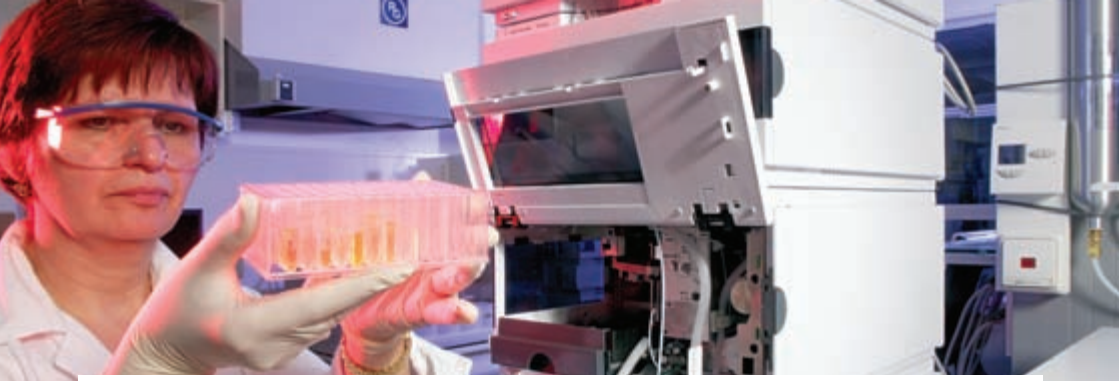
Aquapolis

Apart from the cultural attractions there are important sport events as well. Every year the National Olympic Watersports Centre of Szeged is the host of different world championships like kayak-canoe, rowing and dragonboat. The present life of the city is always changing, reviving. After river Tisza totally destroyed the city which, with the help of Europe and by the hard work of the citizens, was rebuilt and now has become more beautiful than ever. Szeged of the 21st century as an open city ready to continue and widen partnerships to be referred to as a knowledge and regional centre of Europe.

Water Tower

City Hall

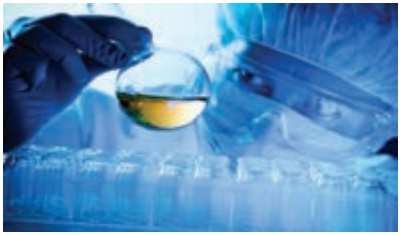
Votive Church



Today, with a 900-strong research and development team in Hungary, the Company has evolved into the Central-Eastern European region's most important center for pharmaceutical research.

The future pharmaceutical industry in Richter today

For any company thinking to the longer term, it is vital to have a presence in new fields that can provide breakout points in the future. For the pharmaceutical sector biotechnology provides such an opportunity. Consequently, in 2007 Gedeon Richter Plc. made the strategic decision to enter this field through the establishment of biotechnological R&D and manufacturing facilities.
The following year the Company commenced the construction of a plant for the development and manufacture of biopharmaceutical products in the Hungarian city of Debrecen, with total expenditure expected to be in the region of HUF 15 billion. The new manufacturing unit is seen as not only a milestone in the realization of Richter's strategic goals but also a significant step forward in enhancing the competitiveness of Hungarian industry and thereby also that of Hungary.
The new biotechnological unit in Debrecen offers a good example of the strategic goals of Richter, a company both based in Hungary and operated by a Hungarian



management team. The Company intends to establish a complex and competitive biopharma line which will help expand its domestic and international product portfolio with products offering high added value.

Gynecology – an area of focus

For Richter gynecological therapy is an area of strategic importance in which the Company is a leading player even by international standards. Its major goal is to be a trustworthy partner for women and physi-

cians alike. Gedeon Richter is one of the few companies in the world which offer a comprehensive gynecology portfolio from the most advanced oral and emergency contraceptives to antifungal and hormone replacement therapy products. Moreover, the Company is determined to expand its product range continuously. Our employees' internationally acknowledged expertise in steroid chemistry and active pharmaceutical ingredient development, the cost-effective large-scale production, together with facilities that comply with the strictest quality standards ensure a

competitive advantage that provides solid ground for further growth.

The acquisition of Swiss pharmaceutical company PregLem was a major milestone in 2010, since PregLem now constitutes Richter's original research and development centre focusing on gynecology, more precisely on gynecological conditions with significant unmet medical needs. On the other hand, the purchase of German Grünenthal's oral contraceptive portfolio offers a unique opportunity for Richter both to strengthen its presence in Western European markets and to further expand the company's existing female healthcare business.

Co-operation

The Company considers it essential to establish research partnerships to facilitate the development and marketing of new molecules. By forming alliances with foreign pharmaceutical manufacturers and research institutions, Richter intends to transfer international experience to serve Hungarian innovation. Richter supports talented youths not only in their positions at the Company but at the research facilities of Hungarian universities and also those of the Hungarian Academy of Science.

The Company runs joint research projects with more than thirty such institutions.

Gedeon Richter Plc.

Chemical Works of Gedeon Richter Plc. is a leading Central-Eastern European pharmaceutical company with its headquarters in Hungary. The multinational company is vertically integrated, Richter is engaged not only in the manufacture but the research and development, and marketing of pharmaceutical products, too. The parent company owns manufacturing

subsidiaries in five countries; its products are available, through its own distribution network, to the inhabitants of nearly 100 different countries.

Richter blends the century-old traditions of Hungarian pharmaceutical production with the high technological standards of modern times. Our main objectives are to deliver advanced medicines to the public, as well as, through our operations, assist in the region's development, help the growth of Hungarian economy and boost the country's competitiveness. Corporate social responsibility is a top priority: environment protection, economic and social sustainability are major concerns in the Company's strategy.

On a mission to improve the quality of human life, Gedeon Richter seeks to serve a healthier future generation in the new millennium.

Unique market network

Unique among Central-Eastern European firms, the market network of Gedeon Richter covers five continents with its products reaching nearly one hundred countries around the world. The Company is present in more than thirty countries thanks to its five production sites, thirty one representative offices, twenty one commercial subsidiaries and wholesale joint ventures. Richter's market network branches into many countries in the European Union; moreover, it extends into the United States, Japan, the CIS countries and the Eastern region.

The Company operates an extensive marketing and distribution network in Hungary, the CIS and Eastern Europe. Furthermore, the acquisition of Swiss PregLem and that of German Grünenthal's oral contraceptive portfolio offers a platform for Richter to establish its sales and marketing teams in key Western European countries. More than 80 per cent of the Company's revenue is generated by exports (978 million euros in 2011). Of key importance in Richter's strategy is the maintenance and enhancement of a strong market position in the traditional markets of the CIS countries, especially Russia, and the Central-Eastern European region, where Richter plays the role of a regional multinational company. However, we also place great emphasis on strengthening our market presence in the US and EU since these remain major contributors to our excellent performance.

Efficient, affordable products – worldwide

The Richter brand is known and respected all over the world. The Company produces about one hundred pharmaceutical products in more than 170 presentations. Among its products are original, generic and licensed preparations, offering effective, advanced and affordable treatments for many therapeutic areas. Whilst our R&D efforts are focused on drugs for the central nervous system, we also attach great importance to the development and production of cardiovascular and gastrointestinal preparations and

oral contraceptives. The product range is constantly expanding as new products are introduced.

In the service of innovation

Original pharmaceutical molecular research and continuous innovation have been defining elements of Richter's strategy since its establishment in 1901.

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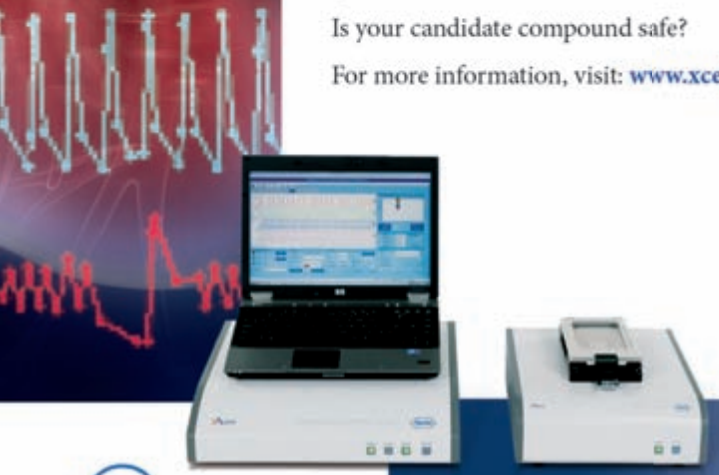

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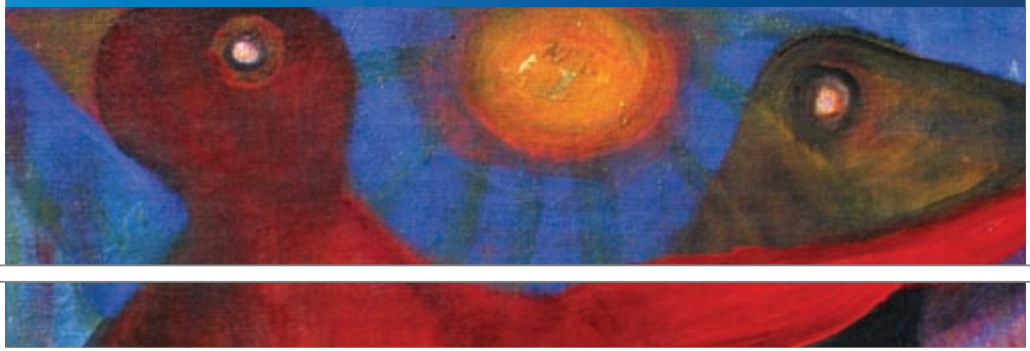

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