MEDICAL UNIVERSITY OF VIENNA

Young Scientist Association

YSA

8th YSA-PhD-Symposium
General Information & Abstracts

Lecture Hall Center of the Medical University of Vienna, General Hospital of Vienna

www.meduniwien.ac.at/ysa

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The YSA thanks the following sponsors for their financial support of the 8th YSA-PhD-Symposium

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8th YSA-PhD-Symposium
General Information & Abstracts

June, 13th - 14th 2012

Programm & Abstracts
General Information

Lecture Hall Center of the Medical University of Vienna
General Hospital of Vienna
Währinger Gürtel 18 - 20
1090 Vienna, Austria

Welcome Address

Wolfgang Schütz,
Rector of the Medical University
of Vienna

Dear colleagues,
Welcome to the 8th YSA-PhD Symosium
of the Medical University of Vienna!

The training of young scientists is one of the Medical University of Vienna's key objectives. Apart from doctoral studies 15 new PhD-programmes have been initiated in recent years. There are now more than 900 early stage researchers enrolled in our doctoral curricula. High impact publications reflect our efforts.
The annual YSA-PhD-Symposium is a major scientific event at the Medical University of Vienna and shall be deemed as an integrative communication platform for basic and applied medical research at our University. The training of young scientists is one of the most important responsibilities a University has to deal with and since 2005, PhD students have the annual opportunity to acquire experience in giving presentations and interacting with colleagues, ranging from undergraduate students to highly renowned international scientists. The growing importance and development of doctoral studies at the Medical University of Vienna is emphasized by the continuously increasing number of submitted abstracts.

I would again like to thank the YSA for organizing this important event and for compiling an exciting program including distinguished speakers from abroad. Clearly, this is the result of YSA's continually improving networking, which is also documented by submissions from young scientists from neighbouring countries.

Karin Gutiérrez-Lobos
Vice-Rector for Education, Gender & Diversity of the Medical University of Vienna

Dear participants,
I warmly welcome you to the 8th YSA-PhD Symposium 2012!

This annual symposium organized by the Young Scientist Association is a major scientific event at the Medical University of Vienna and shall be considered an integrative communication platform for basic and applied medical research at our university. The Medical University of Vienna considers our doctoral students as early stage researchers and training of young scientists is one of the most important responsibilities for this university. At the YSA-PhD-Symposium doctoral students from over 40 thematic programs have the opportunity to gain experience in giving presentations and networking with colleagues from various scientific fields including highly renowned international scientists.

Since 2005 continuously increasing numbers of abstracts have been submitted to the symposium illustrating the growing importance of the doctoral studies at the Medical University of Vienna. This year more than 300 abstracts have been submitted by young scientists reflecting not only the high number of doctoral students but also the increasing impact of the high-level training that the Medical University of Vienna offers our young scientists.

Personally, I would like to thank the Young Scientist Association for organizing this symposium and for compiling this exciting scientific program that includes lectures from distinguished international keynote speakers from a range of scientific fields. I want to highlight the continuous efforts of the Young Scientist Association that improve networking between doctoral students, young scientists, and principal investigators at our university. I thank these outstanding young scientists of the Young Scientist Association for their commitment and their continuous endeavor by which the YSA-PhD Symposium has become both an important part of the scientific career of doctoral students and an integral part of annual scientific program at our university.

Let me conclude by wishing you all an inspiring symposium.

Welcome Address

Stefan Böhm,
Director of the Doctoral School of
the Medical University of Vienna

Dear colleagues,

It is a great pleasure to welcome you at the 8th YSA-PhD-Symposium of the Medical University Vienna (MedUni Vienna). This YSA-PhD-Symposium extends the series of previous YSA-PhD-Symposia very successfully organized by the Young Scientist Association (YSA) of the MedUni Vienna.

In its 8th year, the YSA-PhD-Symposium keeps the high standards developed during its short history! This year, the YSA has received more than 300 abstracts. This witnesses the interest in our YSA-PhD-Symposium, and we are particularly pleased by the fact that we have also received abstracts from neighbouring countries. Of course, we most warmly welcome participants from other universities, whether in Austria or elsewhere in Europe or the world.

The aim of the YSA-PhD-Symposium is to provide a platform for our youngest research fellows, the doctoral students, to present their thesis project in front of academic colleagues other than their supervisors or lab mates. Thereby our young colleagues can hopefully collect novel views and ideas concerning their own research work, not only from fellow students, but also from leading scientists of the Medical University of Vienna. This will certainly help the young researchers to further increase the impact of their scientific work. Moreover, the symposium offers an opportunity to meet doctoral students from other thematic programs, to learn about their work, and to discuss general issues of a doctoral student's daily life with people having an unrelated scientific background.

I would like to thank all those who submitted an abstract for their contribution to this remarkable collection of high level research results that is going to be presented within this symposium. I cordially thank the YSA board for organizing this symposium, the reviewers for evaluating all the abstracts and selecting the oral presentations, and all sponsoring pharmaceutical companies for their support. Special thanks go to Rector Schütz and Vice-Rector Gutiérrez-Lobos for their continuous support of the doctoral school and to the ERSTE Bank and the Vienna Insurance Group; without their help it would not be possible to organize the YSA-PhD-Symposium.

I wish all of you two pleasant days of YSA-PhD-Symposium from both, a scientific and a social perspective.

Hermann Agis,
President of the Young Scientist Association
of the Medical University of Vienna,
On behalf of the YSA Board 2011/2012

Dear colleagues,

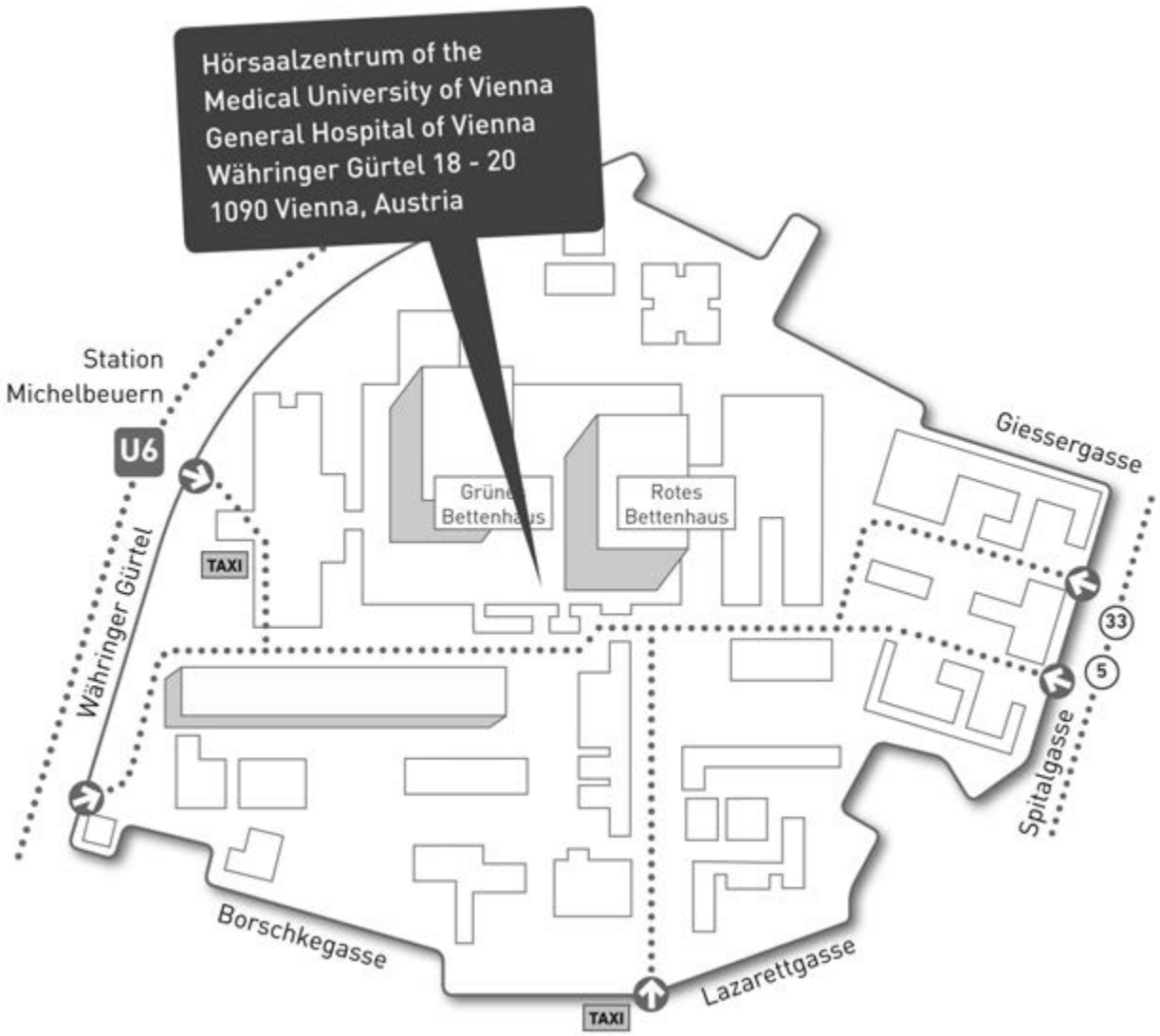
On behalf of the Young Scientist Association (YSA) Board, I welcome all of you to the 8th YSA-PhD Symposium here at the Medical University of Vienna. The first symposium was held in 2005 and has developed into a high performance and interactive scientific platform bringing together doctoral students, academic colleagues and supervisors.

Over the years the YSA-PhD-Symposia have provided opportunities for doctoral students to publically share their data, improve their presentation skills, learn about different methods and discuss their work in a multidisciplinary scientific environment ultimately establishing a scientific national- and international network. Over 1500 scientific projects have been presented by young scientists at the YSA-PhD-Symposia over the last eight years, contributing to the development of each young scientist's scientific career. This year, the current YSA-Board consisting of 7 enthusiastic young scientists, together with their co-workers set out to follow the tradition and organize the 8th YSA-PhD Symposium. We have all learned a lot during this extra-curricular activity and enjoyed the work in the ambitious team of the YSA. We want to encourage all young scientists affiliated with the Medical University of Vienna to join the YSA as members and become thus an important part of this society's future. You, together with over 500 members, help us to follow the vision of this association of young scientists that establish this scientific platform at the Medical University of Vienna.

We all are looking forward to the keynote lectures from four internationally renowned speakers and give a special thanks for their contributions and inspiration throughout the event. In addition, we happily announce that over 300 abstracts were submitted this year. From these abstracts, 24 were chosen to be presented orally and 279 were chosen to be presented as posters during the guided poster sessions. The YSA proudly announces that there will be prices for the best oral- and poster presentation in each session that will be awarded during the closing ceremony at the end of the symposium. The YSA would especially like to thank Rector Schütz, Vice-Rector Gutiérrez-Lobos, Curriculum Director Böhm and Vice-Curriculum Director Lang for their expertise and support during the organization of this years YSA-PhD-Symposium. We would also like to thank all young scientists and supervisors for submitting their abstracts as well as all chairpersons and abstract reviewers for the selection of the abstracts for the oral presentations and the evaluation of the papers for the YSA Publication Award.

Finally, we would like to thank all sponsors of the 8th YSA-PhD Symposium, especially the ERSTE Bank and the Vienna Insurance Group for supporting the Social Event, taking place in the Heurigen Feuerwehr-Wagner (Grinzingerstrasse 53, A-1190 Vienna). All participants who registered for the event are invited to enjoy a relaxing evening with friends and colleagues. We are looking forward to a successful and inspiring event!

Map: Symposium Venue



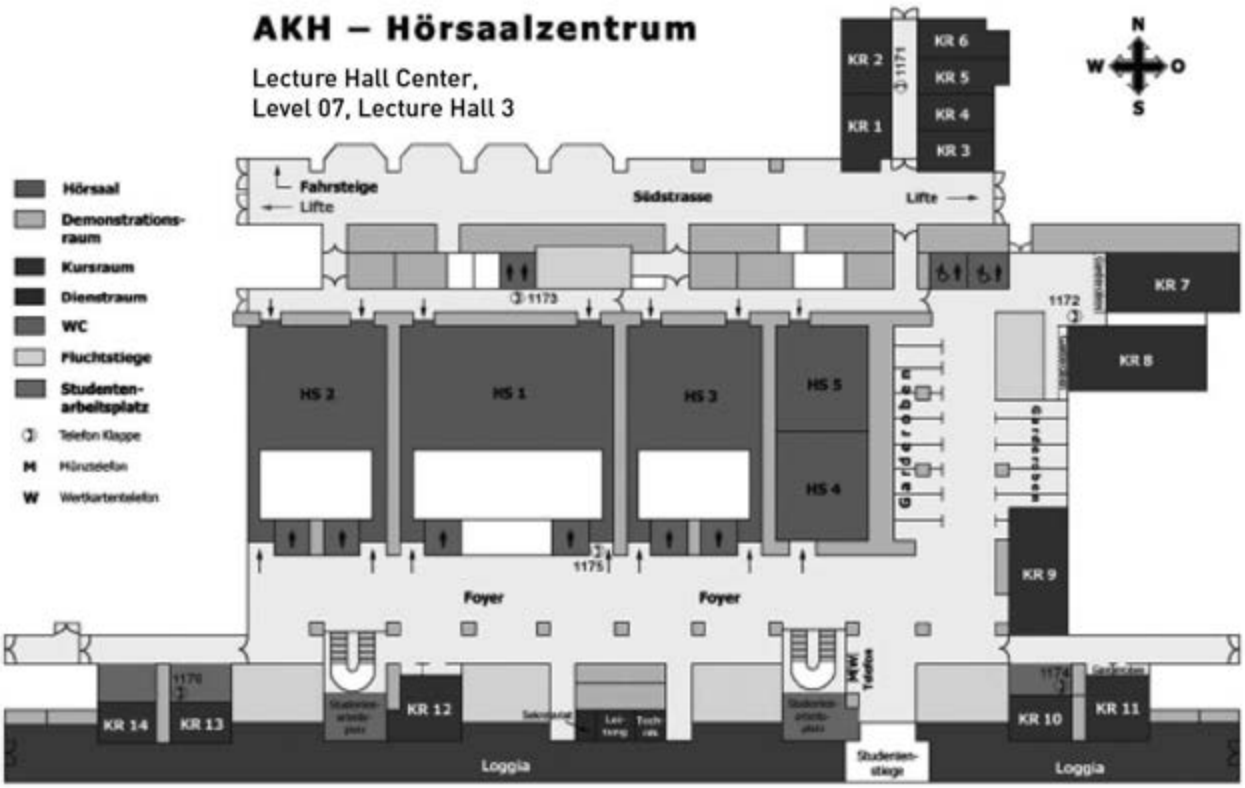


Table of Content

Young Scientist Association of the Medical University of Vienna

Organisation of the YSA-PhD-Symposium 2012

(from left to right): Hermann Agis, Josefine Lindroos, Lilian Kuster, Andreas Heindl, Simone Schlacher, Martina Gaggl, Iris Uras

Networking our way through science

Mission Statement and Activities

1. Facilitating networks of young scientists at the Medical University of Vienna (MedUni Vienna).
2. Establishing a link between young scientists and the board of the MedUni Vienna.
3. Intensifying exchange of information between young scientists and senior researchers.
4. Organizing PhD symposia, scientific workshops and lectures of international speakers.
5. Integrating foreign MSc/PhD students and scientists working at the MedUni Vienna.

Background

YSA was founded in 2005 as a communication platform for young scientists at the Medical University of Vienna.

Organisation

President: Hermann Agis
Vice President: Josefine Lindroos
General Secretary: Martina Gaggl
Treasurer: Lilian Kuster
Communications Officer: Simone Schlacher
IT & Communication: Andreas Heindl
Work Shop Coordinator: Iris Uras

We all would like to thank our Co-Workers for their contribution!

Contact

YSA-Young Scientist Association of the Medical University of Vienna
E-Mail: ysa@meduniwien.ac.at
Homepage: www.meduniwien.ac.at/ysa

Doctoral Programs at the MedUni Vienna

Thematic Programs within the PhD Studies N094

- Cell Communication in Health and Disease (CCHD)
- Endocrinology and Metabolism
- Immunology
- Inflammation and Immunity (IAI)
- Malignant Diseases
- Medical Informatics, Biostatistics & Complex Systems
- Medical Physics
- Molecular Drug Targets (MolTag)
- Molecular Mechanisms of Cell Biology
- Molecular Mechanisms of Cell Signaling
- Molecular Signal Transduction
- Neuroscience
- RNA-Biology
- Structure and Interaction of Biological Macromolecules (SIBM)
- Vascular Biology

Thematic Programs within the Doctor of Applied Medical Science N790

- Biomedical Engineering
- Cardiovascular and Pulmonary Disease
- Clinical Endocrinology, Metabolism and Nutrition
- Clinical Experimental Oncology
- Clinical Neurosciences (CLINS)
- Mental Health and Behavioural Medicine
- POeT - Program for Organfailure-, replacement and Transplantation
- Preclinical Clinical Research for Drug Development
- Public Health
- Regeneration of Bones and Joints

Thematic Programs within the Doctor of Medical Science N090

- Biomedical Engineering
- Molecular Signal Transduction
- Dermatology
- Endocrinology and Metabolism
- Immunology
- Medical Physics
- Mental Health and Behavioral Medicine
- Molecular Biology in Medicine
- Neuroscience
- POeT - Programme for Organfailure, -replacement and Transplantation
- Preclinical and Clinical Research in Pharmaceutic Development
- Psycho-Analysis, Psychotherapy, Psychosomatic Medicine and Ethics
- Regeneration of Bones and Joints
- Tumorbiology - Oncology
- Vascular Biology

Program at a Glance

Wednesday, June 13th 2012

08.30-09.00 Registration and Poster Mounting

09.00-09.30
Opening of the 8th YSA-PhD Symposium

09.30-10.30
Oral Session 1 chaired by Wilfried Ellmeier and Christine Mannhalter

10.30-11.00 Coffee Break

11.00-12.00
Keynote Lecture by Jennifer L. Whistler: Using biased agonism at GPCR Trafficking and Responsiveness to Drugs of Abuse.
Chaired by Michael Freissmuth and Johannes Hainfellner

12.00-12.30 Lunch

12.30-14.30
Oral Session 2 chaired by Maria Sibilia and Rudolf Valenta

14.30-15.00 Coffee Break

15.00-17.00
Guided Poster Session 1

Poster Workshop 1 chaired by Christoph Aufricht and Irma Schabussova
Poster Workshop 2 chaired by Eleonora Dehlink and Winfried Pickl
Poster Workshop 3 chaired by Barbara Scheiber-Mojdehkar and Clemens Scheinecker
Poster Workshop 4 chaired by Mariann Gyongyosi and Egon Ogris
Poster Workshop 5 chaired by Senta Graf and Franz Kainberger
Poster Workshop 6 chaired by Peter Steinberger and Theresia Thalhammer
Poster Workshop 7 chaired by Karlheinz Hilber and Xiaohui Rausch-Fan
Poster Workshop 8 chaired by Peter Pietschmann and Zsolt Szepfalusi
Poster Workshop 9 chaired by Heimo Breiteneder and Elisabeth Förster-Waldl
Poster Workshop 10 chaired by Oleh Andrukhov and Martin Hohenegger
Poster Workshop 11 chaired by Stefan Böhm and Wolfgang Schreiner
Poster Workshop 12 chaired by Johannes Breuss and Barbara Cvikl

17.00-18.00
Keynote Lecture by Anna Teti: Bone Modeling and Remodeling in Health and Disease

Sponsored by the Austrian Society for Bone and Mineral Research (ÖGKM)
Chaired by Katharina Kerschan-Schindl and Michael B. Fischer

19.00 - 23.00

Social Event
sponsored by the
ERSTE Bank and the
Vienna Insurance Group

Thursday, June 14th 2012

08.30-09.00 Poster Mounting

09.00-10.30
Oral Session 3 chaired by Daniela Pollak and Timothy Skern

10.30-11.00 Coffee Break

11.00-12.00
Keynote Lecture by Rashika El Ridi: Novel Chemotherapeutic and Vaccination Approaches for Schistosomiasis

Sponsored by the Austrian Society of Allergology and Immunology (ÖGAI)
Chaired by Ursula Wiedermann and Gerhard Zlabinger

12.00-12.30 Lunch

12.30-14.30
Guided Poster Session 2

Poster Workshop 13 chaired by Martin Bilban and Renate Kain
Poster Workshop 14 chaired by Isabella Ellinger and Gerold Holzer
Poster Workshop 15 chaired by Diana Bonderman and Johannes Schmid
Poster Workshop 16 chaired by Ghazaleh Gouya and Alexander Niessner
Poster Workshop 17 chaired by Johannes Berger and Brigitte Hantusch
Poster Workshop 18 chaired by Kyra Borchhardt and Michael Kundi
Poster Workshop 19 chaired by Gürkan Sengoelge and Jolanta Siller-Matula
Poster Workshop 20 chaired by Gabriela Berlakovich and Sebastian Nijman
Poster Workshop 21 chaired by Ulrike Holzinger and Jürgen Zezula
Poster Workshop 22 chaired by Elisabeth Presterl and Stefan Wagner
Poster Workshop 23 chaired by Diana Mechteriakova and Walter Speidl
Poster Workshop 24 chaired by Enikö Kallay and Johann Wojta

14.30-16.00
Oral Session 4 chaired by Adelheid Elbe-Bürger and Hannes Stockinger

16.00-16.30 Coffee Break

16.30-17.00
YSA Publication Award Lecture

17.00-18.00
Keynote Lecture by J. Andrew Pospisilik: Epigenetic Control of Metabolic Disease

Chaired by Denise Barlow and Stefan Kubicek

18.00 - 18.30
Awards for best presentations

Congress Information

Registration
Registration will take place at the registration desk, which is located in the admission area at Level 7 of the Lecture Hall Centre (AKH-Hörsaalzentrum).

Registration Fee
Admission to all scientific sessions is free.

Name Badges
Delegates are required to wear their name badges throughout the congress.

Language
Official congress language is English.

Congress Venue
Lecture Hall 3 at the General Hospital Vienna

Social Program
On Wednesday evening, June 13th, 7:00 p.m. registered attendees are cordially invited to the Heurigen Feuerwehr-Wagner (Grinzingerstrasse 53, 1190 Vienna) to enjoy an exclusive dinner.

How to get there:
• 10-15 min taxi or take the U6 metro from the Gerneral Hospital „Michelbeuern-AKH“ towards „Floridsdorf“. Get of at station „Spittelau“.

• Follow the signs to tram D (Strassenbahn D). Take tram D towards „Nußdorf“. Get of at „Grinzinger Straße“. Walk to Grinzinger Straße 38 (Weingut Feuerwehr Wagner).

The YSA would like to thank the ERSTE Bank and the Vienna Insurance Group for facilitating the Social Event.

The YSA thanks the following sponsors
for their financial support of the 8th YSA-PhD-Symposium:

Medical University of Vienna

ERSTE Bank

Vienna Insurance Group

Szabo Scandic

Alumni Club Medical University of Vienna

Cell Communication in Health and Disease (CCHD), PhD Program

eBioscience

Life Technologies

PAA

Structure and Interaction of Biological Macromolecules (SIBM), PhD Program

THP Medical Products

Center for Molecular Medicine of the Austrian Academy of Sciences (Ce-M-M Research)

Molecular Mechanisms of Cell Signaling, PhD Program, Max F. Perutz Laboratories, Vienna

Austrian Society for Bone and Mineral Research (ÖGKM)

Austrian Society of Allergology and Immunology (ÖGAI)

Roche

Amgen

Biomedica

Biozym

Inflammation and Immunity (IAI), PhD Program

New England Biolabs

Bartelt

Charles River

Wednesday, June 13th 2012

Oral Session 1

Chaired by Wilfried Ellmeier and Christine Mannhalter

S 1 **The role of the transcription factor MAZR in mast cells**
Abramova, A. , Sakaguchi, S. , Schebesta, A. , Boucheron, N. , Schmidt, U. , Ellmeier, W.

S 2 **Connectional hierarchy in the primary somatosensory cortex of primates**
Ashaber, M. , Pálfi, P. , Palmer, C. , Kántor, O. , Friedman, R. , Roe, A. , Négyessy, L.

S 3 **Genotype of serotonin-1B receptor affects serotonin-1A receptor binding in vivo**
Baldinger, P. , Hahn, A. , Mitterhauser, M. , Kraus, C. , Wadsak, W. , Rujescu

S 4 **The molecular signature of cutaneous graft-versus-host disease:**
IL-22 and Th2 cytokines predominate in the acute disease stage.
Brüggen, C. , Klein , I. , Greinix, H. , Bauer, W. , Kuzmina, Z. , Rabitsch, W. , Knobler, R. , Stingl, G., Stary, G.

Keynote Lecture 1

K 1 **Keynote lecture by Jennifer L. Whistler**
GPCR trafficking and responsiveness to drugs of abuse.
Chaired by Michael Freissmuth and Johannes Hainfellner

Oral Session 2

Chaired by Maria Sibilia and Rudolf Valenta

S 5 **Restoring CGMP levels in diabetic rats attenuates podocyte damage**
Fang, L. , Radovits, T. , Szabo, G. , Mozes, M. , Rosivall, L. , Kokeny, G.

S 6 **Role of epigenetic mechanisms in regulation of the Calcium Sensing Receptor expression in colorectal cancer**
Fetahu, I. , Höbaus, J. , Hummel, D. , Thiem, U. , Manhardt , T. , Kallay, E.

S 7 **A pencil beam algorithm for scanned helium ion beam dose calculation**
Fuchs, H. , Ströbele, J. , Schreiner, T. , Hirtl, A. , Georg, D.

S 8 **Disruption of STAT3 signaling promotes K-Ras induced lung tumorigenesis**
Grabner, B. , Schramek, D. , Zwick, R. , Penninger, J. , Sibilia, M. , Popper, H. , Eferl, R. , Casanova, E.

S 9 **An isolated heart setup to investigate diagnostic and control methods for rotary blood pumps**
Granegger, M., Mahr, S., Eskandary, F., Horvat, J., Zimpfer, D., Schima, H., Moscato, F.

S 10 **The role of iron in oxidative tissue damage in multiple sclerosis**
Hametner, S. , Schuh, C. , Haider, L. , Wimmer, I. , Lassmann, H.

S 11 **Influence of ketamine on resting-state functional connectivity in healthy volunteers-a fMRI study**
Höflich, A. , Hahn, A. , Atanelov, J. , Baldinger, P. , Kraus, C. , Windischberger, C. , Kasper, S., Lanzenberger, R.

S 12 **Sigma-1 receptor agonist treatment is protective against renal ischemia/reperfusion injury**
Hosszu, A. , Banki, N. , Antal, Z. , Koszegi, S. , Prokay, A. , Vannay, A. , Szabo, A. , Fekete, A.

Poster Session 1

Poster boards are sponsored by the following PhD programs:
Cell Communication in Health and Disease (CCHD)
Structure and Interaction of Biological Macromolecules (SIBM)

Poster Workshop 1

Chaired by Christoph Aufricht and Irma Schabussova

P 1 **Deciphering the role of the Calcium Sensing Receptor in Regulating Colorectal Cancer Proliferation**
Aggarwal, A. , Kállay, E.

P 2 **Prolyl hydroxylase inhibitors: A new strategy for periodontal regeneration?**
Agis, H. , Hueber, L. , Schröckmair, S. , Vinzenz, P. , Wehner, C. , Watzek, G. , Gruber, R.

P 3 **Inter-device reliability of Dysphonia Severity Index measurement**
Aichinger, P. , Feichter, F. , Aichstill, B. , Bigenzahn, W. , Schneider-Stickler, B.

P 4 **Mucosal tolerance induction with structurally different antigens: studies on underlying mechanisms**
Akgün, J. , Schabussova, I. , Hufnagl, S. , Wild, C. , Wiedermann, U.

P 5 **Expression of thyroid hormone binding protein CRYM (μ-crystallin) negatively correlates with advanced stages of prostate cancer**
Aksoy, O. , Hassler, M. , Herac, M. , Culig, Z. , Susani, M. , Zielinski, C. , Thallinger, C. , Kenner, L.

P 6 **Alkali treatment of titanium surfaces increases adhesion of stromal cells in vitro**
Al Mustafa, M. , Agis, H. , Watzek, G. , Gruber, R.

P 7 **A Smoothened-Ampk axis rewires metabolism**
Amann, S. , Teperino, R. , Bayer, M. , Loipetzberger, A. , Knauf, C. , Aberger, F. , Pospisilik, J. , Esterbauer, H.

P 8 **Peptide Mimotopes of Malondialdehyde-Epitopes for Clinical Applications in Cardiovascular Disease**
Amir, S. , Hartvigsen, K. , Gonen, A. , Jensen-Jarolim, E., Tsimikas, S. , Wagner, O. , Witztum, J. , Binder, C.J

P 9 **Heme arginate protects Skeletal Muscle against Ischemia Reperfusion Injury: A randomized, placebo controlled Trial in healthy Subjects**
Andreas, M. , Schmid, A. , Doberer, D. , Schewzow, K. , Weisshaar, S. , Heinze, G. , Moser, E. , Wolzt, M.

P 10 **Effects of bacteria and lipopolysaccharide on human megakaryocytes**
Arbesu Cruz, I. , Zhang, J. , Ramanathan, G. , Mannhalter, C.

P 11 **Diversity and projections of ventral hippocampal pyramidal neurons.**
Arszovszki, A. , Borhegyi, Z. , Klausberger, T.

P 12 **Specific interaction of dendritic cells in response to several allergens**
Ashjaei, K., Lengger, N., Smole, L., Bublin, M., Breiteneder, H., Hoffmann-Sommergruber, K., Wagner, S.

Poster Workshop 2

Chaired by Eleonora Dehlink and Winfried Pickl

P 13 **Effects of statins on ABCB1 localisation and on endogenous dolichol level**
Atil, B. , Hohenegger, M.

P 14 **Higher-order principles in distribution patterns of serotonergic receptor subtypes revealed by PET**
Attaripour Isfahani, S. , Wadsak, W. , Bauer, A. , Ding, Y. , Henry, S. , Rattay, F. , Lanzenberger, R. , Savli, M.

P 15 **Platelets directly enhance neutrophil transmigration in response to oxLDL**
Badrnya, S. , Butler, L. , Söderberg-Naucler, C. , Volf, I. , Assinger, A.

P 16 **A rapid and simple electrophoretic approach to screen for glutamine deamidation**
Bae, N. , Yang, J. , Herald, S. , Javier, M. , Gert, L.

P 17 **Der p 11, the mite paramyosin, is an important allergen only in certain geographical areas**
Banerjee, S. , Resch, Y. , Chen, K. , Swoboda, I. , Scheiblhofer, S. , Valenta, R. , Vrtala, S.

P 18 **Altered Neural Activation within the Working Memory Network in Remitted Major Depressive Disorder**
Bartova, L. , Diers, K. , Rabl, U. , Meyer, B. , Scharinger, C. , Moser, E. , Kasper, S. , Pezawas, L.

P 19 **Low biological variation protein characterization by 2D DIGE as new putative Normalization Standards**
Baumgartner, R. , Veitinger, M. , Umlauf, E. , Oehler, R. , Gerner, C. , Volf, I. , Lamont, J. , Zellner, M.

P 20 **Comparison of the cross-protection induced by TBEV vaccines**
Beck, Y. , Fritz, R. , Orlinger, K. , Barrett, P. , Kreil, T.

P 21 **Transmitter release by nicotinic receptors in the spinal cord**
Beiranvand, F. , Schwarz, K. , Huck, S. , Scholze, P.

P 22 **Characterization of the inflammatory response to solid cancer metastases in the human brain**
Berghoff, A. , Lassmann, H. , Höftberger, R. , Preusser, M.

P 23 **Folding of G-Protein Coupled Receptors: The Adenosine-A2A/Molecular Chaperone Connection**
Bergmayr, C. , Gsandtner, I. , Holy, M. , Kudlacek, O. , Nanoff, C. , Thurner, P. , Freissmuth, M. , Gruber, C.

P 24 **Resonance frequency analysis: A pilot study on a new diagnostic tool for dental ankylosis**
Bertl, M. , Weinberger, T. , Schwarz, K. , Gruber, R. , Crismani, A.

Poster Workshop 3

Chaired by Barbara Scheiber-Mojdehkar and Clemens Scheinecker

P 25 **Identification of transient PP2A.RTS1-Substrate interactions by a method called M-TRACK**
Bhatt, B. , Kupka, T. , Mudrak, I. , Schuechner, S. , Kuderer, S. , Frohner, I. , Ammerer, G. , Ogris, E.

P 26 **Dissection of flavivirus entry and assembly by structure-based mutational analysis**
Blazevic, J. , Bilek , G. , Heinz, F. , Stiasny , K.

P 27 **Evaluation of primary meniscus refixation using different magnet resonance sequences – a prospective, clinical cohort study**
Blutsch, B. , Aldrian, S. , Trattnig, S.

P 28 **Inquiring about avian thymic dendritic cells**
Bódi, I.

P 29 **Correlation between Ureaplasma biovars detected by real-time PCR from a single vaginal smear and preterm delivery: preliminary results**
Böhm, J. , Kasper, D. , Schulz, S. , Jatzko, B. , Witt, A. , Hafner, E. , Sliutz, G. , Berger, A.

P 30 **Separation of protein complexes in chronic inflammatory diseases (Juvenile Idiopathic Arthritis) by means of Blue-Native PAGE**
Bohn, A. , Tendl, K. , Herkner, K. , Kenzian, H.

P 31 **Insulin like growth factor binding protein 7 (IGFBP7) is downregulated in multiple myeloma with consequences for myeloma cell growth and bone disease.**
Bolomsky, A. , Hose, D. , Schreder, M. , Heintel, D. , Pfeifer, S. , Ludwig, H. , Zojer, N.

P 32 **Late toxicity after primary external beam radiation therapy in prostate cancer**
Valentin Bombosch , Maximilian P. Schmid , Richard Pötter , Samir Sljivic , Christian Kirisits , Wolfgang Dörr , Gregor Goldner

P 33 **Cross-linked iron oxide particles - synthesis and visualization**
Borny, R. , Popovic, M. , Edelhauser, P. , Gürkan, E. , Priessner, K. , Neumüller, J. , Lammer, J. , Funovics, M.

P 34 **The Arterial Supply of the Long Head of the Biceps Tendon with its Clinical Implications on Superior Labral Anterior to Posterior (SLAP) Lesions**
Bösmüller, S. , Fialka, C. , Pretterklieber, M.

P 35 **Tools for Assessing Protein Regulation Dynamics from Quantitative Mass Spectrometry Data**
Breitwieser, F. , Colinge, J.

P 36 **CDK4 and 6 as therapeutic targets in human melanoma - just redundant proteins?**
Briand, C. , Jerney, W. , Blunder, S. , Schicher, N. , Pehamberger, H. , Hoeller, C.

P 37 **Novel adhesion molecules involved in lymph node metastasis**
Brown, M. , Hantusch, B. , Raab, I. , Bennett, K. , Kerjaschki, D.

P 38 **Angiogenesis is challenged in experimental brain metastases**
Bugyik, E. , Szabó, V. , Dezső, K. , Nagy, P. , Paku, S.

Poster Workshop 4

Chaired by Mariann Gyongyosi and Egon Ogris

P 39 **The enhancement of osteogenesis through the use of dental pulp pluripotent stem cells in 3D**
Caballé Serrano, J., Gil, C., Martínez, E. , Giner, L., Atari, M.

P 40 **A Lab-on-a-Chip for Cell-Based Assays: Continuous and Label-Free Monitoring of Human Fibroblasts**
Charwat, V. , Ertl, P. , Joksch, E. , Kloesch, B. , Kiener, H.

P 41 **The roles of serotonin (5HT) receptors in pain sensation**
Das Gupta, K. , Yousuf, A. , Boehm, S.

P 42 **Ambulatory arterial stiffness index in renal transplant children – Cross sectional study**
Dégi, A. , Kerti, A. , Kis, É. , Cseprekál, O. , Szabó, A. , Reusz, G.

P 43 **Treatment of mallet fractures by K-wire extension block technique - a biomechanical comparison of 4 different methods**
Dietmaier, M.

P 44 **A Spatio-Temporal Latent Atlas for Fetal Brain Segmentation**
Dittrich, E. , Riklin-Raviv, T. , Kasprian, R. , Brugger, P. , Prayer, D. , Langs, G.

P 45 **The role of BMP antagonists in human fracture healing.**
Domaszewski, F. , Sarahrudi, K.

P 46 **The NBD-NBD interface is not the sole determinant for transport in ABC transporters**
Dönmez, Y. , Parveen, Z. , Chiba, P. , Stockner, T.

P 47 **Neurotransmitter Alterations in Ether Lipid Deficiency**
Dorninger, F. , Peneder, T. , Pifl, C. , Forss-Petter, S. , Berger, J.

P 48 **Is chronic low-dose dexamethasone treatment sufficient to induce apoptosis within rat cortex?**
Drakulić, D. , Stanojlović, M. , Grković, I. , Mitrović , N. , Horvat, A.

Poster Workshop 5

Chaired by Senta Graf and Franz Kainberger

P 49 **VGLUT3 expressing primary afferents in neuropathic pain**
Draxler, P. , Honsek, S. , Forsthuber, L. , Maleiner, B. , Sandkühler, J.

P 50 **Renal impairment in premature infants – influence on short-term outcome**
Dufek, S. , Ehringer, T. , Cardona, F. , Aufricht, C. , Arbeiter, K. , Csaicsich, D.

P 51 **Gender, wellbeing and chronic autoimmune diseases: Results of a qualitative Study**
Dür, M. , Sadloňová, M. , Smolen, J. , Dejaco, C. , Kautzky-Willer, A. , Fialka-Moser, V. , Stamm, T.

P 52 **The impact of modification and localization of the Src family kinase Lck on T cell activation**
Eckerstorfer, P. , Paster, W. , Zimmermann, L. , Sonnleitner, A. , Schütz, G. , Stockinger, H.

P 53 **Proteomics study of Kv7 channels**
Erdem, F. , Chen, W. , Lubec, G. , Boehm, S. , Yang, J.

P 54 **Expression of canine immunoglobulins against the tumour antigen EGFR**
Fazekas, J. , Singer., J. , Weichselbaumer, M. , Wang, W. , Mader, A. , Steinfellner, W. , Sobanov, Y. , Mechtcheriakova, D. , Matz, M. , Spillner, E. , Kunert, R. , Jensen-Jarolim, E.

P 55 **A functional pharmacogenetic screen in lung cancer**
Fece de la Cruz, F. , Smida, M. , Nijman, S.

P 56 **Interaction of FimH with Glomerular Endothelial cells and LAMP-2**
Feenstra, T. , Schmidt, M. , Brandes, R. , Aarestrup, F. , Rees, A. , Kain, R.

P 57 **Extending The Field Of View In Adaptive Optics Scanning Laser Ophthalmoscopy**
Felberer, F. , Kroisamer, J. , Hitzenberger, C. , Pircher, M.

P 58 **Antagonizing endosomal Toll-like receptors diminishes inflammatory arthritis**
Fischer, A. , Herman, S. , Pfatschbacher, J. , Hoffmann, M. , Steiner, G.

P 59 **Trabecular Direction and Deformation Distribution in Lung Transplant Patients with severe Osteoporosis Risk**
Fischer, L. , Patsch, J. , Zweytick, P. , Schüller-Weidekamm, C. , Valentinitsch, A. , Kainberger, F. , Langs, G.

P 60 **In vitro study of biological compatibility of different surface coating**
Fleischmann, L. , Rausch-Fan, X. , Crismani, A. , Andrukhov, O.

Poster Workshop 6

Chaired by Peter Steinberger and Theresia Thalhammer

P 61 **The novel IL-1 family member IL-33 is produced during human pregnancy and controls function of primary trophoblasts in vitro**
Fock, V. , Zeisler, H. , Knöfler, M. , Pollheimer, J.

P 62 **Gene expression and bone architecture in men with osteoporotic hip fractures**
Föger-Samwald, U. , Patsch, J. (1,2), Salem, S. , Pail, P. , Schamall, D. , Mousavi, M. , Kainberger, F. , Pietschmann, P.

P 63 **Local circuits in the intermediate CA1 hippocampus**
Forro, T. , Valenti, O. , Lasztoczi, B. , Klausberger, T.

P 64 **IFN gamma inducible GTPase guanylate binding protein 1 negatively regulates T cell receptor activation at a very early stage**
Forster, F. , Paster, W. , Zojer, V. , Schiller, H. , Zlabinger, G. , Naschberger, E. , Stürzl, M. , Stockinger, H.

P 65 **Regulation of Scavenger Receptor Class B, type I by mTOR in vascular endothelial cells**
Fruhwürth, S. , Röhrl, C. , Mikula, M. , Stangl, H.

P 66 **Functional and physical interactions between P2Y receptors and ion channels**
Gafar, H. , Chandaka, G. , Boehm, S.

P 67 **Reducing damages of the lung following lower limb ischemic-reperfusion injury by postcondition**
Garbaisz, D. , Turóczi, Z. , Rosero, O. , Lotz, G. , Rakonczay, Z. , Harsányi, L. , Szijártó, A.

P 68 **Non-responsiveness to certain routine vaccines involves different regulatory immune cell populations and IL10 production**
Garner-Spitzer, E. , Wagner, A. , Paulke-Korinek, M. , Kollaritsch, H. , Heinz, F. , Fischer, G. , Kundi, M. , Wiedermann, U.

P 69 **Probing the first crystal structure of a voltage-gated Na channel**
Gawali, V. , Lukács, P. , Cervenka, R. , Koenig, X. , Rubi, L. , Mike, A. , Hilber, K. , Hannes, T.

P 70 **The ambiguous role of TREM-2 in Escherichia coli peritonitis**
Gawish, R. , Sharif, O. , Doninger, B. , Stich, K. , Knapp, S.

P 71 **Epitope grafting between Bet v 1 and its homologue Api g 1 produces chimeric proteins with different lysosomal stability**
Gepp, B. , Lengger, N. , Briza, P. , Wallner, M. , Smole, U. , Ferreira, F. , Radauer, C. , Breiteneder, H.

P 72 **Centerpoint Replotting And Its Effects On Central Retinal Thickness In Four Prevalent SD-OCT Devices**
Gerendas, B. , Waldstein, S. , Lammer, W. , Montuoro, A. , Bota, G. , Simader, C. , Schmidt-Erfurth, U.

Poster Workshop 7

Chaired by Karlheinz Hilber and Xiaohui Rausch-Fan

P 73 **Facilitation of synaptic strength in the spinal cord induced by prolonged opioid exposure**
Gerhold, K. , Drdla, R. , Sandkühler, J.

P 74 **Mass Spectrometrical Identification of Hippocampal NMDA Receptor Subunits NR1, NR2A-D and Five Novel Phosphorylation Sites on NR2A and NR2B.**
Ghafari, M. , Höger, H. , Pollak, A. , Lubec, G.

P 75 **Protective effect of biliverdin and biliverdin reductase against bile acid-induced toxicitiy in liver cells**
Gonzalez Sanchez, E., M.J. Perez, N.S. Nytofte, O. Briz, M.A. Serrano, M.J. Monte, F. Jimenez, F. Gonzalez-San Martin, J.J.G. Marin

P 76 **Imaging of Endogenous mRNA Variants in Living Plant Cells**
Göhring, J. , Jacak, J. , Barta, A.

P 77 **The role of Stat3 in murine Natural Killer Cells**
Gotthardt, D. , Eva, P. , Birgit, S. , Biaggio, M. , Sexl, V.

P 78 **NeuROn - Neuropsychological Rehabilitation Online**
Grafeneder, J. , Slavc, I. , Leiss, U.

P 79 **Abstract Withdrawn**

P 80 **Serum levels of Dkk-1 in patients with normal and impaired fracture healing**
Gregori, M. , Köttstorfer, J. , Sarahrudi, K.

P 81 **The Role of Natural IgM Antibodies with Specificity for Malondialdehyde-adducts in Atherosclerosis**
Gruber, S. , Tsiantoulas, D. , Ozsvar Kozma, T. , Binder, C. ,

P 82 **An Essay Concerning the Molecular Prediction of Disease Outcome in Melanoma Patients**
Gschaider, M. , Neumann , F. , Peters , B. , Wolf , I. , Wenzel , , Mauch , C. , Schreiner , W. , Wagner , S.

P 83 **IgE cross-reactivity between Bet v 1 and the mung bean proteins cytokinin-specific binding protein and Vig r 1 in patients with birch pollen-associated allergy to mung bean sprouts**
Guhsl, E. , Hofstetter, G. , Hemmer, W. , Ebner, C. , Vieths, S. , Vogel, L. , Breiteneder, H. , Radauer, C.

P 84 **O-GlcNAc modification of STAT5a is essential for its transforming properties**
Hager, M. , Kerenyi, M. , Nivarthi, H. , Gouilleux, F. , Hölbl, A. , Kovacic, B. , Sexl, V. , Moriggl, R.

Poster Workshop 8

Chaired by Peter Pietschmann and Zsolt Szepfalusi.

P 85 **Oxidative Damage to Basal Ganglia in Multiple Sclerosis**
Haider, L. , Steinberger, G. , Hametner, S. , Lassmann, H.

P 86 **Notch signaling plays a role in human placental development: regulation of cell column proliferation and trophoblast invasion**
Haider, S. , Pollheimer, J. , Meinhardt, G. , Knoefler, M.

P 87 **Characteristics, treatment modalities, clinical outcome and evaluation of new treatment techniques in bony avulsion of the FDP**
Halat, G. , Negrin, L. , Hajdu, S. , Erhart, J. , Platzer, P.

P 88 **Microbial analysis in saliva – a new way to determine periodontopathic bacteria**
Haririan , H. , Bertl, K. , Andrukhov, O. , Moritz, A. , Rausch-Fan, X.

P 89 **Evaluation of the mechanism of action of a novel anti cancer compound**
Hebar, A. , Mads, D. , Sorensen, P. , Selzer, E.

P 90 **Expression Level of the Receptor for Advanced Glycated End Products (RAGE) in the Syncytiotrophoblast correlates with the Severity of Per-Eclampsia as demonstarted by a novel Method for automated in-situ Quantifications of Proteins**
Heindl, A. , Dekan, S. , Rogojanu, R. , Ecker, R. , Bises, G. , Thalhammer, T. , Uhrova, H. , Seewald, AK. , Ellinger, I.

P 91 **Follistatin as an inducer of lymph vessel formation in melanoma**
Heinz, M. , Niederleither, H. , Grusch, M. , Petzelbauer, P.

P 92 **Hippocampal levels and activity of the glutamate transporter 1 (GLT-1) and sodium/potassium trans porting ATPase subunit alpha-3 (AT1A3) are paralleling memory training in the multiple T-Maze in the C57BL/6J mouse**
Heo, S. , Csaszar, E. , Beuk, T. , Jung, G. , Hoeger, H. , Lubec, G.

P 93 **Characterization of the MHC-helices' tertiary structure via differential geometric parameters**
Hischenhuber, B., Havlicek, H., Schreiner, W., Knapp, B.

P 94 **Gene Amplification but not epigenetic alterations cause aberrant vitamin D 24-hydroxylase expression in colorectal cancer**
Höbaus, J., Thiem, U., Fetahu, I., Hummel, D., Gober, L., Manhardt, T., Kallay, E.

P 95 **Adipokines in pediatric and adult obesity - TOBI Kids**
Hochbrugger, E., Fritsch, M., Itariu, F., Prager, G., Zeyda, M., Willfort-Ehringer, A., Widhalm, K., Stulnig, T.

Poster Workshop 9

Chaired by Heimo Breiteneder and Elisabeth Förster-Waldl

P 96 **Mechanistic analysis of costimulation blockade-resistant rejection of donor bone marrow triggered by donor T cells**
Hock, K., Pilat, N., Baranyi, U., Gattringer, M., Muehlbacher, F., Wekerle, T.

P 97 **The impact of recipient age on the outcome of bone marrow transplantation-based tolerance induction**
Hock, K., Oberhuber, R., Lee, O., Wekerle, T., Tullius, S.

P 98 **Computertomography-based evaluation of volumetric changes after sinus floor augmentation**
Hof, M., Pommer, B., Girardi, M., Heimel, P., Watzek, G., Zechner, W.

P 99 **Establishment of a new ischemic excision model**
Hofmann, A., Hartinger, J., van Griensven, M., Redl, H., Mittermayr, R.

P 100 **Bacterial Ghosts as Delivery Systems for Foreign Proteins**
Höggerl, F., Schlacher, S., Langemann, T., Hodul, I., Champeimont, J., Mayr, B., Lubitz, W.

P 101 **Topical Application of Glucocorticoids for Hearing Preservation in an Animal Model of Cochlear Implantation**
Honeder, C., Gstoettner, W., Plasenzotti, R., Arnoldner, C.

P 102 **Does the MTHFR 677C>T influence genetic imprinting and FVIII activity in hemophilia A carriers?**
Horvath, B., Male , C., Pabinger-Fasching, I., Reitter-Pfoertner, S., Thom, K., Mannhalter, C.

P 103 **High Dietary Vitamin D Reduces the Load of Chemically-Induced Colorectal Tumors in Mice**
Hummel, D., Thiem, U., Höbaus, J., Fetahu, I., Manhardt, T., Mesteri, I., Kallay, E.

P 104 **Alternative splicing of EGFR ECD in human melanoma**
Imrédi, E., Rásó, E., Tímár, J.

P 105 **A genome-scale collection of gene deletion mutants in C. glabrata**
Istel, F., Schwarzmüller, T., Glaser, W., Willinger, B., Kuchler, K.

P 106 **Ultramicroscopy (UM): 3D reconstructions of vascular networks in mice using lectinstaining**
Jährling, N., Auer, C., Tabatabai, G., Hahn, C., Saghafi, S., Becker, K., Dodt, H.

Poster Workshop 10

Chaired by Oleh Andrukhov and Martin Hohenegger

P 107 **Genetic variability of the human GLA gene in a healthy Austrian population**
Jallitsch-Halper, A., Steinhauser, C., Huber, A., Koizar, D., Födinger, M., Sunder-Plassmann, G.

P 108 **Fine specificity of the human antibody response after tick-borne encephalitis virus infection and vaccination**
Jarmer, J., Zlatkovic, J., Aberle, J., Chmelik, V., Stiasny, K., Heinz, F.

P 109 **Blockade of cellular adhesion mediated by osteopontin and its protease-cleaved forms by monoclonal antibodies**
Jürets, A., Leitner, L., Sarabi, A., Zeyda, M., Stulnig, T.

P 110 **Renin-angiotensin-aldosterone system blockers in diabetic nephropathy: the role of epithelial to mesenchymal transition**
Kőszegi, S., Bánki , N., Wagner, L., Hosszú, Á., Lénárt, L., Gellai, R., Tulassay, T., Fekete, A.

P 111 **The GPCR – associated sorting protein 1 regulates rimonabant-induced downregulation of GPR55**
Kargl, J., Whistler, J., Waldhoer, M.

P 112 **Scopolamine Administration Modulates Muscarinic, Nicotinic and NMDA Receptor Systems**
Keihan Falsafi, S., Höger, H., Pollak, A., Lubec, G.

P 113 **The effect of weight loss and metformin treatment on glycotoxic intermediates in type 2 diabetes.**
Kender, Z., Reismann, P., Grolmusz, V., Rácz, K., Nawroth, P., Bierhaus, A.

P 114 **NPHS2 p.V290M mutation in adult-onset steroid-resistant nephrotic syndrome – should it be screened for?**
Kerti, A., Csohány, R., Szabó, A., Árkossy, O., Sallay, P., Szabó, T., Reusz, G., Tory, K.

P 115 **Alzheimer's disease risk gene-product Lymphocyte-specific Protein Tyrosine Kinase regulates neuritic outgrowth, long-term synaptic strengthening and in vivo hippocampus-dependent spatial learning and memory**
Kim , E., Monje, F., Pollak, D., Li, L., Lubec, G.

P 116 **Anti-tumour effects of combination resveratrol and celecoxib in vitro and in vivo**
Kisková, T., Jendželovský, R., Papčová, Z., Orendáš, P., Bojková, B., Kassayová, M., Fedoročko, P.

P 117 **Implication of interleukin – 1 beta in the formation of neuromyelitis optica – like lesions in the rat brain**
Kitic, M., Hochmeister, S., Pohl, M., Lassmann, H., Bradl, M.

Poster Workshop 11

Chaired by Stefan Böhm and Wolfgang Schreiner

P 118 **Characterising the interaction between the COPII component SEC24C and the human serotonin transporter**
Koban, F., Sucic, S., Freissmuth, S.

P 119 **Evaluation of health-related and legal interventions regarding allegedly delinquent and convicted opioid addicts in Austria**
Koechl, B., Bruckmueller, K., Jagsch, R., Soyer, R., Fischer, G.

P 120 **Macrophage-Colony Stimulating Factor (M-CSF) and Transforming Growth Factor-beta 1 (TGF- ß1) - Predictive Serum Markers for Fracture Healing?**
Koettstorfer, J., Domazsewski, F., Kaiser, D., Kecht, M., Sarahrudi, K.

P 121 **Pharmacological characterization of circular plant peptides with oxytocin-like activity**
Köhbach, J., O´Brien, M., Miazzo, M., Muttenthaler, M., Akcan, M., Craik, D., Freissmuth, M., Gruber, C.

P 122 **Protective effect of a single radiation dose applied before transient ischemia in rat's hippocampal neurons**
Kokošová, N., Burda, J., Šmajda, B.

P 123 **Train the Brain – Neural responses as a measure of therapeutic success in patients with anosmia**
Kollndorfer, K., Krssak, M., Frasnelli, J., Hoche, E., Kowalczyk, K., Mueller, C., Trattnig, S., Schöpf, V.

P 124 **A Normative database of the serotonergic system in healthy subjects using multi-tracer PET**
Kraus, C., Mitterhauser, M., Bauer, A., Ding, Y., Henry, S., Rattay, F., Savli, M., Lanzenberger, R.

P 125 **alpha-Catulin down-regulates E-cadherin and promotes Melanoma Progression and Invasion**
Kreiseder, B., Orel, L., Bujnow, C., Pflüger, M., Hundsberger, H., Schütt, W., de Martin, R., Wiesner, C.

P 126 **Inhibition of pro-inflamamtory effects in human cardiac myocytes and endothelial cells by levosimendan**
Krychtiuk, K., Watzke, L., Kaun, W., Demyanets, S., Huber, K., Maurer, G., Wojta, J., Speidl, W.

P 127 **Rapid ROS generation by the preclinical anticancer ruthenium compound KP1339 predicts cancer cell sensitivity**
Kryeziu, K., Heffeter, P., Pirker, C., Senkiv, Y., Jungwirth, U., Keppler, B., Berger, W.

P 128 **Using statistical measures for automated comparison of in-beam PET data for ion beam therapy verification**
Kuess, P., Birkfellner, W., Helmbrecht, S., Fiedler, F., Enghardt, W., Georg, D.

Poster Workshop 12

Chaired by Johannes Breuss and Barbara Cvikl

P 129 **Detection of short-lived protein-protein interactions - Identification of protein phosphatase 2A (PP2A) substrates**
Kupka, T., Bhatt, B., Mudrak, I., Schüchner, S., Frohner, I., Reiter, W., Ammerer, G., Ogris, E.

P 130 **Pharmacochaperoning of the ER-retained A1-adenosine receptor.**
Kusek, J., Gruber, C., Nanoff, C., Freissmuth, M.

P 131 **Phagocytosis of mesothelial cells in peritoneal dialysis**
Kuster, L., Herzog, R., Böhm, M., Kratochwill, K., Spittler, A., Aufricht, C.

P 132 **Response to the extracorporeal photophersis in patients with bronchiolitis obliterans syndrome according to the new National Institutes of Health (NIH) consensus criteria after allo-HSCT: prospective study.**
Kuzmina, Z., Weigl, R., Petkov, W., Krenn, K., Greinix, H.

P 133 **Firing patterns of identified prefrontal interneurons in freely-moving rats**
Lagler, M., Borhegyi, Z., Klausberger, T.

P 134 **GLI1 as a novel marker for pituitary adenoma stem cells**
Lampichler, K., Ilhan-Mutlu, A., Wolf, F., Vila, G., Knosp, E., Wagner, L., Luger, A., Baumgartner-Parzer, S.

P 135 **Establishment of a fluorescent proliferation assay for fungal cells suitable for flow cytometry analysis**
Langenecker, J., Bourgeois, C., Majer, O., Kuchler, K.

P 136 **Effect of different therapy options on immunoglobulin levels in patients suffering from acute, acute-on-chronic, or chronic liver failure**
Lebherz-Eichinger, D. , Schmidt, E. , Motal, M. , Klaus, D. , Mangold, A. , Ankersmit, H. , Krenn, C., Roth, G.

P 137 **Evaluation of IMRT and VMAT treatment plan quality delivered with and without flattening filter using Pareto optimal fronts**
Lechner, W., Kragl, G., Georg, D.

P 138 **Reduced somatostin production in colorectal cancer with uncontrolled cell proliferation, as compared to controlle cell growth in young and adult normal colonic mucosa**
Leiszter, K. , Sipos, F. , Galamb, O. , Wichmann, B. , Patai, Á. , Valcz, G. , Molnár, B. , Tulassay, Z.

P 139 **Lysosomal Membrane Protein 2 (LAMP-2), a potential new receptor involved in antigen presentation in Dendritic Cell**
Leone, D.

Keynote Lecture 2

K 2 **Keynote Lecture by Anna Teti**
Bone Modeling and Remodeling in Health and Disease

Chaired by Katharina Kerschan-Schindl and Michael B. Fischer
This lecture is sponsored by the Austrian Society for Bone and Mineral Research (ÖGKM)

Thursday, June 14th 2012

Oral Session 3

Chaired by Daniela Pollak and Timothy Skern

S 13 **Polo-like Kinase is necessary for cytoskeletal inheritance of Trypanosoma brucei**
Ikeda, K. , Warren, G. , de Graffenried, C.

S 14 **Keratinocytes but not dendritic cells express TLRs 3, 6 and NOD2 in human prenatal and adult skin in situ**
Iram, N. , Mildner, M. , Prior, M. , Petzelbauer, P. , Fiala, C. , Hacker, S. , Tschachler, E. (2,6), Elbe-Bürger, A.

S 15 **Evaluation of HDL-associated proteins as novel biomarkers for chronic kidney disease**
Kopecky, C. , Eller, P. , Gerner, C. , Michlits, G. , Säemann, M. , Weichhart, T.

S 16 **Osteopontin-induced inflammatory response of human adipocytes**
Leitner, L. , Jürets, A. , Sarabi, A. , Zeyda, M. , Stulnig, T.

S 17 **Cryo-electron tomography of baculovirus-induced actin comet-tails**
Müller, J. , Schur, F. , LeClainche, C. , Narita, A. , Maeda, Y. , Welch, M. , Carlier, M. , Small, V.

S 18 **Angiogenesis in degenerative aortic valve disease**
Panzenböck, A. , Jakowitsch, J. , Seitelberger, R. , Bonderman, D. , Rosenhek, R. , Baumgartner, H. , Lang, I.

Keynote Lecture 3

K 3 **Keynote lecture by Rashika El Ridi**
Novel Chemotherapeutic and Vaccination Approaches for Schistosomiasis.
Chaired by Ursula Wiedermann and Gerhard Zlabinger
This lecture is sponsored by the Austrian Society of Allergology and Immunology (ÖGAI)

Poster Session 2

Poster boards are sponsored by the following PhD programs:
Cell Communication in Health and Disease (CCHD)
Structure and Interaction of Biological Macromolecules (SIBM)

Poster Workshop 13

Chaired by Martin Bilban and Renate Kain

P 140 **Transdifferentiating alpha cells into insulin producing beta cells**
Li, J. , Kubicek, S.

P 141 **Role of mTOR during Differentiation of Human Amniotic Fluid Stem Cells to Schwann Cells**
Li, K. , Preitschopf, A. , Rosner, M. , Shanmugasundaram, B. , Lubec, G. , Hengstschläger, M. , Mikula, M.

P 142 **Targeting cancer epigenetic vulnerabilities using small molecules inhibitors**
Licciardello, M. , Dürnberger, G. , Berg, T. , Markt, P. , Colinge, J. , Kralovics, R. , Nijman, S. , Kubicek, S.

P 143 **Proteomic analysis of cell populations in artificial peritoneal dialysis effluents**
Lichtenauer, A. , Herzog, R. , Aufricht, C. , Kratochwill, K.

P 144 **Heme Oxygenase-1 specifically modulates pro- and anti-adipogenic molecules to inhibit white adipose differentiation.**
Lindroos, J. , Zapf, T. , Jeitler, M. , Husa, J. , Tauber, S. , Esterbauer, H. , Wagner, O. , Bilban, M.

P 145 **Understanding the role of Polo-like kinase in Trypanosoma brucei through chemical genetics**
Lozano, A. , Warren, G. , de Graffenried, C.

P 146 **A calcium channel-like selectivity filter modulates an access pathway for local anesthetic drugs in voltage-gated Na channels**
Lukács, P. , Cervenka, R. , Gawali, V. , Koenig, X. , Rubi, L. , Mike, Á. , Hilber, K. , Todt, H.

P 147 **Characterization of the protein microenvironment of the folate receptor beta**
Machacek, C. , Stockinger, H. , Repic, A.

P 148 **Intracellular recognition machinery for Streptococcus pneumoniae**
Maier, B. , Sigel, S. , Knapp, S.

P 149 **Chemical Genetics to Uncover Breast Cancer Vulnerabilities**
Mair, B. , Müllner, M.

P 150 **Type I Interferons Exacerbate Lethality during Candida-Induced Sepsis Through CCL2-Mediated Recruitment of Inflammatory Monocytes**
Majer, O. , Bourgeois, C. , Zwolanek, F. , Lassnig, C. , Kerjaschki, D. , Mack, M. , Müller, M. , Kuchler, K.

P 151 **Neuronal firing coding of the prefrontal cortex and hippocampus during a rule switching task**
Malagon VIna, H. , Tomioka, R. , Klausberger, T.

Poster Workshop 14

Chaired by Isabella Ellinger and Gerold Holzer

P 152 **Role of ERG in endothelium and its cooperation with NF-κB.**
Malkani, N.

P 153 **Targeting the HIF pathway to inhibit tumour angiogenesis: Focus on HIF-1B**
Mandl, M. , Kapeller, B. , Lieber, R. , Mandlmayr, A. , Macfelda, K.

P 154 **Neutrophils and NETs at the culprit lesion site of ST-elevation acute coronary syndrome**
Mangold, A. , Scherz, T. , Falkinger, A. , Puthenkalam, S. , Distelmaier, K. , Preissner, K. , Lang, I.

P 155 **Specific monocyte subsets are increased at the culprit lesion site of ST-elevation acute coronary syndrome patients**
Mangold, A. , Falkinger, A. , Scherz, T. , Distelmaier, K. , Lang, I.

P 156 **CD4CD28null T-cells are specifically enriched at the culprit lesion site of ST-elevation acute coronary syndrome patients**
Mangold, A. , Scherz, T. , Falkinger, A. , Adlbrecht, C. , Lang, I.

P 157 **The ex vivo and in vivo antioxidant effect of cortisol**
Marczell, I. , Stark, J. , Nagy-Repas, P. , Rácz, K. , Bekesi, G.

P 158 **Prostate Cancer Stem Cells Demonstrate Decreased mTOR Activity and are Resistant to mTOR Inhibition in Hypoxia**
Marhold, M. , Tomasich, E. , Hofbauer, T. , Spittler, A. , Krainer, M. , Horak, P.

P 159 **Unraveling the alternative splicing landscape in Arabidopsis using RNA-seq**
Marquez Ortiz, Y. , WS Brown, J. , Simpson, C. , Kalyna, M. , Barta, A.

P 160 **A single nucleotide polymorphism (rs342293) on chromosome 7q22.3 is associated with mean platelet volume in patients with carotid artery disease**
Mayer, F. , Arbesu, Hoke, M. , Schillinger, M. , Minar, E. , Koppensteiner, R. , Horvath, Mannhalter, C.

P 161 **Liquid chromatography tandem mass spectrometry-based method for the rapid determination of lysosomal enzyme activities for selective metabolic and newborn screening in dried blood spots**
Mechtler, T. , Mueller, H. , Metz, T. , Ostermann, K. , Herkner, K. , Kasper, D.

P 162 **A multigene signature-based approach to uncover the contribution of sphingolipid machinery to the process of epithelial to mesenchymal transition**
Meshcheryakova, A. , Svoboda, M. , Jensen-Jarolim, E. , Mechtcheriakova, D.

P 163 **Cardiomyogenic effects of Cardiogenol C on lineage-committed progenitor cells**
Mike, Á. , König, X. , Todt, H. , Koley, M. , Schnürch, M. , Mihovilovic, M. , Weitzer, G. , Hilber, K.

Poster Workshop 15

Chaired by Diana Bonderman and Johannes Schmid

P 164 **Copper complexation of thiosemicarbazones enhances apoptosis in human colon carcinoma**
Miklos, W. , Heffeter, P. , Kowol, C. , Jungwirth, U. , Keppler, B. , Berger, W.

P 165 **Chromatin- and transcription-assisted inactivation of interferon-activated transcription factors Stat1 and Stat2**
Mikulic, I. , Kovarik, P.

P 166 **Towards subtype selective modulators of GABAA receptors**
Mirheydari, P. , Varagic, Puthenkalam, R. , Ramerstorfer, J. , Sieghart , W. , Ernst, M.

P 167 **Transcriptome analysis of 3T3-L1 preadipocyte differentiation using RNA-seq reveals formerly unidentified differentially regulated genes**
Mitterer, G. , Tauber, S. , Klinglmueller, F. , Husa, J. , Lindroos, J. , Jeitler, M. , Wagner, O. , Bilban, M.

P 168 **Induction of IL-35 in human T cells upon co-stimulation via CD43 and PD-1**
Modak, M. , Seyerl, M. , Aigner, R. , Cejka, P. , Majdic, O. , Zlabinger, G. , Stoeckl, J.

P 169 **Pediatric epilepsy surgery – predictors of (un)favourable outcome**
Muehlebner-Fahrngruber, A.

P 170 **Glucocorticoid receptor function is essential for SOCS2-mediated negative regulation of hepatic GHR signaling**
Mueller, K. , Kornfeld, J. , Schuetz, G. , Hilton, D. , Moriggl, R.

P 171 **TLR-independent recognition of Streptococcus pyogenes is required for successful inflammatory response**
Mühlbacher, C. , Gratz, N. , Kratochvill, G. , Ebner, F. , Sigl, S. , Knapp, S. , Alexopoulou, L. , Kovarik, P.

P 172 **Pharmacokinetic-pharmacodynamic modeling of P-glycoprotein function at the rat blood brain barrier studied with positron emission tomography**
Müllauer, J. , Syvänen, S. , Kuntner, C.

P 173 **Detection of bartonella spp. in ixodes ricinus**
Müller, A., Reiter, M., Stockinger, H., Khanakah, G., Stanek, G.

P 174 **Prolyl hydroxylase inhibitors increase the production of vascular endothelial growth factor in human dental pulp cells**
Müller, H. , Trimmel, K. , Cvikl, B. , Watzek, G. , Gruber, R. , Agis, H.

P 175 **Kinetics of primary and memory IgG1 & IgE antibody responses induced by an allergen derivative in-vivo in a murine model of allergy.**
Narayanan, M. , Focke-Tejkl, M. , Valenta, R.

Poster Workshop 16

Chaired by Ghazaleh Gouya and Alexander Niessner

P 176 **Can serum biomarkers reliably quantify lung contusion in polytraumatized patients?**
Negrin, L. , Halat, G. , Gregori, H. , Schüller, G. , Kettner, S. , Hajdu, S. , Heinz, T.

P 177 **Exploratory Analysis of Multiple fMRI Paradigms**
Nenning, K. , Langs, G.

P 178 **Role of lipid-derived mediators in obesity-induced adipose tissue inflammation**
Neuhofer, A. , Zeyda, M. , Mascher, Z. , Itariu, B. , Legerer, B. , Matzner, E. , Stulnig, T.

P 179 **Humanized Model for Respiratory Allergy Using a Human Mugwort-specific T-cell Receptor and HLA-DR1**
Neunkirchner, A., Leb-Reichl, V., Schmetterer, L., Wojta-Stremayr, D., Rosloniec, E., Jahn-Schmid, B., Bohle, B., Pickl, W.

P 180 **Detailed hemodynamic characterization of athlete's heart using left ventricular pressure-volume analysis in a rat model**
Oláh, A., Lux, Á., Birtalan, E., Hidi, L., Németh, B., Merkely, B., Radovits, T.

P 181 **Prevention of distant organ failure by postconditioning of small intestine on ischemia reperfusion injury model of rats**
Ónody, P., Rosero, O., Hegedűs, V., Pomizs, I., Dániel, Á., Harsányi, L., Lotz, G., Szijártó, A.

P 182 **Pancreatitis-associated protein-ELISA (MucoPAP) as a second-tier test for cystic fibrosis. Results of a four month's period within the Austrian Newborn Screening.**
Ostermann, K., Metz, T., Prusa, A., Herkner, K., Kasper, D.

P 183 **Expression and regulation of Notch pathway members in human decidualization**
Otti, G., Saleh, L., Knöfler, M.

P 184 **A retrospective data analysis of children and adolescents having immigration background in the acute psychiatry – transcultural risk and resilience factors in relation to suicidal and self-harming behaviour**
Özlü, Z., Akkaya-Kalayci , T.

P 185 **Tri a 37, a new wheat food allergen, is a member of plant defence proteins**
Pahr, S., Constantin, C., Papadopoulus, N., Mäkelä, M., Ebner, C., Mari, A., Thalhamer, J., Valenta, R.

P 186 **Development of Therapeutic Attitudes: Teaching and Learning in Psychotherapy**
Pastner, B., Schechtner, C., Billeth, S., Löffler-Stastka, H.

P 187 **IL-10R2 overexpression is restricted to microsatellite-stable colorectal cancer and enhances proliferation upon IL-22**
Paul, G., Movadat, O., Khare, M., Gasche, C.

Poster Workshop 17

Chaired by Johannes Berger and Brigitte Hanusch

P 188 **Clustered regularly interspaced short palindromic repeats: Bacteriophage-defence system in Clostridium difficile**
Pecavar, V., Fiedler, A., Kunczer, V., Hasenberger, P., Indra, A.

P 189 **Lymphangiogenesis in kidney transplants is strikingly different in humans and mice.**
Pedersen, M.

P 190 **Effects of antibodies to lysosomal associated membrane protein 2 on human macrophages**
Peschel, A.

P 191 **The endosomal transporter CD222 - a novel regulator of T cell activation?**
Pfisterer, K., Forster, F., Zojer, V., Eckersdorfer, P., Zwirzitz, A., Stockinger, H., Leksa, V.

P 192 **T cells in Neuromyelitis optica**
Pohl, M., Misu, T., Mader, S., Fujihara, ., Reindl, M., Lassmann, H., Bradl, M.

P 193 **Protein kinases signaling networks involved in learning and memory linked to Alzheimer's disease**
Polyakova, M., Sase, S., Li, L., Lubec, G.

P 194 **The Oral Health Impact Profile (OHIP) to measure Oral Health-related Quality of Life (OHQoL) in clinical oral implant research**
Pommer, B., Dvorak, G., Hof, M., Watzek, G., Palmer, R.

P 195 **Assessment of Batch to Batch Variation in Polyclonal Antithymocyte Globulin Preparations**
Popow, I., Leitner, J., Majdic, O., Saemann, M., Zlabinger, G., Steinberger, P.

P 196 **A novel score of p53 activity increases the accuracy of p53 diagnosis in human breast cancer**
Proestling, K., Glock, A., Marton, E., Suess, D., Vinatzer, U., Schreiber, M.

P 197 **Retargeting T cells to viral glycoproteins for adoptive therapy of human Cytomegalovirus infection**
Proff, J., Lehner, M., Full, F., Besendörfer, M., Ensser, A., Holter, W.

P 198 **Alveolar bone structure of implant sites following either orthodontic tooth movement or tooth extraction**
Pseiner, B., Plenk, H.

P 199 **A mid-infrared sensor system for detecting changes in melanoma cells treated with anti-225D9.2+-TT antibodies**
Pucciarelli, D., van den Driesche, S., Wagner, S., Vellekoop, M., Breiteneder, H., Hafner, C.

Poster Workshop 18

Chaired by Kyra Borchhardt and Michael Kundi

P 200 **Understanding subtype selective allosteric modulation of GABAA receptors**
Puthenkalam, R., Varagic, Z., Mirheydari, P., Sieghart, W., Ernst, M.

P 201 **Angiogenesis in chronic thromboembolic pulmonary hypertension (CTEPH)**
Puthenkalam, S., Panzenboeck, A., Winter, M., Schubert, U., Jakowitsch, J.,

P 202 **Pathway analysis: How does Wnt1 perform its anti-lymphangiogenic function in melanoma**
Puujalka, E., Niederleithner, H., Heinz, M., Petzelbauer, P.

P 203 **The effects of religious conviction/spirituality on the coping strategies of cancer patients**
Rassoulian, A., Gaiger, A., Büssing, A.

P 204 **Genome-wide genotyping of patients with benign childhood epilepsy with centrotemporal spikes**
Reinthaler, E., Lal, D., Zimprich, A., Sander, T., Neubauer, B., Zimprich, F.

P 205 **Genotyping Lyme borreliosis spirochetes**
Reiter, M., Schötta, A., Korschinek, I., Müller, A., Khanakah, G., Stockinger, H., Stanek, G.

P 206 **The coexistence of Huntigton and Alzheimer disease**
Remenyi, V., Miltenberger-Miltenyi, G., Nyiro, G., Kovacs, T., Molnar, M.

P 207 **The effect of in vitro-gastro-duodenal digestion of the major shrimp allergen tropomyosin on IgE reactivity and allergenic activity**
Resch, Y., Weghofer, M., Mari, A., Scheiblhofer, S., Focke, M., Thalhamer, J., Valenta, R., Vrtala, S.

P 208 **The effects of MDR1 polymorphisms on tacrolimus through levels in long-term kidney transplant recipients**
Riegersperger, M., Plischke, M., Sunder-Plassmann, G., Steinhauser, C., Jallitsch-Halper, A., Winkelmayer, W., Huber, A., Födinger, M.

P 209 **Automatic analysis of tumor budding in colorectal cancer specimens**
Rogojanu, R. , Thiem, U. , Mesteri, I. , Ellinger, I. , Heindl, A. , Seewald, AK. , Haisan, A. , Thalhammer, T. , Bises, G.

P 210 **Effects of ischemic postconditioning on reperfusion injury after a short and a long ischemic period**
Rosero, O., Ónody, P., Tamás, J., Garbaisz, D., Kocsis, I., Lotz, G., Harsanyi , L., Szijártó, A.

P 211 **Ca2+ channel impairments in dystrophic cardiomyocytes**
Rubi, L., König, X., Hilber, K., Todt, H., Bittner, R.

Poster Workshop 19

Chaired by Gürkan Sengoelge and Jolanta Siller-Matula

P 212 **Molecular Characterization of the Tumour-Stroma Crosstalk Using a Novel 3D Co-Culture In Vitro Model**
Rudisch, A., van der Kuip, H., Dolznig, H., Garin-Chesa, P., Sommergruber, W.

P 213 **LRET-based distance measurements in the mammalian glutamate transporter EAAC1**
Saha, K., Bulling, S., Sandtner, W., Stockner, T., Ecker, G., Sitte, H.

P 214 **Arginase I as a key mediator of the innate immune system at the interface to autoimmunity**
Sahin, E., Brunner, J., Kral, J., Schabbauer, G.

P 215 **Phosphorylation of Kv7.2 determines its regulation via G Proteins**
Salzer, I., Chen, W., Kubista, H., Lubec, G., Boehm, S., Yang, J.

P 216 **Silk spider Nephila clavipes - Proteome and posttranslational modifications of the spidroins**
Santos Pinto, J., Heo, S., Garcia Caviquioli, A., Santos, L., Palma, M., Lubec, G.

P 217 **Intraperitoneal injection of saline modulates hippocampal brain receptor complex levels but does not impair performance in the Morris Water Maze**
Sase, A., Khan, D., Höger, H., Lubec, G.

P 218 **Decoding transmembrane immunoglobulin-like glycoprotein CD147 inside the cell**
Schatzlmaier, P., Zojer, V., Stockinger, H.

P 219 **Intermediate Monocytes but not TIE2 Expressing Monocytes are Biomarkers for Colorectal Cancer**
Schauer, D., Starlinger, P., Reiter, C., Jahn, N., Zajc, P., Buchberger, E., Bachleitner-Hofmann, T., Bergmann, M. , Stift, A. , Gruenberger, T., Brostjan, C.

P 220 **Evaluation of Fibroblast Growth Factor Receptor 1 (FGFR1) as potential new therapy target in Malignant Pleural Mesothelioma**
Schelch, K., Hoda, M. , Ghanim, B. , Pirker, C., Hegedus, B., Berger, W., Klepetko, W., Grusch, M.

P 221 **Proteomic analysis of thioredoxin-targeted proteins in Entamoeba histolytica**
Schlosser, S., Leitsch, D., Duchene, M.

P 222 **Feasability of transrectal ultrasound in the assessment of locally advanced cervix cancer in the course of primary adaptive radiochemotherapy**
Schmid, M., Pötter, R., Brader, P., Kratochwil, A., Goldner, G., Kirchheiner, K., Sturdza, A., Kirisits, C.

P 223 **Characterization of the major wheat food allergen Tri a 36**
Schmidhuber, A., Pahr, S., Constantin, C., Giavi, S., Nikos Papadopoulos , N., Ebner, C., Vrtala, S., Valenta, R.

Poster Workshop 20

Chaired by Gabriela Berlakovich and Sebastian Nijman

P 224 **The role of Cingulin in endothelial junctions**
Schossleitner, K., Petzelbauer, P.

P 225 **Prolyl hydroxylase inhibitors decrease formation and activity of osteoclast in murine bone marrow cultures**
Schröckmair, S., Vinzenz, P., Gruber, R., Agis, H.

P 226 **Iron accumulation in models for inflammation/degeneration of the central nervous system: Does iron impact neurodegeneration?**
Schuh, C., Hametner, S., Bradl, M., Lassmann, H.

P 227 **Kinematic changes in patients with double arthrodesis of the hindfoot for realignement of planovalgus deformity.**
Schuh, R., Hofstaetter, J., Wanivenhaus, A., Trnka, H.

P 228 **Quantitative reproducibility assessment of RPE atrophy lesions in patients with choroidal neovascularization related to neovascular age-related macular degeneration using polarization-sensitive OCT**
Schütze, C., Bolz, M., Teleky, K., Baumann, B., Pircher, M., Götzinger, E., Hitzenberger, C., Schmidt-Erfurth, U.

P 229 **Predicting the Effects of Stent-Grafting in the Aortic Arch onto Vessel Mobility**
Schwartz, E., Holfeld, J., Czerny, H., Loewe, C., Langs, G.

P 230 **Angiogenic properties of different Ti surface evaluated by cell co-culture with endothelial cells and osteoblasts**
Shi, B., Andrukhov, O., Berner, S., Schedle, A., Rausch-Fan, X.

P 231 **Angiogenic potential of fibrin biomatrix delivered VEGF165 in a model of angiogenesis**
Slezak, P., Hartinger, J., Mittermayr, R., Redl, H.

P 232 **Measurements of intramolecular distance changes at atomic level in LeuTAa using Luminescence Resonance Energy Transfer.**
Sohail, A., Stolt-Bergner, P., Ecker, G., Freissmuth, M., Stockner, T., Sitte, H., Sandtner, W.

P 233 **A Transcutaneous Energy Transmission System Delivering up to 45 Watt to an Artificial Heart**
Sommer, C., Finocchiaro, T., Steinseifer, U., Schima, H., Lanmüller, H.

P 234 **Influence of gliptins on endostatin, glucose and HbA1c in 35 NIDDM patients**
Sponder, M., Dangl, D., Sabri, A., Kosi, L., Kautzky-Willer, A., Kampf, S., Hammer, A., Strametz-Juranek, J.

P 235 **Influence of sex and etiology on Endostatin serum levels in patients with chronic heart failure (CHF)**
Sponder, M., Pacher, R., Hülsmann, M., Gwechenberger, M., Knoth, J., Kampf, S., Fritzer-Szekeres, M., Strametz-Juranek, J.

Poster Workshop 21

Chaired by Ulrike Holzinger and Jürgen Zezula

P 236 **Pharmacochaperone-mediated rescue of trafficking deficiency in the ABCB-transporter subfamily**
Spork, M., Parveen, Z., Mastalir, M., Gstach, H., Stockner, T., Ecker, G., Chiba, P.

P 237 **Molecular characterization of wheat antigens involved in celiac disease**
Srinivasan, B., Focke-Tejkl, M., Swoboda, I., Constantin, C., Mittermann, I., Pahr, S., Vogelsang, H., Huber, W.

P 238 **Time-dependent expression of pro- and anti-apoptotic proteins in cortex of adult male Wistar rats after permanent bilateral occlusions**
Stanojlović, M., Drakulić, D., Grković, I., Mitrović, N., Horvat, A.

P 239 **Assessment of intrafraction prostate and patient motion for IMRT patients with rectal balloon**
Steiner, E., Stock, M., Goldner, G., Georg, D.

P 240 **Calmodulin kinase II modulates amphetamine-induced reverse transport in the dopamine transporter**
Steinkellner, T., Eisenrauch, B., Konrad, L., Freissmuth, M., Pollak, D., Sitte, H.

P 241 **EVI1 is a potent modulator of transcriptional and biological responses of human myeloid cells to all-trans retinoic acid**
Steinmetz, B., Heilos, D., Hackl, H., Soucek, K. , Slabakova, E. , Bennett, K., Grebien, F., Wieser, R., Hartl, K. , Rommer, A.

P 242 **Role of GHR in liver fibrosis**
Stiedl, P., Blaas, L., Stanek, V., Zollner, G., Esterbauer, H., Eferl, R., Trauner, M., Casanova, E.

P 243 **PathogenPCR for improved diagnosis of neonatal sepsis**
Straub, J.

P 244 **The prevalence of root resorption of maxillary incisors caused by impacted maxillary canines**
Strbac, G., Foltin, A., Gahleitner, A., Bantleon, H., Vasak, C. , Watzek, G., Zechner, W., Bernhart, T.

P 245 **In skin, sensitization to the house dust mite allergen Der p 2 is not solely dependent upon TLR-4 activation**
Stremnitzer, C., Szalai, K., Starkl, P., Willensdorfer, A., Schrom, S., Mildner, M., Reichart, U., Jensen-Jarolim, E.

P 246 **Signalling network between ERG, cMyc and NF-KB is critical in prostate cancer.**
Sughra, K., Ilyas , M., Malkani, N., Kozakowski, N., Hoesel, B., Schmid, J.

Poster Workshop 22

Chaired by Elisabeth Presterl and Stefan Wagner

P 247 **Genomics effects of 9-cis-retinoic acid in an adrenocortical cell line**
Szabo, D., Szabo, P., Zsippai, A., Eder, K., Patocs, A., Falus, A., Racz, K., Igaz, P.

P 248 **Signalling pathways of the calcium sensing receptor in colonocytes**
Tennakoon, S., Schmid, J., Kallay, E.

P 249 **Visualization of Dendritic mRNA Localization and its Interacting RNA-Binding Proteins in Living Neurons**
Tolino, M., Kiebler, M., Doyle, M.

P 250 **Role of Adenosine A2A Receptors in the Modulation of GABA(A) Receptor Function**
Treven, M., Vasiljevic, M., Milenkovic, I., Sieghart, W.

P 251 **Histone deacetylases HDAC1 and HDAC2 control Cd8 silencing in CD4 lineage T cells**
Tschismarov, R., Boucheron, N., Lagger, S., Moser, M., Göschl, L., Sakaguchi, S., Zupkovitz, G., Winter, M., Matthias, P., Seiser, C., Ellmeier, W.

P 252 **Factors determining outcome in patients with heart failure and normal ejection fraction**
Tufaro, C., Mascherbauer, J., Marzluf, B., Binder, T., Lang, I., Bonderman, D.

P 253 **Moleculare connectivity in patients with unilateral temporal lobe epilepsy investigated with [18F]FDG PET**
Vanicek, T., Hahn, A., Asenbaum-Nan, S., Assem-Hilger, E., Savli, M., Kranz, G., Baldinger, P., Lanzenberger, R.

P 254 **Chemoresistance can be induced by bile acid-independent activation of FXR in liver and intestinal cancer cells**
Vaquero, J., Monte,, M.J., Serrano, M.A., Herraez, E., Gonzalez-Sanchez, E., Romero, M.R., Rosales, R., Blazquez, A.G., Perez, M.J.,. Macias, R.I.R., Sanchez-Vicente, L., Lozano, E., Jimenez, F., Gonzalez-San Martin, F., Muntane, J., Briz, O., Marin, J.J.G.

P 255 **Expression of α-SMA in Hepatic Stellate Cells as Their Activation Biomarker with Down-Regulated Immune Response in Human Hydatid Infection.**
Vatankhah, A., Halasz , J., Piurko, V., Gregor, V., Schaff , Z., Timar, J.

P 256 **LRET-based distance measurements in a bacterial homolog to mammalian glutamate transporter GltPh**
Venkatesan, S., Sohail, A., Sandtner, W., Stockner, T., Ecker, G., Sitte, H.

P 257 **Popliteal artery trauma: Treatment and Outcome**
Vielgut, I., Gregori, M., Platzer, G.

Poster Workshop 23

Chaired by Diana Mechteriakova and Walter Speidl

P 258 **A proteomic approach to identify novel interactors of the Tec family kinases that play a crucial role in T-cell development and differentiation**
Vitko, D.

P 259 **Does periodic coronary venous pressure elevation promote regenerative processes through SAFE pathway initiation?**
Wadowski, P., Andreas, M., Khazen, C., Vukovich, T., Aumayr, K., Jusic, A., Pfisterer, N., Mohl, W.

P 260 **Heme oxygenase 1 in brown adipose tissue**
Wagner, G., Lindroos, J., Mitterer, G., Esterbauer, H., Wagner, O., Bilban, M.

P 261 **Quantitative Comparison Of Segmentation Performance In Four Spectral Domain Optical Coherence Tomography Instruments**
Waldstein, S., Gerendas, B., Montuoro, G., Lammer, J., Simader, C., Schmidt-Erfurth, U.

P 262 **Simvastatin reduces IL-6 mediated migration of human metastatic melanoma cells**
Wasinger, C., Minichsdorfer, C., Hohenegger, M.

P 263 **Preclinical studies towards a novel pharmacological therapy for X-linked Adrenoleukodystrophy**
Weber, F., Forss-Petter, S., Wiesinger, C., Muneer, Z., Stockinger, H., Berger, J.

P 264 **Prolylhydroxylase inhibitors decrease plasminogen activation in periodontal fibroblasts**
Wehner, C., Watzek, G., Gruber, R., Agis, H.

P 265 **Effects of ischemia and inflammatory mediators on liver cell functions: a comparative in vitro study**
Weidinger, A., Dungel, P., Ghebes, C., Duvigneau, J., Müllebner, A., Redl, H., Kozlov, A.

P 266 **Extracellular ATP release as a potential benefit in shockwave treatment enhanced wound healing**
Weihs, A., Junger, W., Schaden, W., Sitte, H., Ruenzler, D.

P 267 **Attention in children - the necessity of guidelines for assessment and intervention of attentional disorders**
Weiler, L. , Slavc, I. , Leiss, U.

P 268 **Three-dimensional optical coherence tomography for dermatologic in vivo tumor diagnosis.**
Weingast, J. , Alex, A. , Drexler, W. , Binder, M.

Poster Workshop 24

Chaired by Enikö Kallay and Johann Wojta

P 269 **Fluid replacement with colloids and its impact on platelets, hemostasis and renal function**
Wetzel, L. , Scharbert, G. , Kozek-Langenecker, S.

P 270 **Is iron a key player in neurodegeneration?**
Wimmer, I. , Hametner, S. , Schuh, C. , Bradl, M. , Lassmann, H.

P 271 **Characterization of long-term survivors on subcutaneous treprostinil**
Winter, M. , Sadushi-Kolici, R. , Skoro-Sajer, N. , Bonderman, D. , Klepetko, W. , Lang, I.

P 272 **Biological activity of cytokines applied as ‚natural adjuvants' bound to Virus-like nanoparticles is critically influenced by their membrane-anchor characteristics**
Wojta-Stremayr, D. , Küng, H. , Neunkirchner, A. , Schmetterer, K. , Pickl, W.

P 273 **Persistence of allergen-specific IgE responses in HIV infected patients with low CD4+ cell counts**
Wollmann, E. , Marth, K. , Gallerano, D. , Valenta, R., Sibanda, E.

P 274 **Interaction of the transmembrane serine protease testisin with the serpin Protein C Inhibitor (PCI)**
Yang, H. , Wahlmueller, F. , Geiger, M.

P 275 **The influence of local cooling on capsaicin induced pain and epidermal nerve fibre density reduction**
Zadrazil, M. , Kovacs, G. , Scharbert, G. , Hainfellner, J. , Schemper, M. , Knolle, E.

P 276 **Hypertension in children is underdiagnosed. Can education help?**
Zaller, V. , Dufek, S. , Csaicsich, D. , Aufricht, C.

P 277 **Overproduction of Th-1 and Th-17 cytokines in the peripheral blood of psoriatic patients is not confined to the skin-homing T cell subset**
Zhou, J. , Koszik, F. , Brunner, P. , Stingl, G.

P 278 **Human allergen-specific T cell clones show high heterogeneity and plasticity within the Th2 lineage**
Zulehner, N. , Nagl, B. , Walterskirchen, C. , Zlabinger, G. , Pickl, W. , Bohle, B.

P 279 **Lipid-protein interactions in cell membranes**
Zwirzitz, A. , Eckerstorfer, P. , Stockinger, H.

Oral Session 4

Chaired by Adelheid Elbe-Bürger and Hannes Stockinger

S 19 **Probing Network Fragilities in Embryonal Tumors by Synergistic Drug Combinations**
Radic, B. , Rix, U. , Kubicek, S. , Superti-Furga, G.

S 20 **Stat3 in the tumor microenvironment of colitis associated cancer**
Rampetsreiter, P. , Musteanu, M. , Gotthardt, D. , Svinka, J. , Müller, M. , Strobl, B. , Sexl, V. , Eferl, R.

S 21 **IL-13 plays a critical role during bacterial pneumonia.**
Saluzzo, S. , Joanna Warszawska, J. , Doninger, J. , Lakovitz, K. , Mesteri, I. , Knapp, S.

S 22 **Role of EGFR in inflammation induced colorectal cancers**
Srivatsa, S. , Bissonnette, M. , Sibilia, B.

S 23 **Lipocalin 2 modulates inflammation and impairs host defense against Streptococcus pneumoniae**
Warszawska, J., Gawish, R., Sharif, O., Doninger, B. , Mesteri, I. , Schenk, P. , Weiss, G. , Knapp, S.

S 24 **High-speed, wide-field polarization sensitive OCT for measuring retinal nerve fiber layer birefringence, retardation and thickness**
Zotter, S. , Pircher, M. , Torzicky, T. , Yoshida, H. , Hirose, F. , Vass, C. , Schmidt-Erfurth, U. , Hitzenberger, C.

YSA Publication Award Lecture

David Weismann
Complement factor H binds malondialdehyde epitopes and protects from oxidative stress

Keynote Lecture 4

K 4 **Keynote Lecture by J. Andrew Pospisilik**
Epigenetic Control of Metabolic Disease
Chaired by Denise Barlow and Stefan Kubicek

Abstracts – Thematic Overview

Biomedical Engineering (3 abstracts)
S 9, P 16, P 233

Cardiovascular and Pulmonary Disease (13 abstracts)
S 18, P 8, P 42, P 154, P 155, P 156, P 160, P 180, P 201, P 234, P 235, P 252, P 271

Cell Communication in Health and Disease (17 abstracts)
S 21, P 10, P 37, P 66, P 70, P 95, P 118, P 130, P 133, P 144, P 148, P 215, P 218, P 226, P 250, P 260, P 270

Clinical Endocrinology, Metabolism and Nutrition (1 abstract)
P 182

Clinical Experimental Oncology (4 abstracts)
P 31, P 179, P 219, P 222

Clinical Neurosciences (13 abstracts)
S 3, S 11, P 14, P 22, P 78, P 123, P 124, P 169, P 177, P 206, P 253, P 269, P 275

Dermatology (5 abstracts)
S 4, S 14, P 82, P 268, P 277

Endocrinology and Metabolism (11 abstracts)
S 16, P 7, P 107, P 109, P 113, P 134, P 140, P 157, P 161, P 167, P 178

Immunology (37 abstracts)
S 1, S 15, P 4, P 12, P 17, P 20, P 26, P 28, P 29, P 30, P 51, P 52, P 56, P 64, P 68, P 71, P 83, P 108, P 132, P 139, P 147, P 162, P 175, P 185, P 189, P 190, P 191, P 195, P 197, P 207, P 221, P 223, P 245, P 251, P 255, P 272, P 279

Inflammation and Immunity (13 abstracts)
S 22, S 23, P 58, P 81, P 96, P 97, P 135, P 150, P 168, P 237, P 243, P 273, P 278

Malignant Diseases (22 abstracts)
S 6, S 8, S 19, S 20, P 1, P 5, P 36, P 46, P 55, P 89, P 103, P 127, P 141, P 149 ,P 153, P 164, P 170, P 187, P 196, P 220, P 241, P 242

Medical Informatics, Biostatistics and Complex Systems (3 abstracts)
P 35,P 93,P 229

Medical Physics (12 abstracts)
S 7, S 24, P 44, P 57, P 59, P 72, P 128, P 137, P 199, P 228, P 239, P 261

Mental Health and Behavioral Medicine (4 abstracts)
P 119, P 184, P 186, P 203

Molecular Biology in Medicine (6 abstracts)
S 12, P 102, P 110, P 111, P 146, P 216

Molecular Drug Targets (1 abstract)
P 240

Abstracts – Thematic Overview

Molecular Mechanisms of Cell Biology (5 abstracts)
S 17, P 69, P 90, P 105, P 236

Molecular Mechanisms of Cell Signaling at the MFPL (6 abstracts)
S 13, P 25, P 129, P 145, P 165, P 171

Molecular Signal Transduction (21 abstracts)
P 13, P 23, P 40, P 41, P 53, P 61, P 77, P 84, P 86, P 101, P 121, P 142, P 163, P 183, P 211, P 232, P 246, P 258, P 262, P 265, P 266

Neuroscience (30 abstracts)
S 2, S 10, P 11, P 18, P 21, P 47, P 48, P 49, P 63, P 73, P 85, P 106, P 112, P 115, P 117, P 122, P 151, P 166, P 192, P 193, P 200, P 204, P 213, P 217, P 238, P 249,P 256, P 263, P 74, P 92

POeT - Programme for Organfailure, -replacement and Transplantation (5 abstracts)
P 50, P 131, P 143, P 208, P 276

Preclinical and Clinical Research in Pharmaceutic Development (1 abstract)
P 172

Regeneration of Bones and Joints (23 abstracts)
P 43, P 6, P 24, P 27, P 34, P 39, P 45, P 60, P 62, P 80, P 87, P 88, P 98, P 99, P 120, P 174, P 176, P 194, P 198, P 227, P 230, P 244, P 257

RNA-Biology (2 abstracts)
P 76, P 159

Tumorbiology – Oncology (10 abstracts)
P 38, P 94, P 104, P 116, P 125, P 138, P 158, P 212, P 247, P 248

Vascular Biology (14 abstracts)
P 9, P 15, P 19, P 65, P 91, P 126, P 136, P 152, P 202, P 214, P 224, P 231, P 259, P 274

Other (21 abstracts)
S 5, P 2, P 3, P 32, P 33, P 54, P 67, P 79, P 100, P 114, P 173, P 181, P 188, P 205, P 209, P 210, P 225, P 254, P 264, P 267, P 75

Jennifer L. Whistler

University of California San Francisco, Ernest Gallo Clinic and Research Center, USA.

Dr. Whistler received a Bachelor of Science degree in Genetics from the University of California, Davis. After working in the biotech industry for several years, she obtained her Ph.D. in Molecular and Cell Biology from the University of California, Berkeley. She then did postdoctoral work studying the cell biological mechanisms that regulate the trafficking of membrane proteins. She has been a Principal Investigator at the Ernest Gallo Clinic and Research Center at UCSF since 2000. Her research is focused on the pharmacology and trafficking of G protein coupled receptors (GPCRs). She is particularly interested in assessing how manipulating ligand bias through GPCR trafficking alters in vivo adaptations to clinically important drugs, including those for neuropsychiatric disease and pain. More recently she has expanded her research back to her genetic roots to examine how common allelic variation in GPCR drug targets confers disease risk or alters drug responses. Dr. Whistler is a Professor in residence in the Department of Neurology at UCSF and a member of the Neuroscience Graduate Program. She is a Kavli fellow, a recipient of four NIH R01s from diverse institutes (NIDA, NIMH and NIAAA) and a NIDA Cutting Edge Basic Research Award. She is also the recipient of an American Asthma Foundation Early Excellence Award and a Novo Nordisk Innovation Award. Dr. Whistler is a member of the Editorial Board for the Journal of Biological Chemistry.

Keynote Lecture 1

GPCR Trafficking and Responsiveness to Drugs of Abuse.

My laboratory studies the trafficking of G protein coupled receptors (GPCRs) important in pain, addiction and other neuropsychiatric disease. We examine the trafficking of GPCRs in response to endogenous ligands and exogenous drugs at these GPCRs. We have found that endocytosis and post-endocytic sorting of GPCRs in response to endogenous ligands and various exogenous drugs often differ and that receptor trafficking plays a crucial role in regulating signal transduction, neuronal activity and rodent behavior.

Anna Teti

Bone Biopathology Laboratory, University of L'Aquila, Italy

Studied biology at the University of Bari, Italy graduating in 1977. Post-doc research fellow at the Institute of Anatomy at the University of Bari from 1997-1981, Italy. Training in bone cell biology from 1983-1987 at the University of Bari, Italy, University of Berne, Switzerland, MRC Nuffield Orthopaedic Centre in Oxford, UK, and Washington University at St. Louis, MO, USA. Associate Professor at the University of Bari from 1987-1993 and at the University of L'Aquila from 1993-2000. She is now Professor of Histology and Embryology at the University of L'Aquila, Italy since 2000.

Her scientific interests include basic and translational research to discover the molecular and cellular pathogenesis of osteoporosis, bone cancer, inflammatory bone diseases and osteopetrosis. She has published 158 peer review articles in the field of osteobiology and osteopathology and her work has been recognized in receipt of the Research Prize from the Austrian Society for Bone and Mineral Research in 1991, the Prix André Lichtwitz from the Institut National de la Santé et de la Recherche Médicale in 1993, and the Swiss Bridge Award from the Swiss Bridge Foundation in 2008.

Keynote Lecture 2

Bone Modeling and Remodeling in Health and Disease.

Vertebrates are characterized by an internal skeleton made by more than 200 bones and cartilages articulated with each other. By this complicated array of hard structures, the skeleton fulfills the important functions of locomotion, organ protection, lodging of hematopoiesis, mineral homeostasis and endocrine regulation of kidney function, energy metabolism and male fertility. Ossification starts in the fetus along with marrow hematopoiesis, and evolves post-natally through modeling and remodeling processes that permit skeletal mass buildup. The essential role of bone cells in keeping the hematopoiesis lifelong has recently been discovered and is subject of intense research aimed at understanding the role of the hematopoietic niche in physiological and pathological contexts. After accrual, preservation of skeletal mass is implemented by concerted bone remodeling, which ensures continuous renovation of the tissue with no changes in bone mass. This balanced process maintains the mechanical, structural and metabolic properties of the skeleton unaltered, until ageing or diseases disrupt this equilibrium. Skeletal homeostasis is fulfilled by specialized bone cells, the osteblasts that construct the bone and the osteoclasts that destroy it, in association with systemic and local regulators. We will review landmark discoveries which clarified the intricate net connecting bone cells among themselves and with other systems, representing the cellular basis of normal and abnormal bone development and homeostasis.

Rashika El Ridi

Zoology Department, Faculty of Science, Cairo University, Egypt.

Rashika El Ridi received her Professorship in Immunology in 1986 and has since been working at the Zoology Department at the University of Cairo in Egypt with visiting-scientist positions at both Harvard University in the USA (1995) and Hirosaki University in Japan (1996). Between 1990 and 2000 Rashika El Ridi was also Director of Schistosomiasis Research at the BioMedical Research Center at the Egyptian Organization for Sera and Vaccines in Egypt. Throughout her career she has received many rewards to mention a few is the L'Oreal-Unesco for Women in Science Award in 2010 and the Egyptian State Award of Merit and Recognition in High-Tech Sciences in 2010.

She has dedicated her scientific career on unravelling the biology behind the disease of schistosomiasis, ultimately leading to a vaccine candidate. Sometimes referred to as "snail fever" schistosomiasis is a chronic parasitic disease causing severe illness especially in children. The parasite is estimated to infect 200 million people in the developing countries in Africa, Latin America and Asia. In addition, schistosomiasis facilitates co-infection with malaria, tuberculosis, and viruses such as HIV.

Keynote Lecture 3

Novel Chemotherapeutic and Vaccination Approaches for Schistosomiasis.

Schistosomiasis is a debilitating disease affecting approximately 600 million people in 74 developing countries, with 800 million, mostly children at risk. To circumvent the risk of having praziquantel (PZQ) as the only drug used for treatment, several PZQ derivatives were synthesized, and drugs destined for other parasites were used with success. A plethora of plant-derived oils and extracts were found to effectively kill juvenile and adult schistosomes, yet none was progressed to pre- and clinical studies except an oleo-gum resin extracted from the stem of Commiphora molmol, myrrh, which action was challenged in several trials. We have proposed an essential fatty acid, a component of our diet and cells, the polyunsaturated fatty acid arachidonic acid (ARA) as a remedy for schistosomiasis, due to its ability to activate the parasite tegument-bound neutral sphingomyelinase, with subsequent hydrolysis of the apical lipid bilayer sphingomyelin molecules, allowing access of specific antibody molecules, and eventual worm attrition. This concept was convincingly supported using larval and adult Schistosoma mansoni and S. haematobium worms in in vitro experiments, and in vivo studies in inbred mice and outbred hamsters. Even if ARA proves to be an entirely effective and safe therapy for schistosomiasis, it will not prevent reinfection, and accordingly, the need for developing an effective vaccine remains an urgent priority. Our studies have supported the status of S. mansoni calpain, glutathione-S-transferase, aldolase, triose phosphate isomerase, glyceraldehyde 3-phosphate dehydrogenase, enolase, and 2-cys peroxiredoxin as vaccine candidates, as they are larval excreted-secreted products and, contrary to the surface membrane molecules, are entirely accessible to the host immune system effector elements. We have proposed and shown that the use or these molecules, in conjunction with Th2 cytokines-inducing adjuvants for recruiting and activating eosinophils and basophils, will likely lead to development and implementation of a sterilizing vaccine in a near future.

J. Andrew Pospisilik

Department of Epigenetics, Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany

J. Andrew Pospisilik studied Human Physiology at the University of British Columbia (Vancouver, Canada). There he joined Ray Pederson on the footsteps of the discovery of DPIV inhibition as a therapeutic strategy. A trio of works during his PhD documented for the first time the long-term benefits of specific dipeptidyl peptidase IV (DP IV)-inhibitors on types -1 and -2 diabetes, including direct concerted improvements in insulin-secretory function, insulin sensitivity, and β-cell turnover. Joining Josef Penninger as post-doc at IMBA in late 2003, he exploited gene-targeting strategies in mice and tackled a long enigmatic link between mitochondrial dysfunction and diabetes and obesity. These findings broke dogma and changed our understanding of the etiology of insulin resistance. Another landmark contribution from Dr. Pospisilik's work was the first genome-wide RNAi screen for obesity in adult drosophila, a finding which lead to the discovery of Hedgehog signaling as one of only several pathways capable of differentially regulating the lineage commitment of brown and white adipose tissues in mammals. In spring of 2010, he joined the MPI in Freiburg as an founding junior member of the institute's Epigenetic Focus, the Max Planck Society's commitment to foster excellence in Epigenetic research.
Andrew is well recognized in the field of metabolic disease regulation with a number of contributions in top ranking journals, and with several highlights as must-read's by the Faculty of 1000. He was nominated for Vienna's "Future Prize", and in 2011, he was awarded W2 ('associate professor' equivalent) by the Max Planck Society. He is the holder of numerous awards, patents, and high profile grants and is fortunate to be embedded in a hotspot of epigenetic research in the Freiburg, Basel, Strasbourg area. His relatively new lab is currently tackling the enigmatic role of a number of epigenetic control systems in the development of disease.

Keynote Lecture 4

Epigenetic Control of Metabolic Disease.

Recent estimates place the global incidence of obesity beyond 1 billion by the year 2030. Perhaps most disturbing, rates of childhood obesity have more than doubled in the last decade. This rapid rise in early life incidence of obesity and the long-term health implications with respect to heart disease, diabetes and stroke, make obesity one of the world's chief economic and health care challenges of the day. While numerous studies have established a genetic framework for our understanding of obesity, the contribution of several critical regulatory layers, in particular epigenetic regulation, remain poorly understood.

What is epigenetic regulation? C.H. Waddington coined the term in 1942 to conceptualize how one DNA blueprint confers multiple phenotypes through development. In fact, the term was appeared even prior to our understanding of what genes physically were. The contemporary definition, while often debated, has narrowed to include the study of how stably inherited phenotypes arise from a single DNA blueprint and typically converges on chromatin regulatory systems that have the potential to alter transcriptional state. My lab is interested in understanding exactly how these chromatin regulatory systems impact disease. Here, I would like to outline the burgeoning young field of metabolic disease epigenetics with some of our recent work and with seminal examples from the field.

8th YSA-PhD-Symposium Abstracts

Oral Presentations (S)
Poster Presentations (P)

S 1 The role of the transcription factor MAZR in mast cells

Abramova, A.* (1), Sakaguchi, S. (1), Schebesta, A. (1), Boucheron, N. (1), Schmidt, U. (2), Ellmeier, W. (1)

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We recently identified the transcription factor MAZR as an important regulator of Cd8 gene expression and demonstrated that MAZR is part of the transcription factor network that controls CD4/CD8 cell fate choice of DP thymocytes. However, MAZR is a newly identified factor and essentially nothing is known about the role of MAZR in other immune cell lineages. MAZR is also expressed in mast cells (MCs), which are key players in normal and pathophysiological type I hypersensitivity reactions. MCs are classically activated via FcεRI stimulation although alternative modes of activation exist. The FcεRI-induced signaling pathways that regulate MC activation are well described. However, much less is known about transcription factor networks that regulate MCs. Using conditional "floxed" MAZR mice and Vav-Cre deleter strains, we are performing a detailed analysis to reveal the role of MAZR during MC development and function. Although MAZR-sufficient and MAZR-deficient bone marrow-derived MCs (BMMCs) have a similar phenotypic appearance, total cell numbers of mucosal type IL-3-generated MCs is 2-3 fold reduced in the absence of MAZR. The analysis of FcεRI-induced early MC effector functions revealed a mild reduction of β-hexosaminidase release, while LTB4 production was not affected in the absence of MAZR. There was also no major difference in IL-4, IL-5, IL-6, TNFα, and GM-CSF cytokine production (late effector function) after simulation of MazrF/F and VavCre x MazrF/F BMMCs. Together, our results indicate that MAZR is required for efficient differentiation of MCs in vitro. However, MAZR appears to have no major role in the regulation of effector functions induced by FcεRI-mediated MC activation. To further characterize the type of MC that are generated in the absence of MAZR, we are currently performing a transcriptome analysis using Agilant arrays. Moreover, we are determining MC function in vivo. Results of these additional experiments will be presented.

Topic: Immunology

S 2 Connectional hierarchy in the primary somatosensory cortex of primates

Ashaber, M.* (1), Pálfi, P. (1), Palmer, C. (2), Kántor, O. (1), Friedman, R. (3), Roe, A. (3), Négyessy, L. (1,4)

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Primary somatosensory cortical area 3b (A3b) and adjacent area 1 (A1) exhibit similar functional organizations. However, it is thought that area 1 represents a higher processing stage than area 3b. The somatotopic representations of the two areas are not equivalent: cortical magnification factors and neuronal receptive field properties are different. We studied underlying connectional properties of the functional hierarchical relationship of A3b and A1. These questions were investigated by the combination of electrophysiological and bidirectional neuronal tract tracing methods in the primary somatosensory cortex focusing on A3b and A1, of the squirrel monkey. Laminar distribution of retrograde and anterograde labeling, which were mostly localized to the superficial layers, did not reveal specific hierarchical relationship between the two areas. Anisotropy index, computed for the intrinsic connections indicated a mediolaterally oriented distribution suggesting strong cross-digit connectivity within the areas. Intrinsic connections of area 1 were spatially more extensive than that of A3b, which support widespread integration between fingertip representations in A1. Notably, the distribution of intrinsic connections in A3b was modular exhibiting several peaks of densities, while in A1 the density of intrinsic connections peaked around the site of injection and gradually decreased with increasing distance from the injection site. This observation suggest that modular organization is more prevalent in A3b then in A1. The size of the region with the strongest retrograde labeling in A1 tended to be smaller than that found in A3b. However, consistent with previous studies on cortical magnification factor, the size of skin areas represented by these heavily labeled cortical fields was similar or greater in A1 than in A3b. This finding supports previous observations suggesting a larger convergence of somatosensory information on neurons of area 1 as compared to that of 3b.

Topic: Neuroscience

S 3 Genotype of serotonin-1B receptor affects serotonin-1A receptor binding in vivo

Baldinger, P.* (1), Hahn, A. (1), Mitterhauser, M. (3), Kraus, C. (1), Wadsak, W. (2), Rujescu, D. (3), Kasper, S. (1), Lanzenberger, R. (1)

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The serotonin-1A (5-HT1A) receptor is the main inhibitory receptor of the serotonergic system. Using positron emission tomography (PET), numerous studies showed a reduced 5-HT1A receptor density in depression. Genetic factors modulating the 5-HT1A receptor density are of high clinical relevance and as serotonin release is mediated by 5-HT1B receptors, changes of the latter are expected to affect the 5-HT1A receptor expression. Here, we investigate the effect of 5-HT1B receptor gene single nucleotide polymorphism (SNP) rs6296 on 5-HT1A receptor binding in vivo using PET. Subjects: 52 healthy subjects (38 female, ageSD=40.48±14.87 years) Measurement: 1 PET using radioligand [carbonyl-11C]WAY-100635 Data analysis: normalization of scans to MNI-space, quantification of receptor binding potentials (BPND) in PMOD 3.3, computation of voxel-wise whole-brain 5-HT1A BPND maps Genotyping: Periphery blood drawing (9ml EDTA blood), DNA isolation using the QIAGEN® QiaAMP DNA Mini Kit, genotyping using SEQUENOM® Statistics: ANOVA and posthoc T-test in SPM8 13 subjects were homozygote CC carriers, 16 were homozygote GG carriers and 23 were heterozygote CG carriers for rs6296. We found a significant effect of genotype on 5-HT1A receptor binding BPND in several brain areas (CC+CG<GG, T>2.18, p<0.05 FDR-corrected). GG carriers had significantly higher 5-HT1A receptor binding in the posterior cingulate cortex, the precuneus, cuneus, amygdala, hippocampus, basal occipito-temporal areas, orbitofrontal cortex, insula, lateral prefrontal and superior temporal cortices. Our finding demonstrates an effect of a genetic variation of the serotonin-1B receptor on the 5-HT1A receptor binding potential, an index of receptor density, in several brain regions in the healthy human brain. As reduced 5-HT1A receptor binding has been frequently associated with major depression, C allel carriers of the 5-HT1B SNP rs6296 might have an increased vulnerability for affective disorders.

Topic: Clinical Neurosciences

S 4 The molecular signature of cutaneous graft-versus-host disease: IL-22 and Th2 cytokines predominate in the acute disease stage

Brüggen, C.* (1), Klein , I. (1), Greinix, H. (2), Bauer, W. (1), Kuzmina, Z. (2), Rabitsch, W. (2), Knobler, R. (3), Stingl, G. (3), Stary, G. (1)

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Graft-versus-host disease (GvHD) is the major clinical complication of allogeneic hematopoietic stem cell transplantation (HCT) occurring in an acute and chronic form. It is unclear whether the same or different pathomechanisms lead to these distinct clinical presentations. To address this issue, we collected lesional skin biopsies of patients suffering from acute (aGvHD) (n=22) and chronic inflammatory (cGvHD) (n=15) GvHD patients as well as serial biopsies of non-lesional skin from HCT recipients at different time points prior and after HCT (n=14). The cellular infiltrate was assessed by immunofluorescent in situ analysis; interleukins and chemokines were analyzed by real-time RT-PCR. Results obtained revealed striking differences between aGVHD and cGvHD lesions. Surprisingly, we found considerably increased levels of IL-22 but not IL-17 in aGvHD skin biopsies compared with samples from cGvHD patients. It further appears that the immune response occurring in aGvHD skin lesions is skewed in the Th2 direction, as evidenced by a relative increase of Th2 cytokines (IL-4, IL-13) and Th2 chemokines (CCL17, CCL22) that was paralleled by a downregulation of Th1 cytokines (IFN-gamma) and Th1 chemokines (CXCL9, CXCL10). CD4+ and CD8+ T-cells dominated the inflammatory infiltrate in both, aGvHD and cGvHD lesions. Epidermal TSLP and T-cells were significantly upregulated as early as at day 20 after HCT in non-lesional skin of subjects who had no clinical signs of GvHD at this time point but would later develop aGvHD compared to those who would not. Our data suggest that diverse mechanisms are operative in acute and chronic forms of skin GvHD with IL-22- and Th2 cytokine-producing cells as candidate effector cells in aGvHD lesions.

Topic: Dermatology

S 5 Restoring cGMP levels in diabetic rats attenuates podocyte damage

Fang, L.* (1), Radovits, T. (2), Szabo, G. (2), Mozes, M. (1), Rosivall, L. (1), Kokeny, G. (1)

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Introduction: Diabetic nephropathy leads to glomerulosclerosis (GS). In animal models, the progression of GS is associated with increased cyclic 3',5'-nucleotide phosphodiesterase (PDE) isoenzyme activity (PDE3, 4, 5) leading to cGMP depletion. We hypothesized that restoring cGMP levels may attenuate podocyte damage in diabetic nephropathy. Therefore, selective inhibition of PDE5 with vardenafil may ameliorate the progression of GS in the rat model of streptozotocin (STZ) diabetes. Methods: Male Sprague-Dawley rats (250-300g) were divided into 2 groups after induction of diabetes with 60 mg/kg STZ: 1) STZ control (non treated, STZ, n=6) and 2) STZ + vardenafil treatment (10 mg/kg/day, STZ-vard, n=8). Non diabetic rats of the same age served as negative control (Co, n=8). Rats were treated for eight weeks, when kidney function was determined (urine protein/creatinine ratio, PC) and kidneys were removed for histology, immunohistochemistry, and mRNA expression analysis. Results: cGMP levels were restored due to vardenafil treatment (Co: 14±9; STZ: 8±1; STZ-vard: 26±15 pmol/mL, p<0,05). Proteinuria improved significantly in treated rats (PC: Co: 3,6±0,9; STZ: 22,5±9,2; STZ-vard: 11,2±4,9, p<0,05), accompanied by less GS (GSI: Co: 1,0±0,1, STZ: 2,1±0,1, STZ-vard: 1,3±0,3, p<0,05), less TGF-ß1 immunostaining (score: Co: 0,1±0,1, STZ: 2,1±0,3, STZ-vard: 1,2±0,3, p<0,05), and lower TGF-ß1 mRNA expression levels (Co: 0,7±0,1, STZ: 1,1±0,1, STZ-vard: 0,6±0,1, p<0,05) compared to STZ-Co group. Vardenafil treatment decreased desmin expression of podocytes (score: Co: 0,08±0,03, STZ: 0,49±0,13, STZ-vard: 0,31±0,08, p<0,05), but did not influence eNOS and nNOS mRNA expression of the kidneys. Conclusion: Restoring cGMP levels with selective PDE5 inhibitor vardenafil effectively attenuated podocyte damage and glomerulosclerosis in diabetic rats. We conclude that increased renal PDE5 activity in diabetes may play an important role on podocyte damage and progression of nephropathy.

Topic: Other

S 6 Role of epigenetic mechanisms in regulation of the Calcium Sensing Receptor expression in colorectal cancer

Fetahu, I.* (1), Höbaus, J. (1), Hummel, D. (1), Thiem, U. (1), Manhardt , T. (1), Kallay, E. (1)

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Introduction: Epidemiological studies suggest a role for calcium in prevention of colorectal cancer (CRC). The calcium sensing receptor (CaSR) probably mediates the antiproliferative action of calcium in colon. The CaSR expression decreases during tumor progression in human CRC. We hypothesized that epigenetic mechanisms like DNA hypermethylation and histone deacetylation might be responsible for silencing the expression of the calcium sensing receptor in colorectal tumors. Material and methods: We analyzed CaSR mRNA and protein expression in CRC tumors and cell lines by real time qRT-PCR and immunofluorescence. Bisulfite sequencing was used to determine the methylation pattern of two regions in the second promoter of the CaSR. We treated colon tumor cell lines with 5-aza-2-deoxycytidine (5-aza-dC), a DNA methyltransferase inhibitor, and/or Trichostatin A (TSA), a histone deacetylase inhibitor to induce the expression of the CaSR. Results and discussion: In CRC patients we observed a significant downregulation of CaSR mRNA expression (P<0.0001) in tumor tissues compared with the respective adjacent mucosa from the same patient. Immunofluorescence staining confirmed downregulation of the CaSR protein in tumors also. Bisulfite sequencing of two regions in the second promoter of the CaSR in CRC cell lines showed dense methylation of the second region. Although the methylation ranged from 2-70% among patients, there was no difference in the methylation pattern between tumor and the respective adjacent mucosa in any of the analyzed regions. Treatment with 5-aza-dC and TSA caused only modest increase of the CaSR expression, despite the presence of densely methylated CpG islands in the promoter of the CaSR. Conclusion: In our patient cohort the loss of CaSR expression in colon cancer is independent of DNA hypermethylation and histone deacetylation.

Topic: Malignant Diseases

S 7 A pencil beam algorithm for scanned helium ion beam dose calculation

Fuchs, H.* (1), Ströbele, J. (2), Schreiner, T. (3), Hirtl, A. (4), Georg, D. (1)

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Purpose: To develop a pencil beam (PB) algorithm enabling fast dose calculation for scanned ion beam therapy. Material and Methods The algorithm was based on fluence weighted elemental PB kernels. Using a new real-time splitting approach, a minimization routine selects the optimal shape for each sub-beam. Dose depositions along the beam path were determined using a look-up table (LUT). LUT data was derived from MC simulations in water using GATE6.1. For other materials, dose depositions were calculated using a water-equivalent depth. Lateral beam spreading due to multiple scattering was accounted for by the Highland-Lynch formula. An improved nuclear correction was modelled using a Voigt function. Validation simulations were performed using phantoms filled with homogeneous materials or heterogeneous slabs. Initial particle energies ranged from 50 to 250MeV/A. For comparison a special evaluation software was developed calculating gamma-indices. Results: Deviations increased with material density, but even for a phantom completely filled with bone, the agreement was good, resulting in range deviations of less than 0.9% and differences in the shape of the Bragg-Peak of less than 0.2mm for 250MeV/A. Lateral beam spreading showed good conformity, with deviations of the central dose of less than 0.87% for water. Results of the gamma index evaluation were in good agreement. As expected, sharp borders of materials with highly different properties, lateral to the beam path, imposed differences slightly above the gamma-index criteria of 2%/2mm at the material borders. Although only data for helium beams was presented above, the performance of the algorithm for proton beams was comparable. Conclusion: The PB algorithm developed for helium ions presents a suitable tool for dose calculation. It allows easy customisation to measured depth-dose distributions and accommodation of varying beam shapes, therefore making it a promising candidate for integration into treatment planning systems.

Topic: Medical Physics

S 8 Disruption of STAT3 signaling promotes K-Ras induced lung tumorigenesis

Grabner, B.* (1), Schramek, D. (2), Zwick, R. (3), Penninger, J. (4), Sibilia, M. (5), Popper, H. (6), Eferl, R. (1), Casanova, E. (1)

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Lung cancer related and unrelated to smoking is still leading cause of cancer deaths worldwide with an overall survival rate of 15%. Several genetic alterations have been associated with lung cancer: loss of tumor suppressor genes such as p53, INK4a, LKB1 and mutations/amplifications in several oncogenes like K-Ras, EGFR, or c-Myc. Most frequent smoking-related mutations impair GTP hydrolysis in K-Ras, an oncogene downstream of the EGFR pathway, causing persistent cell growth and proliferation in 20-30% of lung adenocarcinomas. Another important downstream effector of EGFR signaling is the signal transducer and activator of transcription (STAT)-3. STAT-3 regulates important pathways in tumorigenesis, through upregulation of genes encoding apoptosis inhibitors (Bcl-XL, Bcl-2, Mcl-1, survivin). In patient samples and NSCLC cell lines nuclear, phosphorylated STAT3 is enhanced and correlates with subsequent suppression of apoptosis of NSCLC tumors. However, the role of STAT-3 in lung cancer in vivo has not yet been established. In order to investigate the role of STAT-3 in lung tumors, we have established a genetic mouse model that allows inducing K-RasG12D-dependent lung tumors and simultaneously genetically ablating STAT-3. Survival analysis of this model showed a significant advantage of K-RasG12D male mice harbouring STAT-3 compared to K-RasG12D mice lacking one or both alleles of Stat-3, indicating a haploinsufficient tumor suppressor role in oncogenic K-RasG12D tumor formation. Furthermore, animals with deleted STAT-3 and activated K-RasG12D have a significant increase in tumor burden and develop more low grade tumors and in situ adenocarcinomas than age-matched animals, harbouring only oncogenic K-RasG12D. This data suggest that disruption of STAT-3 signaling promotes tumorigenesis in K-Ras induced tumors. We are currently investigating the molecular and cellular mechanisms responsible for this observation.

Topic: Malignant Diseases

S 9 An isolated heart set up to investigate diagnostic and control methods for rotary blood pumps

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Currently rotary blood pumps (RBPs) are driven at constant speed and provide only limited information about the interaction between heart and pump. With newly developed diagnostic and control algorithms, reliable methods for validation become important. However, whereas simulations simplify the complexity of the heart/pump interaction, in animals isolated hemodynamic changes can be hardly achieved. Aim of this work was to establish an isolated heart setup, which allows the validation of diagnostic and control methods by means of defined and isolated changes in pre-, afterload, contractility and pump support. Pig hearts from the abattoir as well as left-overs from animal experiments were used (n=5). The hearts were arrested, cooled down and prepared for the connection to the isolated heart apparatus. After pressure-controlled coronary reperfusion with warm oxygenated blood the apparatus was switched to „working-mode", which allowed left ventricular ejection into a Windkessel afterload. This afterload could be adjusted by a valve mimicking the arterial resistance; left atrial pressure was controlled by a roller pump. Ventricular contractility could be reversibly reduced by applying halothane with a vaporizer. Hemodynamic conditions similar to heart failure were achieved. Depending on pre-, afterload and halothane concentration, the cardiac output (CO) generated by the ventricle itself was up to 4L/min and the mean arterial pressure (MAP) up to 90mmHg. With the RBP implanted, CO and MAP increased substantially, equivalent to the assisted heart hemodynamics observed in patients. Summarizing, an isolated heart setup was developed, which reproduces the hemodynamics of the assisted circulation. By allowing isolated, defined and reversible changes of hemodynamic parameters, this tool provides new possibilities for the development of diagnostic and control methods for RBPs.

Topic: Biomedical Engineering

S 10 The role of iron in oxidative tissue damage in multiple sclerosis

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Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system with destruction of oligodendrocytes and myelin, relative preservation of axons and astrocytic scar formation. While inflammation and newly forming lesions together with remyelination predominate the early stages of MS, later stages are characterized by expansion of preexisting lesions, exhaustion of remyelinative capacity and neurodegeneration. However, the pathogenic mechanisms of demyelination and neurodegeneration remain poorly understood. Iron and its ability to induce oxidative damage have been implicated in various neurodegenerative conditions including MS. The few pathological studies addressing iron in MS were done on poorly characterized material and often with insensitive iron detection methods. We present a pathological study on formalin-fixed material of 35 autopsy cases of MS and 18 controls. The DAB-enhanced Turnbull blue method was used for detection of iron. Immunohistochemistry was performed for evaluating microglia and macrophages with the markers Iba-1 and CD68. The main iron storage protein ferritin and one of its subunits, ferritin light polypeptide, were detected together with hephaestin and ceruloplasmin, which mediate cellular iron export. The stainings were evaluated both by manual counting and digital optical densitometry. Double-stainings were done to confirm the cellular localization of ferritins, hephaestin and ceruloplasmin. Most of the iron in the human brain is stored within ferritin in oligodendrocytes and myelin. However, upon destruction of myelin and oligodendrocytes during demyelination in MS, this iron is released into the extracellular space and taken up by microglia and macrophages, which upregulate ferritin in response. In demyelinated lesion centers, oligodendrocytes and microglia have largely vanished. Thence the iron is found within astrocytes and axons, where it is likely to promote oxidative-stress induced neurodegeneration.

Topic: Neuroscience

S 11 Influence of ketamine on resting-state functional connectivity in healthy volunteers-a fMRI study

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Objectives: Ketamine is known to exert its schizophrenia-like effects through modulation of the glutamatergic system via the NMDA-receptor. Therefore, the aim of the present study was to investigate the impact of this specific transmitter system on resting state functional connectivity of the default-mode network (DMN). Methods: 10 healthy volunteers (23.2±3.4 years, 6 males) underwent resting state fMRI during esketamine hydrochloride (mean dose 15.12±2.76mg) intravenous maintenance infusion, lasting for 20 minutes. Functional MRI measurements were performed at 3 Tesla using single-shot gradient-recalled EPI (TE=38ms; TR=1800ms matrix size 128x128 voxel; 23 slices; FoV 190x190mm). Data sets were normalized to MNI-space and analysed in SPM8. To avoid seed selection bias, a recently developed approach for the computation of functional connectivity was applied which allows for the definition of the whole DMN as seed region. A repeated-measures ANOVA was performed to assess differences between baseline connectivity values and each 5-minutes block beginning at the start of the ketamine infusion (t=3.2; p<0.001 uncorrected voxel level). Results: Functional connectivity analysis showed a consistent ketamine-induced increase in the precuneus (0-5min: t=3.95; 15-20min: t=4.38; 30-35min: t=4.6) and the posterior cingulate cortex (PCC, 15-20min: t=3.59; 30-35min: t=3.44, see figure). For the later time points (15-20min and 30-35min) the cluster in the precuneus withstands correction for multiple comparisons (p<0.05 FWE-corrected cluster level). Conclusions: The application of a subanaesthetic dose of ketamine leads to a significant increase of the functional connectivity of the precuneus and the PCC, which represent key areas of the default-mode network. These results are consistent with findings in schizophrenic patients, which propose a hyperactivity of the DMN, pointing toward a possible implication of the NMDA-receptor on resting-state functional connectivity.

Topic: Clinical Neurosciences

S 12 Sigma-1 receptor agonist treatment is protective against renal ischemia/reperfusion injury

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Introduction: The activation of the Sigma-1 receptor (S1R)-Akt-endothelial nitrogen monoxide synthase (eNOS) signal transduction pathway has been shown to be protective against cardial ischemia/reperfusion (IR) injury. However there is no data concerning the link between renal IR injury leading to acute renal failure and S1R. Aim: To study the effect of S1R agonist fluvoxamine (FLU) and antagonist NE-100 in renal IR on the postischemic survival, the structural and functional damage and the S1R-Akt-eNOS signaling pathway. Methods: Uninephrectomized male Wistar rats were treated i.p. with FLU (20mg/bwkg; FLU), FLU and NE-100 (20mg/bwkg and 1mg/bwkg; FN) and vehicle (VEH) 30 minutes before the 50 minute renal ischemia. Sham-operated animals served as controls (C) (n=10/group). We observed postischemic survival and the deterioration of renal function in the 24th hour of reperfusion. We studied the alteration of renal capillary diameter and structural damage in vivo using muliphoton microscopy. The amount of renal S1R-Akt-eNOS proteins was determined by Western blot. Results: FLU treatment improved postischemic survival. Deterioration of renal function and renal structural damage was milder in FLU treated animals. This was characterized by the more preserved integrity of the tubular brush border and nuclei as well as less prominent hyaline accumulation. The reduced capillary diameter after IR was increased by FLU treatment (C: 9,86 ± 1,23 µm; VEH: 8,29 ± 1,29 µm; FLU: 10,73 ± 0,67 µm). The increased amount of renal S1R-Akt-eNOS proteins after IR was reduced by FLU treatment to the same level as in control animals. NE-100 suspended all effects of FLU. Discussion: The S1R agonist FLU –used as an antidepressant chronically without notable side-effects – could have a renoprotective effect in IR. We suspect the improvement of renal perfusion and the role of the S1R-Akt-eNOS pathway behind this effect.

Topic: Molecular Biology in Medicine

S 13 Polo-like Kinase is necessary for cytoskeletal inheritance of Trypanosoma brucei

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Eukaryotes have evolved specialized compartments to allow specific reactions to occur in discrete biochemical environments and exert biological processes efficiently. A functional cell depends on a set of strategically arranged organelles which number can vary depending on the need. This opportunity implies a fundamental biological problem: the duplication and the arrangement of organelles require additional intracellular space, then how can these events take place without damaging the efficiency of the pre-existing system. Trypanosoma brucei is a flagellated protist parasite vectored by the hematophagous Tsetse fly to mammals during blood meals, and the causative agent of human Sleeping Sickness and livestock's Nagana disease. T. brucei is an ideal model organism to investigate how eukaryotic cells solve the problems related to the duplication and arrangement of organelles. At every cell cycle, the cell prepares for organelle inheritance by duplicating and rearranging the organelles present in single copy within a restricted volume. Our results shows how T. brucei has evolved a highly ordered event of duplication and segregation of structures to assure the inheritance of a cytoskeletal complex associated to the flagellum called flagellar complex. In most model organisms, the motile kinase, Polo-like kinase regulates the cytoskeleton involved with centriole segregation, chromosome congression, and cytokinesis. The sole Polo-like Kinase homolog in T. brucei (TbPLK) regulates the duplication and segregation of individual components of the flagellar complex. TbPLK is associated to the growing tip of the new set of four parallel microtubules which contacts all organelles which require the kinase to duplicate. These results show how microtubules are involved in the duplication and segregation of structures by interacting with a motile kinase. This can explain how eukaryotic cells regulate the coordination of duplication and segregation of organelles in a limited space.

Topic: Molecular Mechanisms of Cell Signaling at the MFPL

S 14 Keratinocytes but not dendritic cells express TLRs 3, 6 and NOD2 in human prenatal and adult skin in situ

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Pattern recognition receptors (PRRs) initiate innate immune responses and direct subsequent adaptive immunity. They play a major role in cutaneous host defense against a variety of (pathogenic) microorganisms as well as in the pathophysiology of several inflammatory skin diseases. To understand the role played by PRRs in the acquisition of immunological competence, we conducted a comprehensive study to quantify mRNA expression and evaluate protein levels as well as function of PRRs in the developing human skin before and after birth and compared it with adults. We found that already prenatal skin expresses the same spectrum of PRRs as adult skin. Strikingly, many PRRs were significantly higher expressed in prenatal skin (TLRs 1-5, NODs 1/2, NALPs 1/3, DECTIN-1) and skin after birth (TLRs 1, 3, DECTIN-1) than in adult skin. Immunohistologic and immunofluorescence assessment of prenatal skin demonstrated that basal keratinocytes express TLRs 3 and 6, while suprabasal keratinocytes express NOD2 in prenatal as well as in adult skin. Surprisingly, neither dendritic cell precursors in prenatal skin nor epidermal Langerhans cells and dermal dendritic cells in adult skin co-expressed CD1c and TLRs 3, 6 and NOD2 proteins. Stimulation of primary human keratinocytes with selected agonists revealed that specifically the synthetic TLR3 ligand poly (I:C), mimicking viral double-stranded RNA, induced a significantly enhanced secretion of CXCL8, CXCL10 and TNF-alpha in fetal and neonatal keratinocytes as compared to adult keratinocytes. Our data indicate quantitative age-specific modifications in the PRR expression and functional profile of skin cells and innate skin immune reactivity in response to PRR stimulation. Thus, antiviral innate immunity already in prenatal skin may contribute to the protection of developing human skin from viral infections in utero in a scenario where the adaptive immune system is not yet fully functional.

Topic: Dermatology

S 15 Evaluation of HDL-associated proteins as novel biomarkers for chronic kidney disease

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Chronic kidney disease (CKD) is characterized by the development of uremia, progressive loss of renal function and can ultimately lead to kidney failure (end-stage renal disease; ESRD). Patients are characterized by dyslipidemia affecting the quantity and quality of high density lipoproteins (HDL). Proteomic analysis revealed differences in the protein composition of ESRD-HDL compared to healthy HDL. Several proteins, such as serum amyloid A (SAA), surfactant protein B (SP-B) or apolipoprotein CII (Apo-CII) are enriched in ESRD-HDL and can already be detected in earlier stages of CKD. We have found that these qualitative differences in the protein composition of HDL influence its anti-inflammatory function. Therefore, we want to establish HDL-associated proteins as novel biomarkers for individual prognosis, disease progression and an improvement in monitoring therapeutic responses in CKD. At present there is no simple test for determining the amount of HDL-bound proteins. Here we present an easy applicable assay based on an ELISA principle using an HDL catching antibody to directly capture HDL from plasma. Detection antibodies are directed against proteins specifically enriched on uremic HDL, such as SAA, SP-B or Apo-CII and protein quantity is measured by colorimetric reaction using a biotinylated secondary antibody and streptavidin-HRP to ensure highest sensitivity and signal detection. We show that our assay exclusively captures HDL without binding of other lipoprotein fractions and, importantly, without the requirement of a preliminary extensive isolation of HDL from plasma. Biobank samples will be tested for subsequent correlation of the assay data with several laboratory parameters, disease progression and mortality. Finally, we intend to analyze the HDL proteome from other chronic diseases with inflammatory characteristics and accompanying dyslipidemia to categorize disease-specific HDL profiles and identify more potential biomarkers.

Topic: Immunology

S 16 Osteopontin-induced inflammatory response of human adipocytes

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It is now recognized that a basis for the pathogenesis of obesity-associated diseases is a chronic low-grade inflammation in metabolic tissues, particularly adipose tissue, where it causes insulin resistance, contributing to the development of type 2 diabetes (T2D). Osteopontin (OPN) is an inflammatory cytokine involved in these processes and its activity may in part be controlled by post-translational processing via proteolytic cleavage by thrombin and matrix metalloproteases (MMP). Inflammatory conditions as induced by obesity are known to elevate the activity of these proteases and induce their gene expression directly in adipocytes. In recent studies we could show that full-length OPN not only plays a role in activation of human adipose tissue macrophages but also directly interferes with adipocyte function such as insulin-stimulated glucose uptake and induction of inflammatory pathways. The aim of this study is a detailed elucidation of the impact of intact and cleaved OPN on human adipocyte signalling, and obesity-induced adipose tissue inflammation and insulin resistance. Therefore we established an in-vitro human adipocyte differentiation protocol for the investigation of differentiation, gene expression, metabolic alteration and inflammatory activation of human adipocytes in presence or absence of OPN and its cleavage products. Compared to full length OPN, stimulation with a cleaved form increased activation of MAP kinases in adipocytes, a mechanism that is linked to adipose tissue inflammation and remodelling. Further work is ongoing to provide more detailed information on how OPN interacts with adipocytes in order to better understand the pathomechanisms leading to T2D.

Topic: Endocrinology and Metabolism

S 17 Cryo-electron tomography of baculovirus-induced actin comet-tails

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We aim to elucidate the structural organization of the pushing machineries based on actin filaments, to contribute to an understanding of how actin filament polymerization is harnessed to produce motion. Various pathogens, like Listeria and Rickettsia, as well as Vaccinia virus, hijack the actin machinery of cells to propel themselves in cytoplasm and to move from one cell to another, to propagate their infection. Recently identified members of this class of pathogens are baculoviruses, which likewise move inside cells by polymerizing a tail of actin filaments at their rear end (Ohkawa et al., J Cell Biol., 190;187-95, 2010). Since baculoviruses are small, around 250 by 50nm in size, the comet tails are correspondingly smaller and filament arrangements more easily resolved by electron microscopy. We used two systems to study the structure of the actin tails: First, an in vitro assay, including the isolated virus, Arp2/3 complex, actin and other components (Loisel et al., Nature, 401; 613-6, 1999). Second, suitable cell types like B16 melanoma cells were infected with concentrated virus and the tails observed in situ. Initial results from electron tomography of negatively stained preparations, indicate that the virus is propelled by a fish-bone–like array of branched actin filaments, with only a few filaments at any time in contact with the virus. Filament polarity analysis revealed the orientation of individual filaments in the tail structure (Narita, Maeda, J Mol Bio., 365(2):480-501, 2007). Current efforts focus on employing plunge-freezing and cryo-electron tomography of infected cells as well as in vitro samples to achieve better preservation in the Z-direction. This improvement is necessary to resolve the complete actin structure. Alternative, immuno-EM approaches are being used to establish the localization of the Arp2/3 complex in the actin comet tail.

Topic: Molecular Mechanisms of Cell Biology

S 18 Angiogenesis in degenerative aortic valve disease

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Purpose: Aortic valve disease is the most frequent native valve disease in Europe, and the third most frequent cause of cardiovascular death. Because aortic valve degeneration and prosthetic valve degeneration share common features, we hypothesized that a loss of small vessels in the course of an atherosclerotic process is underlying aortic valve stenosis. Methods: 232 aortic valves were collected during aortic valve surgeries and at autopsies, and corresponding patient and echocardiographic data were recorded. 112 aortic valve leaflets were analyzed by X-ray computed tomography and the calcium level was evaluated using the Agatston score. Representative tissue samples were used for immunohistochemical analysis. Gene expression profiles of leaflets and rings were analyzed with Microarrays, and confirmed by Real-time PCR. Results: Aortic jet velocities were 1.3±0.3m/s in healthy controls, 1.9±0.2m/s in aortic sclerosis and 4.7±0.9m/s in aortic stenosis ($p \leq 0.001$). Looking at leaflet weight and calcification we observed an increase of both in correlation with disease progression ($p=0.001$). vWF stains showed that blood vessel density within the valve rings decreased in parallel with increasing severity of aortic stenosis. Microarray cluster analysis of valve rings showed profound differences of normal against stenotic aortic valve rings, independent of stenosis severity. Several blood and lymphatic vessel markers including VE-cadherin and PDPN, as well as VEGFA and CTGF were significantly down-regulated in stenotic rings. Conclusion: Our data show significant reductions in blood and lymphatic endothelial cell markers, as well as pro-angiogenic growth factors in valve rings from patients with aortic stenosis. Loss of microvessels within the valve ring may lead to an under-supply of the valve leaflets giving way to protein accumulation and degradation, apoptosis, cholesterol accumulation, matrix degradation and calcification, thus contributing to stenosis progression.

Topic: Cardiovascular and Pulmonary Disease

S 19 Probing Network Fragilities in Embryonal Tumors by Synergistic Drug Combinations

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Embryonal tumors (ETs) occur early in life and may reveal pathogenetically relevant lesions clearer than adulthood tumors with accumulated passenger mutations. Furthermore, there is an urgent need for new therapeutic approaches to these aggressive ETs and the aim of this project is to approach new targeted therapies. We are using a defined panel of core cell lines representing the three highly aggressive ET entities: neuroblastoma, medulloblastoma and Ewing sarcoma. These genetically engineered isogenic ET cell line pairs are tested against the selected panel of clinically applicable drugs in proliferation assays. Based on the respective IC50 values we divided the compounds in 3 groups: (i) potent; (ii) measurable and (iii) not active (these are excluded from the binary combinatorial screening list). The remaining compounds were screened for synergistic antiproliferative effects through pair wise combinations using a robotics-assisted platform and validated by three-dimensional dose response matrices. Despite the starting hypothesis that these ETs share common functional aberrations the data suggests there are major differences between disease types in response to certain drugs. In TrkA inducible SH-SY5Y neuroblastoma cell line we observed highly synergistic pairs in a concentration range acceptable for the clinical use in pediatric patients. Strikingly, it seems that these three synergistic combinations might follow the same mechanism, although their main targets are very distinct and they show different potencies. Interestingly, the HDAC inhibitor panobinostat is highly potent in all ET cell lines tested, suggesting at least partial overlap of oncogenic pathways in these tumors. These drug synergies might reveal critical crosstalk between aberrant signaling pathways and networks that can be therapeutically exploited, providing the information on the radius of efficacy of these compounds by assessing their protein targeting and global effects on network functions.

Topic: Malignant Diseases

S 20 Stat3 in the tumor microenvironment of colitis associated cancer

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The cytokine-induced transcription factor Stat3 mediates heterotypic signaling between tumor cells and cells in the tumor stroma. However, Stat3 functions in individual cell compartments of the tumor stroma are poorly characterized. We employed a conditional approach to abolish Stat3 in macrophages and granulocytes. Stat3 Δm/ Δm conditional knock-out mice were treated with AOM/DSS to induce colitis-associated colorectal tumors. Interestingly, a significant reduction of tumor burden was observed in mutant Stat3 Δm/ Δm mice after AOM/DSS treatment when compared to littermate controls. This indicates that reduced Stat3 activity in macrophages/granulocytes enhances their immunogenic potential thereby suppressing CRC formation. Next, we addressed the question if enhanced activity of T cells and NK cells can also account for suppression of CRC formation. Therefore, MC38 adenocarcinoma cells (tumor cell-killing is dependent on T cells) and p185 lymphoma cells (tumor cell-killing is dependent on NK cells) were transplanted into Stat3 Δm/ Δm and control recipients. Both cell types showed reduced tumor burden in Stat3 Δm/ Δm recipients indicating that Stat3 deletion in macrophages/granulocytes enhances the antitumor activity of T- and NK cells via a cellular crosstalk. Albeit present at lower numbers, colorectal tumors of Stat3 Δm/Δm mice were more invasive and displayed a significantly enhanced tumor stromalization with increased numbers of infiltrating macrophages, mast cells and NK cells. This suggests that "surviving" tumors in Stat3 Δm/ Δm mice underwent immunoediting and developed mechanisms to render immune cells anergic and tumortolerant. Consistently, the macrophage population present in the stroma of Stat3 Δm/ Δm CRCs was mainly polarized towards a M2 phenotype, which is known to promote tumor progression. Our data suggest that a Stat3-dependent crosstalk between immune cells in the stroma modulates initiation and progression of colitis-associated colorectal cancers.

Topic: Malignant Diseases

S 21 IL-13 plays a critical role during bacterial pneumonia

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Streptococcus pneumoniae (S. pneumoniae) is the leading etiological agent of community-acquired pneumonia (CAP). Asthma and influenza are the two main risk factors for pneumococcal pneumonia. These two rather different diseases share a common feature, which is IL-13 mediated airway hyper reactivity (AHR). IL-13 is secreted by Th2 cells during asthma and by type-2 innate lymphoid cells during influenza infection. Because IL-13 is furthermore known to polarize macrophages towards a so-called anti-inflammatory M2 phenotype, we hypothesized that IL-13 might contribute to the enhanced susceptibility to S. pneumoniae pneumonia.To investigate this idea, we decided to study the role of IL-13 in primary pneumococcal pneumonia and post-influenza pneumonia in mice. To start with, we examined the presence of IL-13 in lung tissue before and upon infection with S. pneumoniae. To our surprise we identified substantial amounts of IL-13 in healthy lungs, thus indicating an innate source of IL-13. Neither respiratory epithelial cells, nor alveolar macrophages produced IL-13 at steady state, thus pointing towards the possible involvement of innate lymphoid cells as the source of this cytokine. By flow cytometry we managed to identify a previously reported small subset of natural helper cells (Lin-CD45 Thy1.2 Sca1) in lungs, their ability to secrete IL-13 is currently being investigated. Upon pneumococcal infection, IL-13 gene deficient animals showed an improved bacterial clearance and survival, which was found to be associated with an enhanced early induction of the chemokine KC and resulting increased influx of neutrophils. Our data thus far point towards a critical role of IL-13 during pneumococcal pneumonia, as IL-13 prevented the early inflammatory response and hence led to impaired bacterial clearance and survival. Further studies will address the cellular origin of IL-13 and the molecular mechanisms that explain the anti-inflammatory role of this cytokine during primary and post-influenza pneumonia.

Topic: Cell Communication in Health and Disease

S 22 Role of EGFR in inflammation induced colorectal cancers

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Colorectal cancer (CRC) is one of the major causes of mortality in the western world. Inflammatory bowel diseases such as Ulcerative Colitis, Crohn's disease are associated with an increased risk of CRC suggesting that immune system activation contributes to tumor promotion. The Epidermal Growth Factor Receptor (EGFR), a member of the tyrosine kinase receptor super family, is known to be overexpressed in CRC, but its molecular functions in this disease are not fully known. This project employs a transgenic murine colitis associated cancer model, wherein EGFR expression is specifically abrogated in the intestines of these mice using the Cre-loxP system. Azoxymethane (genotoxic carcinogen) is administered to these mice, to produce random genetic alterations, followed by Dextran Sulphate Sodium Salt which causes chronic inflammation in distal and intermediate colon. Both treatments mimic the environment for induction of colorectal tumors. The project aims at establishing a concrete role of EGFR in colon carcinogenesis. Our current observations show no difference in tumour incidence in mice lacking EGFR in intestinal epithelial cells (EGFR Δint mice) compared to controls (EGFRf/f). However, when high fat food is administered during carcinogenesis, the opposite phenomenon is observed in the EGFRf/f and EGFR Δintmice. Molecular analysis show higher inflammatory markers in EGFRΔint mice. Interestingly tumor incidence is reduced in mice lacking EGFR inthe myeloid cell lineage. Furthermore, in other intestinal tumor model mice, which are heterozygous for the Adenomatosis Polyposis Coli gene Apc and spontaneously develop polyps in the gut, the loss of EGFR leads to significant reduction in life span likely because of higher tumor incidence. These observations suggest a tissue specific protective role of EGFR that is contrary to the current belief that EGFR is a tumor promoter. Further molecular investigations currently underway will reveal the mechanisms leading to the observed phenotypes.

Topic: Inflammation and Immunity

S 23 Lipocalin 2 modulates inflammation and impairs host defense against Streptococcus pneumoniae

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Background: Lipocalin 2 (LCN2) is an antibacterial protein, known to interfere with the siderophore-dependent iron acquisition of pathogens such as E. coli and Mycobacteria. However, most bacterial species constituting the lung microbiome do not utilize siderophores. Likewise, Streptococcus pneumoniae, a leading colonizer of the respiratory tract and a major cause of community acquired pneumonia, utilizes siderophore-independent mechanisms of iron acquisition. Despite this fact, both colonization and infection with S. pneumoniae induces tremendously high lung levels of LCN2 in mice and humans – and the biological role of this finding remains elusive. We therefore investigated the function of LCN2 during pneumococcal pneumonia. Results: Studying a murine pneumonia model, we found LCN2-/- mice to display an enhanced bacterial clearance, accelerated resolution of lung inflammation and improved survival. As an explanation for this improved host defense we found the early inflammatory response (6h after induction of pneumonia) to be augmented with a significantly increased neutrophil influx in LCN2-/- animals. Mechanistically, LCN2-/- alveolar macrophages exhibited an enhanced secretion of pro-inflammatory cytokines and decreased induction of anti-inflammatory mediators in response to TLR ligands. Consistently, recombinant mouse LCN2 dampened the macrophage's response to TLR ligands, and overexpression studies disclosed that LCN2 mediated these effects via induction of IL-10. Conclusions: We postulate that LCN2 modulates the early inflammatory response upon S. pneumoniae infection through induction of IL-10 and deactivation of lung macrophages, which ultimately results in impaired bacterial clearance and survival.

Topic: Inflammation and Immunity

S 24 High-speed, wide-field polarization sensitive OCT for measuring retinal nerve fiber layer birefringence, retardation and thickness

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We present a new polarization sensitive OCT system (PS-OCT) for measuring the retinal nerve fiber layer (RNFL) birefringence, retardation and thickness in vivo. The system supports scan angles of up to 40°x40° with an A-scan rate of 70kHz. In this study 10 eyes of 5 healthy volunteers were measured 5 times consecutively. The polarization sensitive data was compensated for anterior segment birefringence and 2D en face maps of RNFL birefringence, retardation and thickness were calculated for each dataset. The resulting images were registered with respect to each other, averaged and standard deviation maps were calculated. High quality RNFL birefringence, retardation and thickness maps were obtained and the standard deviation maps prove the high reproducibility of our system. Each volunteer was further imaged with scanning laser polarimetry (SLP). For quantitative comparison between SLP and the new PS-OCT system a circumpapillary evaluation within 2 annular segments (superior and inferior to the optic disc) similar to SLP was performed. Superior and inferior to the optic nerve head the mean retardation within the annular segments was ranging from 19.2° to 26.7° and 16.8° to 27.7° respectively. The standard deviation within the quadrants between the 5 individual measurements was ranging from 0.1° to 0.71°. The mean birefringence varied from 0.106°/µm to 0.137°/µm superiorly and 0.102°/µm to 0.139°/µm inferiorly with a quadrant precision ranging from 0.001°/µm to 0.005°/µm. The mean RNFL thickness superior and inferior varied between 110.4µm to 145.6µm and 108.7µm to 139.4µm with a quadrant precision ranging from 3.5µm to 8.2µm. With our new PS-OCT system we found a high reproducibility and also a high image quality of RNFL birefringence, retardation and thickness measurements. PS-OCT might therefore be a valuable tool for future glaucoma diagnostics.

Topic: Medical Physics

P 1 Deciphering the Role of the Calcium Sensing Receptor in Regulating Colorectal Cancer Proliferation

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With an incidence rate of about 15% of all newly diagnosed cancers, colorectal cancer needs effective prevention strategies. Epidemiological studies suggest an inverse correlation between dietary Calcium (Ca^{2+}) and colorectal cancer risk. The Calcium Sensing Receptor (CaSR), a G-protein coupled receptor first cloned in the parathyroid, regulates extracellular levels of Ca^{2+}. The CaSR is present in numerous tissues independent of their role in calcium homeostasis. In the colon, expression of the CaSR increases along the colonic crypt in parallel with the concentration of Ca^{2+}, suggesting the CaSR to be the molecular switch that turns off proliferation in these cells and promotes differentiation. This may be explained by the effect of Ca^{2+} on the replication machinery in colon cancer. Hence we hypothesized that the CaSR mediates the anti-proliferative effect of Ca^{2+} by regulating the expression of replication licensing factors. Our aim is to establish if there is any correlation between expression levels of key licensing factors and the expression of CaSR in colon cancer specimens. We will examine if manipulation of the CaSR expression in colon cancer cell lines would affect the impact of Ca^{2+} on proliferation. This would be measured by proliferation assays using colorectal cell lines transfected with CaSR constructs with activating or dominant negative mutations. To further test our hypothesis we will examine the expression pattern of licensing factors in colon of different CaSR-specific mouse models (NUF mouse model – activating mutation of CaSR, CaSR/PTH(-/-) knock-out (KO) and smooth muscle specific CaSR KO). Our project would clarify how Ca^{2+} regulates proliferation in colorectal cancer at the molecular and cellular level. Correlation of the expression of the CaSR with the replication licensing process could elucidate why colon tumours lose their responsiveness to calcium.
This project is funded by the Marie Curie Initial Training Network grant number FP7-264663.

Topic: Malignant Diseases

P 2 Prolyl hydroxylase inhibitors: A new strategy for periodontal regeneration?

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Regeneration of the periodontal tissue is only to a limited extent supported by current periodontal therapies. Strategies that stimulate periodontal regeneration require angiogenesis and reduction of catabolic processes in the tissue. Here we follow this approach by using prolyl hydroxylase (PHD) inhibitors to stabilize the transcription factor hypoxia-inducible factor (HIF)-1alpha to induce a pro-angiogenic response in periodontal cells. We evaluated the impact of PHD inhibitors, also when released from bone substitutes, on intracellular HIF-1 levels, production of vascular endothelial growth factor (VEGF) and on viability and proliferation of periodontal fibroblasts. Moreover, we assessed the impact of PHDs on plasminogen activation by periodontal fibroblasts and the effect on osteoclastogenesis and resorption activity of osteoclasts in murine bone marrow cultures. Our results show that PHD inhibitors increase the production of VEGF while cell viability and proliferation was only slightly reduced. In addition bone substitute materials were successfully supplemented with PHD inhibitors to induce a pro-angiogenic response. This pro-angiogenic effect is paralleled by reduced plasminogen activation in periodontal fibroblasts and a decreased osteoclastogenesis and resorption activity. Overall our results show that PHD inhibitors stimulate the pro-angiogenic capacity of periodontal cells while reducing the catabolic activity. In addition our results suggest that bone substitutes can be used as carriers for PHD inhibitors. If PHD inhibitors can be applied to stimulate periodontal regeneration will be addressed in upcoming preclinical studies.

Topic: Other

P 3 Inter-device reliability of Dysphonia Severity Index measurement

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The Dysphonia Severity Index (DSI) is a measure that quantifies the overall vocal quality. Aim of the study is to evaluate the reliability of DSI measurements. The DSIs of 30 subjects were therefore measured using LingWAVES (WEVOSYS) and DiVAS (XION). To evaluate the inter-device reliability of DSI measurements, subject corresponding results were compared. The DSI values of both devices showed great differences. The calculated DSI differences of 95 % of the subjects were within the limits of +2.39 and – 2.82, what makes a clinical interpretation of severity of voice disorder using different devices questionable. Technical and methodical aspects of measurement divergences are discussed, the needs to define hardware and software standards are shown.

Topic: Other

P 4 Mucosal tolerance induction with structurally different antigens: studies on underlying mechanisms

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In a mouse model of poly-sensitized mice, we have shown that intranasal administration of a linear synthetic hybrid, composed of immunodominant T-cell epitopes of the major birch and grass pollen allergens Bet v 1, Phl p 1 and Phl p 5, led to tolerance induction in mice, sensitized with the 3 allergens. Immunosuppression was associated with reduced allergic inflammation and increased IL-10 levels in the lungs. Using the whole recombinant (r) Bet v 1 as allergen chimer for tolerance induction, immunosuppression within the lung was however not associated with increased IL-10 levels. Thus we assumed that mucosal tolerance induction with the hybrid or rBet v 1 depends on the conformation of the allergen. Hybrid and rBet v 1 conjugated with the fluorescence dye 5(6)-Carboxyfluorescein (FAM), were intranasally administered in vivo. After various time points (0, 1, 6, 24 and 48 hours) the antigen uptake in the nasal-associated lymphoid tissue, lung, bronchial lymph nodes, spleen and blood was investigated. After 6 hours, macrophages and dendritic cells were those cell populations with the highest antigen uptake capacity. Interestingly, more FAM-hybrid than FAM-rBet v 1 molecules were detected within the lung cells. Furthermore, FAM-hybrid and FAM-rBet v 1 were used to stimulate isolated murine lung cells in vitro in a time dependent manner (0, ½, 1, 6 and 24 hours) and cells capturing these antigens were identified and characterized via flow cytometry. In vitro, macrophages, dendritic cells and additionally B cells were internalising the antigens, and B cells displayed the population with the highest antigen uptake capacity. With respect of the time kinetic these B cells internalised the FAM-hybrid about 6 hours earlier than the FAM-rBet v 1.Our data show that there are differences in antigen uptake and kinetic in vivo and in vitro, between the linear multipeptide and the conformational allergen. Further studies on internalisation pathways are ongoing.

Topic: Immunology

P 5 Expression of thyroid hormone binding protein CRYM (μ-crystallin) negatively correlates with advanced stages of prostate cancer

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Prostate cancer is the most common cancer in men worldwide and millions of men are diagnosed with this disease each year. Androgens play an important role in early stages of prostate cancer and androgen receptor (AR) signalling has been shown to be involved in differentiation and apoptosis of prostate cancer cells, but the underlying molecular mechanisms are incompletely understood. In this study, we are interested in the role of the thyroid hormone binding protein μ-crystallin (CRYM) and its relation to androgen receptor signalling and thyroid hormone regulation in the development and progression of prostate cancer. We evaluated CRYM expression levels in androgen-dependent LnCAP and androgen-insensitive PC-3 cell lines and human patient samples. CRYM expression was detected in androgen-dependent LnCAP, but not in PC-3 cells. Furthermore, CRYM expression negatively correlated with advanced stages of prostate cancer and completely disappeared in androgen-insensitive human tissue samples. Treatment of CRYM expressing LnCAP cells with thyroid hormone triiodothyronine (T3) resulted in increased proliferation and migration, but no significant difference after T3 treatment was observed in CRYM negative PC-3 cells. We are currently performing CRYM knockdown experiments in the presence and absence of T3 in order to study CRYM-mediated effects on proliferation and migration and plan to carry out chromatin immunoprecipitation (ChIP) experiments to analyse AR-regulated CRYM function in prostate cancer cells.

Topic: Malignant Diseases

P 6 Alkali treatment of titanium surfaces increases adhesion of stromal cells in vitro

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Background: Adhesion of osteogenic cells to titanium surfaces is a prerequisite for osseointegration. Alkali treatment can increase the hydrophilicity of titanium implant surfaces thereby supporting the adhesion of blood components. However, it is unclear if alkali treatment also supports the adhesion of osteogenic cells to titanium surfaces. Material and Methods: Here we have used titanium surfaces produced by alkali treatment and the corresponding controls to demonstrate the impact of hydrophilicity on the adhesion of mesenchymal cell lines. Cell adhesion was determined by scanning electron microscopy. To evaluate the impact of the surface on the cellular response we measured viability, proliferation and protein synthesis. Results: We report that alkali treatment increased the adhesion of L929 and MG63 cells seeded onto with titanium surfaces for 1 to 3 hours, an effect that was restricted to these early time period. Cells grown for 24 hours on the titanium surfaces produced by alkali treatment and the corresponding controls behave similar with regard to viability, proliferation and protein synthesis. Conclusion: Based on these results, we conclude that alkali treatment can support early adhesion of mesenchymal cells to titanium implant surfaces while their biological response remains unchanged.

Topic: Regeneration of Bones and Joints

P 7 A Smoothened-Ampk axis rewires metabolism

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Diabetes, obesity and cancer affect upwards of 15% of the world's population. Interestingly, all three diseases juxtapose dysregulated intracellular signaling with altered metabolic state. However, exactly which genetic factors define stable metabolic setpoints in vivo remains poorly understood. Recently, we have shown that (i) Hedgehog (Hh) signaling blocks white but not brown adipogenesis in vivo (Pospisilik et al., 2010; Cell 2010), and (ii) that this biological effect is sensitive to inflammatory state (Todoric et al., Diabetes 2011). In one of our current research projects we identified a novel Ca++/G-protein coupled Hh-Smoothened (Smo)-Ampk axis that triggered a rapid Warburg-like aerobic glycolysis state within minutes of activation. Of note, small molecule Smo-modulators uncoupled this novel Hh-Smo-Ampk axis from canonical signaling and identified cyclopamine as one of a new class of Smo "partial agonists". Furthermore, Smo-Ampk axis activation in vivo drived robust glucose clearance through activation of muscle and brown adipose tissue glucose disposal. Intriguingly, we could demonstrate that activation of this pathway bypassed any requirement for insulin in type-1 diabetic STZ mice. Finally, acute and chronic treatment of mice with cyclopamine consistently increased body temperature, most likely via direct brown adipose tissue activation. These findings identify a new Hedgehog signaling pathway and provide a unique new therapeutic avenue for obesity and diabetes.

Topic: Endocrinology and Metabolism

P 8 Peptide Mimotopes of Malondialdehyde-Epitopes for Clinical Applications in Cardiovascular Disease

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Introduction: Autoantibodies (autoAbs) specific for malondialdehyde modified LDL (MDA-LDL) represent potential biomarkers to predict cardiovascular risk. However, the generation of MDA-LDL results in the formation of many different epitopes with high variability. The aim of this study was to identify and characterize peptide mimotopes of MDA-LDL that could be used as antigens to improve the reproducible detection of MDA-specific autoAbs. Methods and Results:Peptide phage display libraries were screened for phages binding to the MDA-LDL specific natural IgM Abs LR04. After biopanning two consensus sequences (P1 & P2) of binding phages were synthesized which were specifically bound not only by LR04 but also by other MDA specific murine (EN1) and human (IK17) Abs. Furthermore, the binding of LR04 to late apoptotic cells was completely inhibited by both peptides, identifying them as mimotopes of naturally occurring epitopes on dying cells. Immunization of C57BL/6 mice with P2 conjugated to BSA resulted in the robust induction of Abs against MDA-LDL. Moreover, serum IgG of immunized mice specifically stained epitopes in atherosclerotic lesion of rabbits and humans. Finally, we measured anti-mimotope Abs titers in serum samples previously collected from healthy subjects (n=17) and from patients (n=140) with stable angina pectoris undergoing percutaneous coronary intervention. In patients and in healthy subjects a significant positive correlation was observed between anti-MDA-LDL and anti-mimotope IgM and IgG Abs. Conclusions: Thus, we have identified specific mimotopes of MDA-LDL that serve as highly reproducible antigens to assess autoantibody titers in patients with cardiovascular disease.

Topic: Cardiovascular and Pulmonary Disease

P 9 Heme arginate protects Skeletal Muscle against Ischemia Reperfusion Injury: A randomized, placebo controlled Trial in healthy Subjects

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Objectives: Heme arginate can induce heme oxygenase-1 to protect tissue against ischemia-reperfusion injury. Blood oxygen level dependent (BOLD) functional magnetic resonance imaging measures changes in tissue oxygenation with a high spatial and temporal resolution. BOLD imaging was applied to test the effect of heme arginate on experimental ischemia reperfusion injury in the calf muscles. Methods: A two period, controlled, observer blinded, crossover trial was performed in 12 healthy male subjects. Heme arginate (1 mg/kg body weight) or placebo were infused 24 hours prior to a 20 minutes leg ischemia induced by a thigh cuff. 3 Tesla BOLD-imaging of the calf was performed and signal time courses from soleus, gastrocnemius and tibialis anterior muscle were analyzed. Results: Peak reactive hyperemia signal of the musculature was significantly increased and occurred earlier after heme arginate compared to placebo (106.2±0.6% at 175±16s vs. 104.5±0.6% at 221±19s; p=0.025 for peak reperfusion and p=0.012 for time to peak). Conclusions: A single high dose of heme arginate improves reperfusion patterns during ischemia reperfusion injury in humans. BOLD sensitive MRI is applicable for the assessment of experimental ischemia reperfusion injury in skeletal muscle.

Topic: Vascular Biology

P 10 Effects of bacteria and lipopolysaccharide on human megakaryocytes

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Background: During megakaryopoiesis and thrombopoiesis megakaryocytes mature and form platelets which are then released into the blood stream. Platelets express functional toll like receptor 4 (TLR4) and can modulate TNFα; and IL1-β; production. Importantly, TLR4 is already present in megakaryocytes, and LPS can affect platelet production from megakaryocytes. In this project we analyzed whether bacteria and LPS influence megakaryocytic differentiation and proliferation, and which molecules are affected. Material and methods: CD34+ cells were isolated from cord blood using magnetic beads. The cells were cultured, in the presence of SCF, IL-3 and Tpo to promote megakaryocytic differentiation. Once mature, the megakaryocytes were exposed to buffer, heat killed bacteria (E.coli K118) or LPS. Cell culture supernatants were analyzed for the presence of cytokines using a cytokine proteom profiler (R&D). RNA from megakaryocytes was tested for the presence of the corresponding gene transcripts. The transcripts (pre-mRNA versus mature RNA) were evaluated by an intron spanning PCR. Results: Incubation of megakaryocytes with bacteria lead to upregulation of C5a (1´8 fold), IL8 (4´5 fold), serpin E1 (1´4 fold), and MIF (1´3 fold) in the culture supernatants after 6 and 20 hours. RNA analyses showed that C3, C5 and Serpin E1 transcripts are present as pre-mRNA and mature RNA already on day 6. Pre-mRNA is continuously generated and is maintained until day 12. The addition of bacteria seems to enhance transcription and splicing of pre-mRNA, resulting in a more stable amount of pre-mRNA and increased concentration of mature RNA. Conclusion: Our data show for the first time the presence of complement C3 and C5 RNA in megakaryocytes and their translation to protein in these cells. We could demonstrate that megakaryocytes continuously transcribe C3 and C5 pre-mRNA which they sustain in considerable amount and splice upon contact with bacteria.

Topic: Cell Communication in Health and Disease

P 11 Diversity and projections of ventral hippocampal pyramidal neurons

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The pyramidal neurons of the CA1 area in the ventral hippocampus have numerous diverse and distant connections to other brain regions including the temporal and parietal association areas, visual, auditory, olfactory, somatosensory, gustatory, and visceral areas, and inputs to the amygdalar and prefrontal-orbital-agranular insular region. In addition their differential expression of proteins like calbindin and norbin provide further indications for cellular diversity. This raises the possibility that the pyramidal cells could differ in their activity patterns and information content as well, and they could be part of different brain circuitries. To address this hypothesis we recorded extracellularly the spontaneous firing activity of pyramidal cells in the ventral CA1 area in anaesthetized rats and filled the recorded neurons with neurobiotin by the juxtacellular labeling technique. In order to achieve a filling of distant axonal arborizations, perfusion/fixation was delayed by up to 20 hours after labelling. We determined the soma location, axo/dendritic arborization, expression of selective proteins, firing patterns, correlation with theta and sharp wave -associated ripple activity from the dorsal and ventral hippocampus for the labeled neurons. We observed neurons with different firing patterns; regular spiking, bursting or mainly silent. Other variance was that cells were activated or silent during dorsal CA1 ripple activity. Differences were observed in their neurochemical expression profile and in the path of the axons emitted by these pyramidal cells running to the subiculum or with direct collaterals to cortical areas. We also observed diversity in terms of long-range projections. Our results indicate a diversity of pyramidal neurons in the ventral CA1 hippocampus.

Topic: Neuroscience

P 12 Specific interaction of dendritic cells in response to several allergens

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BACKGROUND: Recent studies focused on the response of dendritic cells to allergens and demonstrated ability of DCs to stimulate a Th2 immune response but did not clarify whether these observations are based on specific interactions between the allergens and the respective allergic people. We elucidate the influence of several allergens on DCs from different groups of allergic and healthy individuals. METHOD: Heparinised blood was obtained from 24 individuals with a history of allergy to birch pollen, grass pollen and 5 individuals as healthy control group. Monocyte-derived immature dendritic cells were stimulated with Bet v 1, Phl p 5 and Act d 10. Surface expression of dendritic cells markers were analysed by flow cytometry. Proliferation assays were performed through co-culture with stimulated MoDCs and autologous naive CD4 T- cells. Quantification of cytokines in supernatants of stimulated DCs and co-cultured naive CD4 T-cells were performed by ELISA. RESULT:The results indicated that stimulated DCs of allergic individuals induce proliferation of naive CD4 T-cells, but Bet v 1 stimulated DCs gave rise to a significantly proliferation in birch allergic individuals which depends on Bet v 1.We demonstrated that maturation of dendritic cells only depended on the maturation inducing factors, IL-1β and TNF-α and there were no differences in co-stimulatory molecules expression by stimulated dendritic cells with different allergens. Furthermore we showed increased level of IL-5 and IL-13 in supernatant of both allergic groups but with high increased level in birch group in comparison to the respective Non-allergen group. In contrast the level of IL-10 and IFN-γ were unaltered. CONCLUSION: Our experiments resulted to significant up-regulated secretion of Th2 cytokine and unaltered secretion of Th1 cytokine in matured DCs stimulated by Bet v 1. we have shown the ability of birch pollen allergic individuals to induce augmented Th2 cytokine profile in respect to Bet v 1.

Topic: Immunology

P 13 Effects of statins on ABCB1 localisation and on endogenous dolichol level

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Cardiovascular are still the leading cause of death worldwide which are causally linked to high blood cholesterol levels. Statins are fungi metabolites and able to block the rate-limiting enzyme HMG-CoA reductase in the mevalonate pathway. Based on this feature, they are introduced as safe and successful drugs in the treatment of hypercholesterolemia. Interestingly, statins have also shown some pleiotropic effects, like anti-inflammatory, anti-thrombogenic, and anti-proliferative actions. The latter effect is observed in higher concentrations leading to apoptosis in several tumor types and direct inhibition of ABCB1 [1-3]. Simvastatin treatment leads to downregulation of ABCB1 on protein as well as on mRNA level in human neuroblastoma cell line SH-SY5Y. Additionally, simvastatin exposure affected also the localisation of ABCB1 in the cell with enhanced accumulation in the cytosol. Previously, our Western blot analyses have proved that simvastatin reduced the amount of mature-glycosylated form of ABCB1 (180 kDa) in the cell while increasing the core-glycosylated form (140 kDa). Interestingly, this is reversed by addition of dolichol, which is also a product of mevalonate pathway and plays key role in N-glycosylation of plasma membrane proteins. To investigate the abolishing potential of dolichol, we have performed caspase 3 fluorescence assay to detect the activation of intrinsic apoptosis after simvastatin treatment. Whereas simvastatin induced caspase 3 activation in a concentration-dependent manner, coadministration with dolichol reduced this activity significantly to the basal level. Moreover, lipid extracts from simvastatin-treated SH-SY5Y cells exhibited a significant depletion of endogenous dolichol level, even with concentrations used in the clinic.
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Topic: Molecular Signal Transduction

P 14 Higher-order principles in distribution patterns of serotonergic receptor subtypes revealed by PET

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Enough evidence is available to support the hypothesis of a local and systemic interplay of the subsystems within the serotonergic system. In this study, we hypothesize that there are also feedback mechanisms modulating the expression of different receptor subtypes and their global interplay. We quantified important receptor subtypes 5-HT1A, 1B and 2A (major inhibitory and excitatory receptors) and 5-HTT (target of many antidepressants) to investigate associations within the serotonergic system. 83 healthy subjects assigned into 4 groups (age=27.7±6.8y; 55% males). After motion correction and spatial normalization in SPM8 dynamic PET scans were done, using the selective radioligands [carbonyl-11C]WAY100635, [18F]Altanserin, [11C]P943 and [11C]DASB, respectively. Binding potential values were quantified by a multi-linear reference tissue model (MRTM2; 5-HT1A, 5-HT1B, 5-HTT; BPND) and a bolus/infusion approach (5-HT2A; BPP) in PMOD 3.4. A standard template in MNI space served for ROI delineation according to the anatomical parcellation scheme of Brodmann (41 regions: BAs). Partial correlations of mean BA BP values were calculated between two sets of receptors controlling for the third and 5-HTT. We found significant correlations between 5-HT1A and 2A controlled for 1B and 5-HTT ($r=0.70$, $p<0.001$), 1A and 1B controlled for 2A and 5-HTT ($r=-0.69$, $p<0.001$), and 1B and 2A controlled for 1A and 5-HTT ($r=0.84$, $p<0.001$). Strong positive correlations of both inhibitory subtypes (5-HT1A and 5-HT1B) with the excitatory 5-HT2A receptor suggest a balanced distribution of these opposing proteins both locally and throughout the entire cortex. The negative correlation between the inhibitory receptors 5-HT1A and 1B is also remarkable. On a global scale they complement each other in the inhibitory performance. Our findings highlight the importance to evaluate interactions between multiple receptor subtypes to improve our understanding of neurotransmitter systems.

Topic: Clinical Neurosciences

P 15 Platelets directly enhance neutrophil transmigration in response to oxLDL

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Beyond their primary role in haemostasis and tissue repair, platelets are causally involved in the onset of inflammatory reactions, cell proliferation and immune response. Platelet activation and platelet binding to the endothelium, results in release of chemokines and increased expression of adhesion molecules, which promote the recruitment of leukocytes that will eventually migrate across the endothelium into the tissue. Here, we provide the first evidence that platelets stimulated with oxidised LDL (oxLDL) directly enhance recruitment and transmigration of neutrophils, via cell-cell interaction. Oxidised LDL immediately activate platelets, which then rapidly bind to neutrophils, foster their activation and facilitate transmigration through an endothelial monolayer. The observed effects of oxLDL on platelet-neutrophil aggregate formation depended on incubation time, lipoprotein concentration and the degree of oxidative modification of LDL. Platelet-neutrophil aggregates form within minutes following stimulation by oxLDL, and sustain for up to one hour post stimulation, while native LDL are unable to induce platelet-neutrophil interactions. Furthermore, we demonstrate that the PI3K pathway is critically involved in platelet-neutrophil aggregate formation and efficient neutrophil transmigration in response to oxLDL. Consequently, platelets enhance neutrophil transmigration in response to oxLDL and might thereby contribute essentially to the amplification of inflammatory processes within the vessel wall, which fosters the development of atherosclerosis.

Topic: Vascular Biology

P 16 A rapid and simple electrophoretic approach to screen for glutamine deamidation

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Protein deamidation is a posttranslational modification with important implications in physiology and medicine. There is, however, no simple technique for a rapid screening of protein deamidation. The deamidating activity of transglutaminase was applied to establish a simple method for the screen of protein deamidation using recombinant human growth hormone, a rat hippocampal membrane fraction and a cell homogenate enriched in 5-hydroxytryptamine-1A (5HT1A-R) receptor as model systems. Here we report a simple, economic and fast approach to assess protein deamidation by two electrophoretic methods: differential cleavage on SDS-PAGE via in situ V8 protease digestion and the principle of spot shifting via BN-PAGE/2D-SDS-PAGE/immunoblotting.

Topic: Biomedical Engineering

P 17 Der p 11, the mite paramyosin, is an important allergen only in certain geographical areas

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Background: More than 20 allergens have been identified in house dust mites (HDM), but little is known about the clinical relevance of the high molecular weight mite allergens. Objective: To study the importance of the high molecular weight allergen, Der p 11 for HDM allergic patients. Methods: A synthetic gene coding for Der p 11 was expressed in E. coli and rDer p 11 purified to homogeneity. The secondary structure of the protein was determined by circular dichroism analysis and its localization in mites by immunogold electron microscopy. The IgE reactivity of rDer p 11 was tested with sera from HDM allergic patients from Europe and Africa in dot-blot assays and the allergenic activity was evaluated by the up-regulation of CD203c expression. Results: rDer p 11 is a thermally stable, alpha helical protein, which shows homology to paramyosins from invertebrates. The allergen is located in the muscle beneath the skin of mite bodies but not in the feces of mites. The IgE-binding frequency of rDer p 11 (Austria 12%, France 5%, Italy 7% and Sweden 11%) and its allergenic activity were low in European populations. A considerably higher IgE binding frequency of rDer p 11 (36%) was found in Zimbabwe. Conclusion: The low IgE-reactivity and allergenic activity of Der p 11 in European populations indicate that Der p 11 needs not to be included into a vaccine for the treatment of HDM allergic patients in Europe. The importance of Der p 11 for other populations needs further investigations.

Topic: Immunology

P 18 Altered Neural Activation within the Working Memory Network in Remitted Major Depressive Disorder

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Major Depressive Disorder (MDD) is characterized by highly varying severity and course as well as a broad spectrum of clinical features including emotional, vegetative, psychomotor and cognitive deficits. Whereas previous neuroimaging studies repeatedly observed altered activation within the working memory (WM) network in acutely depressed patients, imaging data examining neural correlates of these findings in states of clinical remission are still largely unknown. Hence, we initiated a cross-sectional functional magnetic resonance imaging study with the goal to investigate if WM function and associated neural activation differ between 61 remitted medication-free MDD patients and 84 healthy subjects without any life-time history of psychiatric illness, while performing the classical digit variant of the n-back task. In absence of any significant behavioral differences, elevated activation in extended frontal, parietal and cingulate areas, with punctum maximum in the frontal gyrus, was found in both groups during performance of the 2-back versus 0-back condition. Furthermore, in comparison to the control group, rMDD patients showed increased activity in the left frontal cortex, including inferior and middle frontal gyrus as well as adjacent areas such as the medial frontal and insular cortex (cluster significance $p=0.010$), with the peak of activation differences between the left inferior frontal and precentral gyrus (BA 44; $Z=3.71$, $p<0.001$; $x=-62$, $y=12$, $z=12$). Our preliminary findings of increased WM-related neural activation in rMDD patients in absence of any behavioral differences between the groups resemble previous reports in symptomatic MDD, and indicate that rMDD patients might compensate underlying cognitive deficits by increasing neural processing in order to maintain a comparable level of WM performance. Furthermore, the present results point towards persisting functional alterations within cognitive networks even after a full recovery of depression and withdrawal of antidepressant treatment.

Topic: Neuroscience

P 19 Low biological variation protein characterization by 2D DIGE as new putative Normalization Standards

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Platelets are megakaryocyte-derived anucleated cell fragments which play a major role in hemostasis, inflammation and immunology. They are easily obtained and purified from whole blood and beyond their representative characteristics for the vascular cell system, they are also considered as model for the neuronal cells. Some companies – including "Randox Laboratories" – started trails to develop diagnostic methods based on platelets and many of these rely on protein biomarkers which distinguish with their quantitative presence between biological and pathological conditions. Nevertheless, quantification of a protein biomarker requires reliable normalization proteins (NPs) though there are increasing numbers of publications showing that the expression of "traditional" NPs (eg. GAPDH) can be influenced by certain diseases. This can limit the power of a biomarker; therefore we analyzed the platelets proteome with 2D DIGE for proteins with low variability from a huge (n=204) heterogeneous study population - including healthy young, elderlies and even centenarians, but also patients suffering from either Alzheimer's (AD), Parkinson (PD), Schizophrenia or other dementias. A hit list of proteins with stable expression levels was created by calculating the total variations of 890 different platelet proteins and sorted by their total variations. Selected proteins were subsequently identified by tandem mass spectrometry (MS/MS) fragmentation analysis. Keeping in mind that the biological variation (CVtot) is the difference of the total variance and the technical variance (CVtech ~7% Winkler et al 2008), six proteins out of 890 showed a midget CVbio of only 1-2%. Next to these novel normalisation candidates like CapZ□, Prxn6 and isoforms of 14-3-3, the traditional NPs GAPDH and Tubulin emerge in the midfield of our list. 14-3-3gamma was a top ranked protein (CVtot of 8%) and its normalisation capability was tested on 1D Western Blot where it was superior to GAPDH.

Topic: Vascular Biology

P 20 Comparison of the cross-protection induced by TBEV vaccines

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The Mammalian Tick-borne Virus Group of the genus Flavivirus comprises a European-, Far Eastern- and Siberian Tick-borne encephalitis virus (TBEV) subtype. TBEV is the causative agent of tick-borne encephalitis (TBE), an infection of the central nervous system. The highest TBE incidence worldwide is found in Russia where locally produced as well as Western European vaccines for the prevention of TBE are available. The Western European vaccines are based on the European subtype, while the Russian vaccines are based on Far Eastern subtype viruses. The question of to which extent neutralization capacity of vaccine-induced antibodies, based on the European prototype TBE strain, is effective in protecting against the heterologous Far Eastern virus subtype – and vice versa – has not been answered conclusively. Therefore mice were immunized with TBE vaccines based on European and Far Eastern subtype viruses, and an unbiased hybrid virus test system, i.e. virus consisting of a consensus backbone and carrying the structural proteins (prM, E) of different TBE strains, was used to determine cross-neutralizing antibody titers and cross-protective efficacy. All vaccines tested elicited cross-protective responses against the heterologous strains, similar to those induced against the respective homologous vaccine strains.

Topic: Immunology

P 21 Transmitter release by nicotinic receptors in the spinal cord

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Background and purpose: Nicotine and related substances are potent analgesics, which upon systemic application inhibit pain by acting on spinal cord as well as on the supraspinal structures. The effects are mediated by nicotinic acetylcholine receptors (nAChRs) ligand-gated ion channels made up of 5 identical (homopentameric) or 5 different subunits (heteropentameric). In the nervous system, receptors are assembled from 9α (α2-10) and 3β (β2-4) subunits, which determine their pharmacological and biophysical properties. Though we have made substantial progress in understanding the function of nAChRs in the brain we know little about nAChRs in the spinal cord. This lack of knowledge is a serious shortcoming for the development of new drugs that target distinct receptors for an efficient relief of pain. Experimental approach: In the proposed project I will focus on the release of [3H]-labeled transmitter from synaptosomes and slices upon activation of presynaptic nAChRs. All principal neurotransmitters have been implicated in the processing of pain in the spinal cord therefore I will test different neurotransmitter systems. The experiments will be performed on wild type mice and on mice lacking distinct nAChR subunit genes in order to identify the subtype of nAChRs which is involved in mediating transmitter release. Progress and first results: For the first experiments in spinal cord synaptosomes we used [3H]-MPP+ (1-methyl-4-phenylpyridinium) as substrate for labeling the monoamine neurotransmitter system. Nicotine-evoked outflow of [3H]-MPP+ was detectable, however much smaller than in synaptosomes from brain regions. In order to improve the results we are currently developing a method to isolate and purify synaptosomes using a discountinous Percoll gradient. Preliminary experiments using purified synaptosomes are promising.
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Topic: Neuroscience

P 22 Characterization of the inflammatory response to solid cancer metastases in the human brain

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Background: New immunomodulatory agents, like ipilimumab, showed promising activity in brain metastases (BM). However, little is known about the inflammatory response in BM and new insights are needed to further guide the development of treatment strategies. Methods: We investigated 17 human autoptic tissue specimens of BM from breast cancer (n=3), non-small cell lung cancer (NSCLC; n=5), small cell lung cancer (SCLC; n=3) and melanoma (n=6). Immunohistochemical staining for a comprehensive panel of 21 inflammation-associated markers was performed. Results were quantified by manual counting of the various cell populations in three areas of 0.5 mm^2 (intratumoral, peritumoral, control region). Results: Profound microglia activation with marked peritumoral accumulation and some intratumoral infiltration of HLA-DR-positive microglia/macrophages was found. A high proportion of these cells showed strong immunoreactivity for phagocytosis associated markers and MHC class 1, while a smaller subgroup of cells expressed molecules involved in radical production (inducible nitric oxide synthase or NADPH oxidases). Only few B- and T-lymphocytes were observed in and around BM. The number of CD8-positive T-cells was not correlated to MHC class 1 expression on tumor cells. Melanoma BM had significantly less accumulation of peritumoral microglia than NSCLC BM. The inflammatory pattern was independent from treatment of patients with glucocorticoids or radiation. Conclusions: Inflammatory reaction to BM is mainly characterized by activation of microglia/macrophages and shows pronounced upregulation of markers involved in phagocytosis, but seems to be insufficient in activating T-cell response. Treatment strategies aimed at activating specific immunity may potentiate immune attack on tumor cells.

Topic: Clinical Neurosciences

P 23 Folding of G-Protein Coupled Receptors: The Adenosine-A2A/Molecular Chaperone Connection

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The A2A adenosine receptor is a prototypical G protein coupled receptor. It is expressed in a wide variety of cells including as different types as neurons, platelets, cells of the immune system and muscle. An outstanding structural feature of the A2A-receptor is its extended carboxy-terminus made up of a peptide of more than 120 amino acids; its flexibility is unrestrained due to the lack of a membrane-proximal cystein residue that can be modified by palmitoylation. Heterologous overexpression of the A2A adenosine receptor in various cell types results in the intracellular retention of a significant portion of the receptor protein indicating that folding intermediates of the native receptor are recognized by executors of cellular quality control. Using the TAP nano-LC-MS/MS approach we identified a number of candidate interaction partners, several of which belong to the class of molecular chaperones. Focused on Hsp90 alpha, we were able to verify its interaction with the A2A-receptor. Furthermore, inhibition of the chaperone led to increased surface expression of the receptor. Chaperones are the key in controlling the fate of retained receptors; tight interaction may direct proteins to degradation; if eventually released, proteins can be exported suggesting that chaperone molecules be exchanged for the cargo acceptor of the coat protein complex-II (COPII).

Topic: Molecular Signal Transduction

P 24 Resonance frequency analysis: A pilot study on a new diagnostic tool for dental ankylosis

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Ankylosed teeth are considered in orthodontic treatment planning; however, diagnostic tools to quantify the rigidity of the tooth-to-bone connection are rare. Resonance frequency analysis (RFA) can quantify the rigidity of the dental implant-to-bone connection and may thus serve as a potential diagnostic tool to identify ankylosed teeth. To test this assumption, we examined 15 and 30 primary mandibular molars with and without clinical signs of ankylosis by the Osstell™ mentor RFA system. A cut-off implant stability quotient (ISQ) of 43 provided a specificity of 100% and a sensitivity of 53.3% when measured in mesio-distal direction or 20% when measured in bucco-lingual direction. Based on a receiver operating characteristic (ROC), the area under the curve (AUC) of 0.807 showed the mesio-distal direction of measurement to be a test of moderate discriminatory power. Given its non-invasiveness, RFA may serve as a quantitative diagnostic supplement to the clinical examination of potentially ankylosed primary molars.

Topic: Regeneration of Bones and Joints

P 25 Identification of transient PP2A.RTS1-Substrate interactions by a method called M-TRACK

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Protein phosphatase 2A (PP2A) is a phospho-Ser/Thr phosphatase and a typical PP2A holoenzyme consists of a structural A subunit, a catalytic C subunit and a variable, regulatory B subunit, which provides substrate specificity and intracellular localization to the holoenzyme. In yeast 2 functionally non redundant B subunits exist - RTS1 and CDC55. RTS1 is a component of the spindle position checkpoint, regulates sister chromatid cohesion in meiosis, septin dynamics and is involved in global stress response. Its substrates remain elusive due to the transient nature of PP2A-substrate interactions which are difficult to be detected by available methods. In order to study these short-lived interactions we have devised a 2-hybrid method called Methyl-TRACKing (M-TRACK), where the bait consists of a human histone lysine 9 methyltransferase (HKMT) fused to RTS1 and the prey consists of a potential substrate of RTS1 fused to the histone 3 N-terminus (H3 tag). Upon interaction of RTS1 with a potential substrate, the HKMT methylates the lysine K9 on the H3 tag, which can then be identified by methylation-specific monoclonal antibodies. The in vivo functionality and the ability of HKMT-RTS1 to form an active PP2A holoenzyme have been confirmed. Using M-TRACK, we have detected a previously undetected direct physical interaction between RTS1 and one of its putative substrates, KIN4. In control experiments we found that H3-HA-KIN4 methylation was mostly driven by the affinity between RTS1 and KIN4, with a minor contribution of HKMT's affinity for the H3 tag. KIN4 is a specific RTS1 substrate as the other B-subunit, CDC55 failed to detect it in an M-TRACK assay. We also compared the detection abilities and efficiencies of N- versus C-terminally fused RTS1 and KIN4 proteins which revealed that the N - or C-terminal position of the HKMT or H3 tag can influence detection by M-TRACK. Now with proper controls at hand, we are validating other potential RTS1 substrates by M-TRACK.

Topic: Molecular Mechanisms of Cell Signaling at the MFPL

P 26 Dissection of flavivirus entry and assembly by structure-based mutational analysis

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Flaviviruses enter cells via receptor-mediated endocytosis and low-pH-induced membrane fusion, which is mediated by the major surface protein E. Upon exposure of the virion to mildly acidic pH in endocytotic vesicles, E rearranges from the pre-fusion homodimeric state to homotrimers thereby mediating membrane fusion. By the use of non-infectious recombinant subviral particles (RSPs) of tick-borne encephalitis virus (TBEV), it was shown that the low-pH-dependent initiation of fusion is dependent on the protonation of conserved histidine residues at a domain interface in E whereas later steps of the fusion process involve interactions of the transmembrane domains (TMDs) of E. In addition, interactions of TMDs have been hypothesized to be important for the assembly of infectious virus particles. Since RSPs differ in particle geometry, we attempted to study the role of conserved histidines of E and TMDs of all structural proteins in the context of whole infectious TBE virus. For this purpose, we targeted 5 histidines of E that are conserved among all flaviviruses and tested their effect as pH sensors during flavivirus entry and fusion. To investigate the role of TMDs for entry and assembly, we generated chimeric transmembrane anchor mutated TBE viruses, containing different combinations of heterologous Japanese encephalitis virus (JEV) TM segments in their viral proteins. The generated mutants displayed significant reduced specific infectivities compared to wildtype, which may be related to defects in the entry process and specifically membrane fusion. Currently, we investigate whether the reduced infectivity can indeed be ascribed to fusion defects or due to an impairment of other stages of the life cycle, such as virus assembly.

Topic: Immunology

P 27 Evaluation of primary meniscus refixation using different magnet resonance sequences – a prospective, clinical cohort study

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Meniscal tears are commun injuries of the knee joint. The salvage of the meniscus is getting more and more important because of the rising number of osteoarthritis after meniscectomy/partial meniscectomy. In this study all patients between 19 and 59 years with a meniscal tear that was treated operatively with meniscus refixation at the Departement of Trauma Surgery, at the University Hospital of Vienna, Medical University of Vienna, General Hospital of Vienna, between March 2012 and March 2013 will be included. A magnet resonance imaging (7 tesla) using different magnet resonance sequences will be performed 3, 6 and 12 months postoperatively. Additionally, there will be a clinical examination and an interview using a questionnaire at these three time points. A control group of 10 probands will be examined with a magnet resonance imaging (7 tesla) with the same magnet resonance sequences one time only. The objectives of the study will concentrate on the evaluation of the healing success and the differences between clinical and radiological outcome after primary meniscus refixation, as well as the various imaging of the meniscus in the different magnet resonance sequences.

Topic: Regeneration of Bones and Joints

P 28 Inquiring about avian thymic dendritic cells

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Avian thymic dendritic cells histologically are not yet identified, but it is reasonable to assume their existence. Although we have several dendritic cell markers (CD205, 74.3) which identify thymic cells but the distribution and histological appearance of these positive cells showed a high rate similarity thymic epithelial cells. This immunocytochemical observation initiated the introduction of anti-keratin staining into the inquiry of thymic dendritic cells. The distribution of cortical epithelial cells justified the findings obtained by CD205 and 74.3 monoclonal antibodies. Double immunofluorescence staining provided circumstancial evidence for the colocalization of CD205 and 74.3 with the anti-keratin staining. Suprisingly, in the medullary area of the thymus the anti-keratin and the anti- dendritic cell staining are not colocalized, suggesting that in the medulla the CD205 and the 74.3 mAbs recognize a keratin-free dendritic-like cell popoulation. These statements indicate a cytological - and consequently functional - differences between cortical and medullary thymic epithelial cells. Transmission electronmicroscopy of the chicken thymus proved, that the cortical epithelial cells produce cytoplasmic granules, which could be the target of CD205 and 74.3 mAbs. Chick-quail interspecific chimeras confirmed the immunohistochemical data and provided first time an experimental evidence, that thymic cortical epithelial cells and dendritic cells share common intracytoplasmic antigenic epitop (s), which were recognized by CD205 and 74.3 monoclonal antibodies.

Topic: Immunology

P 29 Correlation between Ureaplasma biovars detected by real-time PCR from a single vaginal smear and preterm delivery: preliminary results

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Background and aims: Although numerous studies have associated Ureaplasma spp with preterm delivery and adverse outcome for preterm infants, the proof of a causal relation between vaginal isolation of Ureaplasma species and adverse pregnancy outcome is missing. We hypothesize that it is important to differentiate between Ureaplasma biovars with potentially high and low pathogenicity and that pregnant women with isolation of vaginal Ureaplasma parvum (Biovar 1) are at increased risk for preterm delivery compared to women with isolation of Ureaplasma urealyticum (Biovar 2) or negative results. We report on preliminary results of our ongoing multicenter study. Methods: Vaginal swabs are obtained during routine nuchal translucency screening and are analyzed for Ureaplasma biovars by real-time PCR. Therefore a multiplex assay was established. PCR results are correlated with pregnancy outcome. It is planned to include 4000 pregnant women in the study. Results: Until March 2012, PCR results were available from 2225 women. 1210 swabs revealed negative PCR results. Ureaplasma parvum was found in 900 (40,45%) whereas Ureaplasma urealyticum was found in 170 (7,64%) women. 55 women had a concurrent infection with Ureaplasma parvum and Ureaplasma urealyticum. Pregnancy outcome is available for 886 women. Preterm delivery occurred in 66 (13,7%) pregnancies with negative culture results, in 55 (15,10%) pregnancies with isolation of Ureaplasma parvum and in 10 (14,7%) pregnancies with isolation of Ureaplasma urealyticum (p>0,05). Conclusions: These preliminary data show no statistically significant correlation between rates of preterm delivery and isolation of Ureaplasma biovars in vaginal swabs during first-trimester pregnancy.

Topic: Immunology

P 30 Separation of protein complexes in chronic inflammatory diseases (Juvenile Idiopathic Arthritis) by means of Blue-Native PAGE

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Background: Early diagnosis of juvenile idiopathic arthritis (JIA) remains a challenge for pediatricians. Different electrophoretic methods were compared in this study for their application on serum proteins. Thus, sera from JIA patients were compared to age matched controls in order to find a useful biomarker. Serum samples are mostly analyzed by means of reducing electrophoresis combined with western blotting or MS. By being reduced to their subunits, the higher molecular weight complexes are lost. Since reactive immunoglobulins as well as specific immunocomplexes take both part in the pathogenesis of JIA, the detection, isolation and further analysis of these immunocomplexes is of great importance for the understanding of the disease. Methods: Serum proteins were separated using blue-native PAGE (BN-PAGE), a mild, non-reducing, native electrophoretic method. This led to a high resolution separation even of very high molecular weight protein complexes contained in human serum. This native separation was followed by reducing SDS-PAGE for a further identification of the complexes. Different patients, stages of the disease as well as medications were taken into account. Results: Using the BN-PAGE, a distinct pattern of different protein complexes of high molecular weight could be distinguished in the sera of patients compared to the control group. Moreover, several groups of complex patterns could be observed which could roughly be correlated to clinical manifestations of the disease. The relevant molecular weight range of the observed protein complexes extended from 200kDa to 1200kDa. Thus, they have not been able to be separated sufficiently by SDS-PAGE, which is mainly suited for proteins of up to 250kDa. The new BN-PAGE also allowed tracking of the medication effect on the protein complexes present in the serum. Conclusion: The BN-PAGE seems to be an adequate method for the separation and detection of immunocomplexes apparent in the autoimmune disease JIA.

Topic: Immunology

P 31 Insulin like growth factor binding protein 7 (IGFBP7) is downregulated in multiple myeloma with consequences for myeloma cell growth and bone disease

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Multiple myeloma (MM) is characterized by the clonal accumulation of plasma cells (PCs) in the bone marrow (BM) microenvironment. Similar to long-lived PCs, MM cells reside in the BM where they receive essential survival and growth signals from the surrounding environment. In this context, bone morphogenic proteins (BMPs) were previously shown to play a role in MM. Since there is little information so far about the role of BMP antagonists in myeloma, we sought to analyze the pattern of BMP antagonist expression in MM. In initial experiments we identified insulin like growth factor binding protein 7 (IGFBP7) as a factor with potential relevance in MM. IGFBP7 expression was significantly downregulated in a large set of MGUS and MM patient samples as well as MM cell lines (MMCLs) compared to normal PCs. We therefore aimed to further characterize the role of IGFBP7 in the pathophysiology of MM. Treatment with recombinant IGFBP7 decreased viable cell numbers in 7/7 MMCLs tested. This effect was due to an impairment of proliferation, but not linked to apoptosis and independent of MM growth factor signaling via insulin and IGF-1. Analyzing the mechanism of IGFBP7 silencing in MM demonstrated an upregulation after treatment with 5-aza-2´ and TSA in 5/7 MMCLs, suggesting that IGFBP7 is controlled via methylation. Studying the role of IGFBP7 in the MM microenvironment we observed a significant downregulation of IGFBP7 in BM stromal cells (BMSCs) after co-culture with 4/5 MMCLs. Moreover, IGFBP7 expression increased during osteogenesis and treatment with recIGFBP7 further stimulated osteoblast (OB) activity, suggesting that downregulation of IGFBP7 in BMSCs might contribute to the lack of functional OBs in myeloma. Taken together, these results demonstrate that IGFBP7 is downregulated in MM probably by methylation, which enhances MM cell growth and contributes to MM bone disease. Upregulation of IGFBP7 might be a useful therapeutic intervention in the treatment of MM.

Topic: Clinical Experimental Oncology

P 32 Late toxicity after primary external beam radiation therapy in prostate cancer

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Aim: Late toxicity after primary external beam radiation therapy in prostate cancer are usually reported by actuarial rates. Aim of this study was to show that important aspects in reporting side-effects remain unconsidered. Material and Methods: All 178 patients with primary localized prostate cancer that were treated at the Medical University of Vienna – Department for Radiotherapy within the Austrian-German Multicenter trial were included in this analysis. Treatment consisted of 3D-conformal external beam radiotherapy (EBRT) with a local dose of 70Gy for low and intermediate risk and 74Gy for high risk patients respectively. Gastrointestinal (GI) and genitourinary (GU) late-toxicity were prospectively assessed by the use of EORTC/RTOG protocol. Development in time-course, maximum side-effects, prevalence and actuarial incidence of GI and GU toxicity were recorded and evaluated. Results: Mean follow-up was 74 months. GI and GU toxicity (EORTC/RTOG grade ≥ 2) were documented in 15% (27/178) and 22% (40/178) of cases. The corresponding 5-year actuarial rate (incidence) of GI and GU toxicity was 19% and 23%. As opposed to this prevalence of GI and GU toxicity after 5 years was 1-2% and 2-7%. 100% (27/27) and 85% (24/40) of all GI and GU toxicity occurred within 5 years and lasted less than 3 years in 90% (GI) and 98 (GU) of cases. Conclusion: This report showed that the majority of GI and GU toxicity after primary EBRT are of reversible character. The exclusive report of actuarial rates (incidence) leads consequently to misinterpretation or overestimation of late-toxicity.

Topic: Other

P 33 Cross-linked iron oxide particles - synthesis and visualization

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The purpose of our scientific effort was to create cross-linked iron oxide nanoparticles, with a predefined narrow size distribution. The cross-linked carbonhydrate coating gives stability to cross diffusion barriers, allows surface modification and 3D visualization. To provide a monodisperse solution of the dextran a new method was introduced to pulverize dextran. Nitrogen blasts with a pressure of up to 4 bars were used to carry the dextran molecules into the vigorously stirred water solution. The particles were synthesized within participation of Fe^{2+},Fe^{3+} (ratio 1:1) under dextran excess (Dextran:Fe ratio 35:1). Epichlorohydrin was used for cross-linking. For electron tomography particles were covered with FITCs. The acquisition of the tilt series was performed using a Transmission Electron Microscope. Using the advanced dissolving protocol it was possible to create a single-peak distributed dextran solution with a mean size of 4.519 nm vs. multi-peak with 834.3 nm when the dextran was added stepwise. The core of our particles was 4.02 nm and the overall size 37.12nm. After the cross-linking the hydrodynamic size raised to 49.56 nm. 17 FITC molecules were connected per particle. 3D models of the particles were created from the electron tomography measurements. The dextran coating size was 48.4 nm and verified the DLS measurements. Additionally we were able to detect a zone around the dextran coating with a size of 100.2 nm. We interpret it as a "hydration zone", where single chains of dextran have a high grade of interaction with the surrounding fluid. Within this study, it was possible to create an aminated iron oxide contrast agent. The particles show a narrow distribution. The cross-linking and labeling was efficient and in addition it allowed to visualize the dextran core. An outer hydration zone of the particles was detected, which is probably responsible for the interaction with the surroundings.

Topic: Other

P 34 The Arterial Supply of the Long Head of the Biceps Tendon with its Clinical Implications on Superior Labral Anterior to Posterior (SLAP) Lesions

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Background: Arthroscopic repair of SLAP lesions is often accompanied by a prolonged period of pain during the rehabilitation process. Purpose: The purpose of this anatomical and radiological study is to reinvestigate the arterial supply of the long head of the biceps tendon (LHBT) in terms of a possibly compromised arterial supply by suture anchors used in SLAP repair. Study Design: Descriptive laboratory study. Methods: The anatomic part of the study was performed on 20 human formalin fixed bodies of both sexes. The anterior circumflex humeral artery (ACHA) was located and followed into the intertubercular groove until it reached the fibrous capsule of the shoulder joint. The radiological part of the study was performed on 10 fresh frozen anatomic specimens of upper extremities with equal gender distribution. After injection of contrast agent into the axillary artery a 3D scan was performed and a series of axial cross-sections was taken. By post processing, Maximum Intensity Projection (MIP) reconstructions were generated in different planes as appropriate. Results: All anatomic dissections and radiologic investigations revealed that the proximal part of the tendon of the long head of the biceps brachii muscle received its arterial supply constantly by an ascending branch of the ACHA. There was no reasonable artery supplying the anchor of the LHBT from proximal. Conclusion: Suture anchors used for anatomic reconstruction of SLAP lesions might compromise the complex network of arterioles from the osteotendinous junction but might not obstruct a specific vessel. Clinical Relevance: Various collaterals at the bony origin of the LHBT might be sufficient enough to allow a proper healing of the lesion after SLAP repair. From a functional anatomic point of view the prolonged period of pain might therefore be rather of neuronal origin. Key words: biceps tendon, arterial supply, anterior circumflex humeral artery, SLAP lesion.

Topic: Regeneration of Bones and Joints

P 35 Tools for Assessing Protein Regulation Dynamics from Quantitative Mass Spectrometry Data

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INTRODUCTION: High-throughput quantitative proteomics is able to quantify huge numbers of proteins and posttranslationally modified peptides across multiple samples. Robust statistical models, tested and adapted for proteomics data, can provide adequate answers to questions of protein and PTM regulation. We developed the isobar software library to address the fundamental needs of assessing significance of variation and generating clear and comprehensive user reports for iTRAQ and TMT experiments performed on any MS platform. METHODS: We published statistical models for isobarically tagged proteomics data and extended them the level of modified peptides. All models are implemented in a software which is part of the Bioconductor platform that has emerged as the standard in bioinformatics. Scripts were developed to ensure that reports can be easily generated and automated to provide sensible data for further analysis. The isobar package provides parsers for the most common file formats to read database search results and mass lists (Mascot and Phenyx; MzIdentML and Rockerbox in development). RESULTS: Without much prior R knowledge, analysis reports can be generated to contain quantitation and important information on the identified proteins and sites of modifications. Using resources such as PhosphoSite Plus we annotate reported and unreported sites of phosphorylation, acetylation and methylation. Exploration in R is facilitated by representation of the data in S4 classes and methods. Localization of modification sites in the peptide sequence is facilitated by the integration of Delta Score and PhosphoRS. CONCLUSIONS: We developed a toolkit which is based on robust and precise statistical models of quantitative proteomics data and makes them accessible also for users with minimum R knowledge to allow detection of protein and modification regulation in a reliable and easy manner. Available software for modification localization and public data sources are used to make better reports and thus enable to draw conclusions quicker.

Topic: Medical Informatics, Biostatistics and Complex Systems

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P 36 CDK4 and 6 as therapeutic targets in human melanoma - just redundant proteins?

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The cyclin dependant kinases (CDKs) are proteins that promote the progression of a cell through the cell cycle. CDK4 and CDK6 were mostly seen as redundant proteins but recent evidence points to independent and important functions for CDK6 in various experimental models. The aim of this study was to identify the specific contribution of CDK4 and CDK6 on growth and invasiveness of melanoma in vitro and to explore a possible role for inhibition by the specific CDK4/6 inhibitor PD0332991 as therapeutic targets for melanoma. We first tested the influence of increasing concentration of PD0332991 on growth (MTS assays) and viability of 518A2 (BRAFV600E), M24 (NRAS Q61R) and SKMel28 (BRAF V600E and CDK4 R24C) melanoma cells in vitro. We observed IC50 values at approximately 11nM after 24 and 48 hours, and a growth stop at ~10nM PD033299. After treatment with si- or shRNAs we show a significant downregulation of the protein expression of CDK4 or CDK6, respectively. siRNA treatment led to a significant decrease in cell proliferation after downregulation of each CDK indicating that the loss of one of these G1-kinases is not compensated by the other, as has been indicated in the literature. Furthermore, the migration of cells in scratch assays as well as in transmigration assays was clearly reduced. We believe that CDK4 as well as CDK6 independently, act as important factors in malignant melanoma as shown by our data. Downregulation of CDK4 or CDK6 protein expression resulted in a reduced proliferation and migration in melanoma. Further validation in a human melanoma xenotransplantation mouse model using shRNA transduced melanoma cells, as well as the effect of PD033299 on melanoma in vivo, is being performed. Promising preliminary results of these experiments implicate a possible role of available CDK4/6 inhibitors as a possible treatment strategy for metastatic melanoma.

Topic: Malignant Diseases

P 37 Novel adhesion molecules involved in lymph node metastasis

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Metastases of carcinomas form initially in regional lymph nodes and subsequently in distant organs. The first tumor cells residing in the lymph node form small aggregates in the marginal lymph node sinus and are attached to subcapsular sinus lining endothelial cells. However, the mechanisms by which the intravascular tumor cells select a site in the lymph node for their nidation to form the core for a future metastasis are currently unknown. During the process of tumorigenesis, the specialized sinus lining endothelial cells with a distinct molecular signature from that of blood and lymphatic vessels are transformed into genuine lymphatic endothelial cells that express essential phenotypic lymphatic „markers". We aim to test the hypothesis that the adherence of disseminating carcinoma cells to the subcapsular sinus is a regulated process that involves interaction of tumor-induced lymphatic endothelial surface molecules and as yet unknown interaction partners on carcinoma cells.

Topic: Cell Communication in Health and Disease

P 38 Angiogenesis is challenged in experimental brain metastases

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It is widely accepted that angiogenesis plays an important role in the maintenance of tumor growth. Tumors can acquire their vasculature e.g. by vessel sprouting or inducing intussusceptive microvascular growth. These processes require fibronectin and fibrin containing collagenous matrix. Since collagen I is absent in the brain parenchyma with the exception of the wall of arteries, the significance of these types of angiogenesis during vascularization of brain metastases is questionable. Our aim was to analyze the vascularization of experimental metastases following direct injection of five different tumor lines to the brain parenchyma of mice. Morphometric analysis was performed on methanol fixed frozen sections following various immunofluorescent labeling. No angiogenesis was observed in the peritumoral zone of the lesions. Tumors acquired their vasculature merely by incorporating the host vessels. Incorporated vessels retained their normal structure except that astrocyte foot processes were replaced by the tumor cells. Tumors of epithelial origin showing pushing growth pattern had lower vessel density and elevated vascular cell proliferation, compared to tumors showing invasive growth. A process remarkably similar to intussusceptive angiogenesis was observed in the brain metastases of the fibrosarcoma cell line. Tumor cells attached to the vessel caused the vessel lumen to split, and the pillars formed were filled by tumor cells. However, branching angiogenesis was observed neither in the tumorous lesions nor in the control cerebral wounds. These data suggest that under experimental conditions no sprouting angiogenesis is needed for the incipient growth of metastatic cerebral tumors. According to our results metastases acquire their vasculature exclusively by vessel incorporation in the mouse brain. This phenomenon may also be valid for small metastases in the human brain. Under these conditions revision may needed considering the use of antiangiogenic agents.

Topic: Tumorbiology - Oncology

P 39 The enhancement of osteogenesis through the use of dental pulp pluripotent stem cells in 3D

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The potential for osteogenic differentiation of dental pulp mesenchymal stem cells (DPMSCs) in vitro and in vivo has been well documented in a variety of studies. Previously, we obtained a population of cells from human dental pulp called dental pulp pluripotent stem cells (DPPSCs) that could differentiate into mesodermal, ectodermal and endodermal progenies. We compared the osteogenic capacity of DPPSCs and DPMSCs that had been isolated from the same donors (N=5) and cultivated in the same osteogenic medium in 3D (three dimensions) Cell Carrier glass scaffolds. We also compared the architecture of bone-like tissue obtained from DPPSCs and human maxillary bone tissue. Differentiation was evaluated by scanning electron microscopy, whereas the expression of bone markers such as ALP, Osteocalcin, COLL1 and Osteonectin was investigated by quantitative real time polymerase chain reaction (qRT-PCR). We also used calcium quantification, Alizarin red staining and alkaline phosphatase (ALP) activity to compare the two cell types. New bone tissue formed by DPPSCs was in perfect continuity with the trabecular host bone structure, and the restored bone network demonstrated high interconnectivity. Significant differences between DPPSCs and DPMSCs were observed for the expression of bone markers, calcium deposition and ALP activity during osteogenic differentiation; these criteria were higher for DPPSCs than DPMSCs. This study demonstrates the stability and potential for the use of DPPSCs in bone tissue engineering applications.

Topic: Regeneration of Bones and Joints

P 40 A Lab-on-a-Chip for Cell-Based Assays: Continuous and Label-Free Monitoring of Human Fibroblasts

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Today, cell-based assays are a widely used tool in biological, pharmaceutical and medical research. Despite their wide use and growing market, still some problems are associated with cell-based assays. For example the majority of assays is performed as end-point tests. Consequently, it is difficult and costly to obtain a good time resolution. Since interactions of cells with their environment are complex and typically kinetics are important, monitoring cell behavior over time is often necessary. In an attempt to advance cell-based assays, we develop a cell measurement station capable of continuously and non-invasively monitoring ex vivo living cells. The platform comprises of a microfluidic cell cultivation chamber with integrated electrodes for dielectric spectroscopy, a data acquisition board and external heating and pumping facilities. Human cells growing in the cell-chip are monitored by applying AC of different frequencies (100kHz-20MHz) to the electrodes while the corresponding impedance values are recorded. A passivation layer prevents electrode reactions and current flow through the cells. In order to handle the large amount of recorded data, statistical data analysis methods such as partial least squares models are applied. We have characterized the system using different human cell types and successfully monitored cell behavior such as cell adhesion, growth and apoptosis. We could also identify cell responses to serum starvation, inflammatory cytokines (IL1Î², TNFÎ±) or the Î²-adrenergic receptors agonist isoproterenol. In a next set of experiments the platform will be used to monitor human synovial fibroblasts, which are key players in chronic polyarthritis. Changes of their morphology, cytoskeleton and adhesion properties will be investigated on different substrates and in the presence and absence of inflammatory agents. In addition to dielectric spectroscopy standard cell-based assays will be performed to clearly identify and distinguish cell responses.

Topic: Molecular Signal Transduction

P 41 The roles of serotonin (5HT) receptors in pain sensation

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Nociceptors are primary sensory neurons which are excited by mechanical, thermal, and chemical stimuli. Any tissue injury or inflammation excites nociceptive terminals via mediators such as nucleotides, extracellular protons, bradykinin, nerve growth factor and serotonin (5-HT). These mediators act on sensory neurons via different receptors and signalling pathways. 5-HT is believed to excite sensory neurons via ionotropic 5-HT3 receptors (Julius & Basbaum, Nature 413, 203; 2001), but the role of G protein coupled 5-HT-receptors remained controversial if not enigmatic. To investigate the role of 5-HT-receptors in nociception, dorsal root ganglion neurons (DRG) in primary cell culture were subjected to patch-clamp measurements. In current clamp, such neurons fired 2.6 + 0.92 action potentials in response to 5 consecutive currents injections (0.1 to 0.5 nA; 2 s each; n = 5). The number of elicited action potentials rose to 36.14 + 8.73 in the presence of 10 µM 5-HT (n = 11) and to 25.33 + 4.16 in the presence of 100 µM 5-HT (n = 3). However, in current clamp 10 µM or 100 µM 5-HT failed to cause significant inward currents (69.72 + 25.45 pA and 63.40 + 9.11 pA, respectively), whereas 10µM ATP elicited currents of 606.67 + 147.76 pA (n = 9). Thus, 5-HT did not act via ionotropic receptors (i.e. 5-HT3). The excitatory effect of 10 µM 5-HT was not altered in the presence of the 5-HT3 receptor antagonist tropisetron (30nM), but significantly reduced by the 5-HT2 antagonist ritanserin (10µM). The 5-HT2 agonist 2,5-dimethoxy-4-iodoamphetamine (DOI, 100 µM) also increased action potential firing. An inhibition of Kv7 channels is known to increase the excitability of DRG neurons (Liu et al, JCI 120; 2010) and DOI reduced currents through these channels in a concentration-dependent manner. Thus, 5-HT excites DRG neurons via 5-HT2 rather than 5-HT3 receptors and most likely via an inhibition of Kv7 channels.

Topic: Molecular Signal Transduction

P 42 Ambulatory arterial stiffness index in renal transplant children – Cross sectional study

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Cardiovascular (CV) mortality is four fold higher in renal transplant (Rtx) children compared to the healthy population. In pediatrics hard CV end points are lacking, thus subclinical organ damage as an intermediary end point should be assessed. Ambulatory arterial stiffness index (AASI) is known as surrogate CV marker in adults. Our aim was to establish the factors influencing AASI and its possible relationship to central arterial elasticity. 54 Rtx children (15.5±3.53 years, 38 males) were investigated. AASI was calculated from the results of 24 hour ambulatory blood pressure monitoring (ABPM). Central pulse wave velocity (PWV) was measured by applanation tonometry. and Body composition analysis was done and laboratory values were taken. SD scores (SDS) were calculated. In 54 Rtx, AASI correlated significantly with BMI SDS, SBP SDS (systolic blood pressure, whole period of ABPM), pulse pressure (PP), ECW/TBW and diastolic blood pressure fall (DBPF). (r=0.39; 0.36; 0.49; 0.48; -0.51; respectively p<0.007). By multiple regression analysis, DBPF proved to be the main predictor of AASI. Children diagnosed as hypertensive (n=34) had higher AASI (0.459±0.15 vs. 0.297±0.15) BNP (72.41±72.91 vs. 34.26±25.15), BMI SDS (0.87±1.33 vs. 0.17±0.64), ECW/TBW (0.38±0.01 vs. 0.37±0.01) and they spent longer time on dialysis (7.2±5.3 vs. 11.4±4.13) (p<0.03), but their PWV SDS did not differ from normotensive patients. PWV did not show a relation to AASI. AASI seems to characterize the actual volume and pressure dependent arterial rigidity rather than long term morphological changes of large arteries – as reflected by PWV - in RTX children. Thus, cardiovascular morbidity may be reduced by adequate blood pressure lowering therapy, prevention of obesity and early kidney transplantation. Randomized clinical trials are needed to assess the value of AASI as an intermediary end-point in cardiovascular risk prediction among children after renal transplantation.

Topic: Cardiovascular and Pulmonary Disease

P 43 Treatment of mallet fractures by K-wire extension block technique - a biomechanical comparison of 4 different methods

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Introduction: Mallet deformity of the finger involves avulsion of the extensor tendon from the base of the distal phalanx with a bony fragment. In 1988 Ishiguro first described a new method for operative treatment of mallet finger fractures. It is characterized by closed reduction, extension block pinning and temporary arthrodesis. A few years later, the first modifications of this newly technique were published. The biomechanical principle of the extension block remained, but the used number and way of placement of the K-wires and the fixed position of the distal interphalangeal (DIP) joint changed. Between 2005 and 2011 289 patients with the diagnosis „mallet fracture" were seen in our ambulance. 62 (21%) of them received operative treatment. During this period the proportion of patients treated with the K-wire extension block technique raised. The clinical outcome of the extension block technique in general is slightly better compared to other operative techniques. But up to now there are no biomechanical studies concerning the objective stability of K-wire extension block technique, nor is there any comparison of the mechanical stability of the different modifications which are currently applied. Material and Methods: The original „Ishiguro technique" will be compared to three modifications. The first modification is characterized by changing the insertion-angle of the two K-wires, with the DIP-joint fixed in extension. In the second modification two parallel K-wires are used as extension-pins, while in the third the bony fragment is fixed with an additional K-wire placed right trough the bony fragment.The exact study protocol is being developed. It is planned to compare the mechanical stability of the different methods by repetitive tension and/or evaluation of the tensile failure load. Results and Conclusion In progress.

Topic: Regeneration of Bones and Join

P 44 A Spatio-Temporal Latent Atlas for Fetal Brain Segmentation

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PURPOSE The emergence of novel imaging methods, such as ultra-fast Magnetic Resonance Imaging (MRI), allows the acquisition of high-resolution in utero images. Due to the fact that clinicians and researcher\'s assessment is currently performed qualitatively, there is a need for a quantification of developmental characteristics, and its variability in the population. In this work, we present a spatio-temporal latent atlas that is learnt from a single annotated example and a large number of non-annotated examples. This atlas captures the development of a cerebral structure in healthy fetuses during the 20th-30th gestational week. METHOD AND MATERIALS We perform a spatio-temporal group-wise segmentation of fetal brain structures given a single annotated example. The method is based on a spatio-temporal latent atlas capturing age-dependent characteristics in the training population which aids brain structure segmentation. The emerging atlas segments subcortical structures by integrating information across large number of subjects. It encodes the average development and its variability relevant for diagnosis. RESULTS Experiments show that our proposed spatio-temporal latent (ST) atlas outperforms an existing atlas (AVG) approach without age specificity, since it learns the time dependent shape of the structure during segmentation. Although the segmentation accuracy improves (Dice score ST/AVG atlas: 0.48/0.44), we expect further improvement by including a larger training sample, and more accurate non-rigid initial registration. CONCLUSION We propose a probabilistic spatio-temporal latent atlas for the segmentation of fetal brain structures during early development. From a single annotated example we learn an atlas and segmentations for a set of images. The benefits of the atlas are: 1. It serves as prior during segmentation of large numbers of structures that undergo development. 2. The atlas itself is informative regarding the developmental process.

Topic: Medical Physics

P 45 The role of BMP antagonists in human fracture healing

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Bone Morphogenetic Proteins (BMPs) are known to play an important role in normal bone healing by recruiting bone-forming cells to the area of trauma. Each BMP has a unique role in fracture healing, with specific BMP concentrations occurring at different periods of tissue recovery. It is the complicated balance of BMPs and BMP antagonists that may serve as one intrinsic source for the development of pathologic fracture healing. Current research is dominated by BMP investigation, BMP-2 (Infuse, Fa. Medtronic) and BMP-7 (OP1, Fa. Stryker) are already available for commercial clinical use. Manipulation of naturally occurring BMP inhibitors may offer a promising technique to optimising fracture healing. BMP antagonists can be categorized as (i) pseudoreceptors that compete with signalling receptors, (ii) inhibitory Smads that block signalling, (iii) intracellular binding proteins that bind Smad 1 and 5, and (iv) factors that induce ubiquitination and proteolysis of signalling Smads. A large number of (v) extracellular proteins that bind BMPs and prevent their binding to signalling receptors have emerged. They are the components of the Spemann organizer, noggin, chordin, and follistatin, members of the Dan/Cerberus familiy, and twisted gastrulation. The antagonists tend to be specific for BMPs and are regulated by BMPs, indicating the existence and need of local feedback mechanisms to temper BMP cellular activities. The actions of BMPs are regulated by intracellular and extracellular proteins that bind BMP's or components of the BMP signaling pathways. Often their synthesis is BMP-dependent, pointing to the need of local feedback mechanisms to maintain an ideal balance between BMPs and their antagonists. Future investigation should provide valuable information on the physiological role of BMP antagonists in the skeleton and their role in various skeletal disorders.

Topic: Regeneration of Bones and Joints

P 46 The NBD-NBD interface is not the sole determinant for transport in ABC transporters

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The ABC (ATP binding cassette) transporter superfamily constitutes one of the largest classes of membrane transporters. Their characteristic architecture consists of two transmembrane domains that create the substrate translocation pathway and two cytoplasmic nucleotide binding domains (NBDs), which are the conserved engines that power transport.The majority of ABC transporters, including the multi-specific drug efflux pump P-glycoprotein (P-gp, ABCB1), contains two functional nucleotide binding sites (NBSs) between their NBDs; however, several including the bile salt export pump (BSEP, ABCB11), have one degenerated ATP binding site. Structural alignment shows ABCB1 and ABCB11 to differ in only four residues at the NBD-NBD interface, all of them located in NBS1: E556M, G1178R, Q1180E and S474E. It has been shown that a mutation of the Walker B glutamate (E556), which conducts the nucleophilic attack on ATP via a water molecule, abolishes ATPase and drug transport activity of ABCB1 (Sauna et al., 2002). We tested the hypothesis that function may be restored in ABCB1, when NBS1 is engineered on the basis of ABCB11. For this purpose, these four residues were introduced to ABCB1 either individually or in combination. Function of the transporter was determined by continuous monitoring of rhodamine 123 efflux over five minutes. The P-gp specific MRK16 antibody was used to determine surface expression. As expected, the E556M mutation of the catalytic glutamate resulted in loss of transport function. Whereas the double mutation in the LSGGQ motif (G1178R, Q1180E) reduced transport to below 20%, no measurable rhodamine 123 efflux was observed in either the triple mutant (E556M. G1178R. Q1180E) or the quadruple mutant (E556M. G1178R. Q1180E. S474E). In conclusion, engineering of NBS1 of ABCB1 on the basis of ABCB11 does not seem to result in a functional transporter despite the high homology between the two transporters, indicating that the NBD-NBD interface is not the sole determinant for transport activity.

Topic: Malignant Diseases

P 47 Neurotransmitter Alterations in Ether Lipid Deficiency

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Introduction: Mallet deformity of the finger involves avulsion of the extensor tendon from the base of the distal phalanx with a bony fragment. In Ether phospholipids are a specialized class of lipids that are characterized by an O-alkyl bond at the sn-1 position of their glycerol backbone. The initial steps of their de novo biosynthesis take place in the peroxisome. Plasmalogens constitute the most prominent type of ether lipids in the mammalian body and are a structural component of cellular membranes, which makes them essential constituents of the mammalian body. They perform highly important physiological functions like scavenging of radicals and storage of polyunsaturated fatty acids. Consequently, the lack of ether lipids has drastic consequences, in humans evoking the fatal disease rhizomelic chondrodysplasia punctata (RCDP). Owing to their frequent occurrence in the central nervous system, a role of these compounds in neurotransmission has been repeatedly proposed but never proven. In the present study we show that in the respective mouse model the lack of ether lipids causes hyperactivity, which manifests itself in several behavioral paradigms. Based on this, we analyzed neurotransmitter levels in the brains of these animals using high-performance liquid chromatography and demonstrate a depletion of various neurotransmitters belonging to different transmitter classes. At the same time the turnover of (catecholamine) neurotransmitters appears to be normal, as judged by treatment of ether lipid-deficient animals with the catecholamine biosynthesis blocker alpha-methyl-p-tyrosine. The present results suggest that ether lipids play an important role in synaptic processes and that the lack of these compounds impairs neurotransmitter metabolism and cycle. Additionally, we propose that the observed reduction in neurotransmitter levels contributes to the hyperactive phenotype exhibited by ether lipid-deficient animals. The exact molecular mechanism, by which ether lipid deficiency leads to the reported phenomena, however, demands further investigation.

Topic: Neuroscience

P 48 Is chronic low-dose dexamethasone treatment sufficient to induce apoptosis within rat cortex?

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Objectives: Dexamethasone (DEX), a highly potent and long-lasting anti-inflammatory and immunosuppressive agent, is primarily used to treat certain types of cancer, inflammatory and autoimmune diseases. Its main therapeutic role might be based on glucocorticoid receptor (GR)- mediated mechanisms that control and trigger different processes such as cell cycle progression and apoptosis in hippocampus. The objective of this study was to determine whether chronic glucocorticoid treatment would alter the expression of cell death or cell survival molecules in the cortical brain area. Materials and methods: Adult male Wistar rats were subjected to 8 day course of low-dose DEX (100 g/kg) or saline vehicle i.p. injections. Animals were killed 28h postreatment. The modulation in the expression of signaling molecules was detected by Western blot in whole cell extracts and RT-PCR. Results: DEX treatment caused activation of AKT, augmentation of pro-survival Bcl-2 protein level and Bcl-2:Bax protein ratio. No change of cortical cell death Bax, p53, procaspase 3 and PARP molecule expressions was detected. Results of RT-PCR analysis showed that Bcl-2, Bax and p53 mRNAs were constitutively expressed in the cortex of control rats. In DEX-treated animals only anti-apoptotic Bcl-2 mRNA was increased while no significant alteration in pro-apoptotic Bax and p53 mRNAs expression was observed. Furthermore, DEX treatment altered investigated physiological parameters (corticosterone (CORT) level, body weight, thymus and adrenal gland weight) which confirmed the glucocorticoid action in the periphery and hormone treatment efficacy in this model system. Conclusion: Our findings indicated that this GR agonist could have potential cell's pro-survival effect due to either low DEX concentration used in this study or possible less sensitivity of cortex to chronic DEX administration or both.
Acknowledgment: This study is supported by the Ministry of Education and Science, Project No 173044.

Topic: Neuroscience

P 49 VGLUT3 expressing primary afferents in neuropathic pain

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Diabetes mellitus, the administration of cytokines during chemotherapy or a simple tissue trauma can lead to peripheral nerve damage and further to neuropathic pain. Symptoms experienced by patients likely depend on the aetiology of the underlying disease, and include not only spontaneous pain but also mechanical and thermal hyperalgesia and allodynia. Vesicular glutamate transporter 3 (VGLUT3) is expressed in a subset of primary afferents enabling these fibres to relay external stimuli to postsynaptic spinal neurons through excitatory release of glutamate, which is loaded prior to signalling into presynaptic vesicles via VGLUT3. It has been recently shown that these primary afferents contribute to mechanical allodynia in a mouse model of neuropathic pain. The different symptoms expressed by neuropathic pain patients are also reflected in different animal models of pain. We therefore performed a chronic constriction injury (CCI) in VGLUT3-/- mice and wildtype control, and assessed alterations in mechanical and thermal thresholds through a variety of behavioural tasks. Our results indicate that VGLUT3 may contribute less to mechanical allodynia in the CCI model than in the spared nerve injury (SNI) model. In addition, we could show via immunohistochemical analysis of dorsal root ganglion (DRG) tissue that TRPM8, one of the principle detectors of environmental cold, is co-expressed in a large number of VGLUT3 positive C-fibres, implying a role of VGLUT3-expressing primary afferents in cold transduction. The contribution of VGLUT3-positive fibres to altered signal transduction in neuropathy will be further investigated in different animal models of neuropathic pain.

Topic: Neuroscience

P 50 Renal impairment in premature infants – influence on short-term outcome

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Introduction: Impairment of renal function correlates with poorer outcome of critically ill adults and children treated at intensive care units. For critically ill premature infants the relevance of renal injury on mortality and morbidity is reported only in few studies with small sample sizes. Thus, there is still a lack of knowledge about the role of kidney injury as an independent factor on morbidity and mortality in premature infants. First aim of this study will therefore be to evaluate the role of acute kidney injury on morbidity and mortality. Another aim is to evaluate factors associated with renal impairment during the neonatal period and to detect kidney injury in an early stage or even to detect risk factors for the development of kidney injury in this special population. Methods: We have planned a retrospective study in preterm neonates (borne before gestational age of 37 weeks) who were cared on the NICU of the Medical University of Vienna during a two year period. Primary outcome is mortality itself. Secondary outcome are morbidity factors (i.e. pulmonary failure, development of bronchopulmonary dysplasia, sepsis and other organ failure). Results: The study cohort consists of neonates born before 37 weeks gestational age without major malformations and survival for more than 48 hours (n=510). Out of these neonates 84% (n=430) survived whereas 16% (n=80) died during their treatment at the intensive care unit. The survivors had significant lower serum creatinine and BUN levels (0,59 mg/dl, 16,48 mg/dl) than the nonsurvivors (1,06 mg/dl, 36,68 mg/dl) (p=0,00). Infection parameters of survivors were also significant lower than in the nonsurvivors. There was no significant difference in gender distribution and the number of multiple pregnancies between survivors and nonsurvivors. NEC, incidence of IVH, connatal infection and nosocomial infection were significantly lower in survivors. Further data are currently collected and under analysis.

Topic: POeT - Programme for Organfailure, -replacement and Transplantation

P 51 Gender, wellbeing and chronic autoimmune diseases: Results of a qualitative Study

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Background: Chronic autoimmune diseases are disabling diseases which have a major impact on functioning, health and wellbeing (WHO, 2003). A guidance which of the health and well-being concepts would be important for the patients and therefore worth to be measured within the clinical daily routine is needed. Objectives: This study aimed to provide information which of the health and wellbeing concepts would be relevant for people living with the different autoimmune diseases based on extensive empirical data. Furthermore we wanted to explore gender differences. Methods: A qualitative biographic narrative study was conducted to explore the relevance and meaning of concepts of health and well-being for people living with a chronic autoimmune disease. 75 life stories from people living with Crohn's Disease (CD), diabetes typ1 (D), rheumatoid arthritis (RA), systemic lupus erythematous (SLE) and scleroderma (SSc) were compared to 15 life stories of so called healthy (H) people. Results: 89 (54f/35m) told their life stories. The median age was 51 years. The largest age group (25%) of the sample was between 60 and 69 years old, followed by the 50 to 59 years aged people (24%). Only 8% of the sample were 70 years old or older. The concepts differed between diagnoses and age groups: e.g. social support was more often covered within the life stories of people living with CD, and least often of those living with SLE. Also more women reported about social support than men. Work-life balance and secondary gain from illness were found barely. Conclusions: The different meanings of the several health and wellbeing concepts between diagnose groups and genders have not been identified up to now. Further research should investigate the variety of our findings given in a representative sample. Since the interplay of these concepts and the course of disease resp. its outcome is known, modern medical care, clinicians and other health care professions, are asked to take into account those.

Topic: Immunology

P 52 The impact of modification and localization of the Src family kinase Lck on T cell activation

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During the past decade, great efforts have been made to gain insight into the complex process of antigen-induced T cell activation and the underlying signal transduction pathways. According to common "textbook" knowledge, Lck, the key Src protein tyrosine kinase of T cell activation, is supposed to undergo structural changes from a closed inactive to an open active conformation upon T cell antigen receptor (TCR) engagement. However, previously we could show by biochemical assays and live-FRET imaging that regulation of Lck upon TCR stimulation is rather mediated by other mechanisms, presumably reorganization of localization or domain displacement of intramolecular interactions by ligands than acute structural changes of the molecule. Therefore, we first investigated the determinants mediating binding of Lck to the inner leaflet of the plasma membrane and second determined the ratio between cytoplasmatic and membrane-bound Lck molecules in stimulated and non-stimulated T cells. By using a monomeric enhanced green fluorescent protein (mEGFP)-tagged Lck biosensor and a non-invasive single molecule imaging approach we were able to determine the exact lifetime of Lck in the plasma membrane. Moreover, we found by live-FRET imaging and single molecule analysis Lck molecules forming dimers and higher order structures. Biochemical methods showed that the N-terminal membrane anchor is mediating the intermolecular interaction of Lck. Together, these advanced imaging studies of Lck in the live cell context provide a novel picture of the function and regulation of this key kinase in signaling via TCR. Supported by the GEN-AU Program of the Austrian Federal Ministry of Science and Research, the PhD program Cell Communication in Health and Disease, and the EUROCORES EUROMEMBRANE_project I00300 LIPIDPROD of the Austrian Science Fund.

Topic: Immunology

P 53 Proteomics study of Kv7 channels

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Kv7 channels are a subfamily of voltage-gated K^+ channels, serving as important regulators of neuronal excitability (Fig.1). As mutations in Kv7 channels manifest in serious diseases, it is of particular interest to elucidate their regulatory mechanisms such as phosphorylation. Thus, to characterize the modulation of Kv7 channels by phosphorylation, we first identified in vivo phosphorylation sites through mass spectrometry (MS). Our results indicate the major region of phosphorylation to be the long C-terminal end which is important for channel gating and assembly. Therefore, we will further determine specific phosphoacceptors and kinases necessary for Kv7 channel heteromerization and surface expression.

Topic: Molecular Signal Transduction

P 54 Expression of canine immunoglobulins against the tumour antigen EGFR

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Background: Passive immunotherapy with monoclonal antibodies belongs today to the standard of care in clinical oncology. For instance, cetuximab (Erbitux®) is a chimeric IgG1 antibody being used to target EGFR (ErbB-1) overexpressed in colon cancer, and head and neck cancer of human patients. By contrast, in veterinarian oncology no approved passive immunotherapy is available yet, although cancer is a frequent event in aged dogs (Canis lupus familiaris). We could demonstrate recently that the canine and human ErbB-1 molecules are highly homologous. Harbouring similar biological functions it is also overexpressed in canine mammary cancer where it represents a promising target. Aims: The aim of this diploma study is to optimize the expression procedure of caninized IgG and IgE antibodies with the exact epitope specificity of cetuximab. Consequently, we aim to evaluate their functional effects in vitro and on the long run, to prepare a clinical study in dog cancer patients. Methods & Results: The caninized anti EGFR antibodies were expressed in CHO cells using serum-free media, ensuring later applicability in clinical studies. From over 400 clones (n=x) were selected with the highest production yield according to ELISA. Antibody integrity was controlled via dot blots and western blots detecting heavy and light chains. The specifity of recombinant antibodies was confirmed head to head to the original cetuximab, using human EGFR for coating and as negative control recombinant human HER-2 (ErbB-2) in ELISA. Purification of the canine IgG antibodies was carried out with protein A using the ÄKTATM liquid chromatography system. Antibody binding to the canine mammary carcinoma cell lines P114 and CF41 overexpressing EGFR could be verified in FACS; as next important step we plan to address the tumor growth inhibitory effect of our recombinant antibodies in cell viability and proliferation assays. Conclusion: This is the first approach to generate caninized antibodies for the purpose of a clinical passive anti-cancer immunotherapeutical study in pet dogs. This study demonstrates the importance of comparative medicine to give animals access to state of the art therapies and simultaneously helps to gain new insights for development of better drugs for human oncology.w

Topic: Other

P 55 A functional pharmacogenetic screen in lung cancer

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Background Lung cancer is the leading cause of cancer-related death in western countries, mainly due to late diagnosis. In the past decade, deep-sequencing projects have shed light on the different subtypes of lung cancer based on its landscape of somatic mutations. This has supported the evidence that the genetic background of tumours is highly diverse and complex and this determines the patient response to the treatment. Recently, the first drugs targeting lung cancer specific driven mutations have opened a new era for personalized medicine. Most of the patients still develop drug resistance within the first year of treatment. Thus, it is crucial to develop new approaches and identify more effective drugs. Material and methods We focus on non-small cell lung adenocarcinoma. In order to mimic the molecular heterogeneity of patients we established a genetically tractable cell model. To do so, we generated 100 isogenic cell lines, each one carrying a driven mutation that triggers the tumorigenesis (i.e. EGFR, KRAS, EML4-ALK...) and a second mutation frequently found in lung adenocarcinoma. These cells are screened in a multiplex assay against a panel of drugs, currently used in clinics or in clinical trials. Each cell line is tagged with a unique short DNA sequence called barcode, allowing their identification in a mixed population of cells. This method has recently been employed successfully to identify novel synthetic lethal interactions and mechanisms of resistance in breast cancer cells (Muellner et al., Nat. Chem. Biol. 2011). The abundance of each barcode will be indicative for cell viability. Results We have performed a first screen between our lung cancer-relevant isogenic cell lines and a panel of drugs including kinase inhibitors and classical chemotherapeutics. Currently, we are validating the most significant hits. Conclusions Our system provides a robust method to investigate genotype-specific vulnerabilities upon drug treatment in a high-throughput manner.

Topic: Malignant Diseases

P 56 Interaction of FimH with Glomerular Endothelial cells and LAMP-2

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FimH is a bacterial adhesin located at the tips of the pili of type 1 fimbriated bacteria including pathogenic E.coli. FimH is an essential virulence factor and responsible for bacterial adhesion and activation of cells including uroepithelium, brain endothelium, and neutrophils through a variety of receptors, such as TLR4, CD48 and uroplakin. Recently, FimH has been identified as a molecular mimic for lysosome associated membrane protein-2 (LAMP-2), the target of autoantibodies found in 80-90% of patients with ANCA-associated vasculitis. The purpose of this study was to determine whether LAMP-2 is involved in the interactions between fimbriated bacteria and endothelial cells. First we ascertained the role of FimH in bacterial adhesion to conditionally immortalised human glomerular endothelial cells (GEnC) using FimH-positive and FimH-negative E.coli strains with a GFP signal. FimH-positive bacteria adhered to GEnC, were internalized and trafficked to LAMP-2 positive endosomal or lysosomal compartments. As expected, binding and uptake were inhibited by 2% □-D-mannoside and thus mannose dependent; they were also partially inhibited by antibodies to TLR4. Next we examined the direct interaction of FimH with GEnC using recombinant His-tagged FimH expressed in FimH negative E.coli strain HB101F'. High concentrations of FimH (10 µg/ml) that are known to activate neutrophils rapidly killed GEnC. FimH was internalized in mannose dependent fashion and partly co-localised to LAMP-2 containing compartments. Finally, we affinity-purified native human LAMP-2 from endothelial cells and showed by Western blot overlay, that FimH binds LAMP-2. In summary, we have shown that FimH is essential for binding and uptake of E.coli by GEnC and for the first time we identify it as a ligand for LAMP-2. The results have implications for the pathogenesis of gram negative septicaemias and for autoimmunity in ANCA-associated vasculitis.

Topic: Immunology

P 57 Extending The Field Of View In Adaptive Optics Scanning Laser Ophthalmoscopy

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Purpose: To investigate the influence of the scanning angle on the wave front correction and therefore the quality of images of the human retina acquired in vivo with an adaptive optics scanning laser ophthalmoscope (AO-SLO). Methods: In this study a custom built, lens based AO-SLO instrument operating at a frame rate of 10 fps (scanning angle 1°x1°, 3°x3°, 4°x4° and 5°x5°) is used to record images of the human cone mosaic. The adaptive optics system is operated in closed loop and uses part of the imaging light for wave front sensing. To increase the signal to noise ratio 30 images are registered to each other and averaged prior to data analysis. A possible advantage of the lens based system is that aberrations introduced by the system itself during scanning are reduced in comparison with instruments based on spherical mirrors. The image quality of the same retinal region using different scanning angles is compared. Results: The figure shows representative images recorded from the fovea region in a healthy volunteer. The 4 degree scanning angle shows very similar image quality as images recorded with 1 degree (c.f. comparison of the ROI on the right hand of the figure). The cone mosaic could be resolved down to an eccentricity of 0.25° from the fovea centralis. Conclusions: Similar image quality could be obtained using large scanning angles of up to 4 degrees in healthy subjects. The larger scanning angles reduce artifacts caused by eye motion and the total measurement time, which should allow a broader use of AO-SLO in healthy subjects and patients.

Topic: Medical Physics

P 58 Antagonizing endosomal Toll-like receptors diminishes inflammatory arthritis

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Rheumatoid arthritis (RA), a common chronic inflammatory joint disease, has an autoimmune background. There is evidence that release of endogenous nucleic acids triggers autoimmune reactions important for the onset of RA. Recently, endosomal Toll-like receptors (TLRs) have been implicated in autoimmune processes due to their ability to recognize these nucleic acids. We study the role of TLR7 and TLR9 in the pathogenesis of arthritis by antagonizing them in the pristane-induced arthritis (PIA) model in rats. Different immunoregulatory sequences (IRS) known to inhibit TLR7/9-activity were tested in cultured rat splenocytes. Using the PIA model, these IRS were also tested for their ability to inhibit arthritis development. IRS was applied twice a week subcutaneously at the base tail. Arthritis score and weight changes were assessed during the experiment. Paws, lymph nodes and spleen were taken for protein-, RNA- and histological analysis. The impact of the IRS' was also analyzed in an in vitro osteoclast formation assay using murine bone marrow-derived macrophages. IRS against TLR7, 9 and 7/9 showed a dose-dependent inhibition of pre-activated rat splenocytes and the combined TLR7/9 inhibitor didn't diminish arthritis severity. However, antagonizing TLR9 alone led to reduced arthritis severity, which was also confirmed by reduced protein expression levels in paws and lymph nodes compared to the placebo group and the TLR7 inhibition therapy group. Bone destruction was also strongly reduced in paws of rats treated with the TLR9-antagonist, as revealed by histological analysis. Furthermore, in an in vitro osteoclast formation assay it was shown that treatment with the combined TLR7/9 inhibitor and the TLR9 antagonist led to reduced osteoclastogenesis. We showed that DNA is important in PIA. Despite its role in the rat model, DNA might also play an important role in the pathogenesis of human RA and antagonizing TLR9 might become a useful therapeutic option for RA.

Topic: Inflammation and Immunity

P 59 Trabecular Direction and Deformation Distribution in Lung Transplant Patients with severe Osteoporosis Risk

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Purpose:New onset osteoporosis or progression of a pre-existing disease with bone fractures within the first years after lung-transplantation (LuTX) is a severe but poorly understood problem. High resolution peripheral quantitative computed tomography (HRpQCT) offers novel options for the assessment of disease progression. It provides volumetric BMD, detailed cortical bone geometry and trabecular microarchitecture. We propose and evaluate a novel optical-flow based registration approach to capture trabecular reorganization and observe significant differences between LuTX indications.Methods and Materials:13 patients (Indication groups: (a) vascular diseases, (b) cystic fibrosis, (c) parenchymal diseases) were scanned with HR-pQCT at baseline and 3 months after LuTX. To capture trabecular reorganization the volumes were rigidly registered. Subsequently trabecular deformation was measured using optical-flow. We obtained deformation-field-maps for each case and quantified the increase of trabecular thickness together with trabecular direction. Entropy was used to quantify directional dominance, Kullback-Leibler (KL) divergence between direction and change distribution to quantify the relationship of two measurements. Results:No significant differences between LuTX indication groups were observed when comparing directional dominance at a single time point. However, when including reorganization information by combining trabacular direction and thickness change, significant differences ($p < 0.05$, fdr corrected) were found between indication groups (a)(b) and (b)(c) (mean KL (a) 0.4197, (b) 0.2991, (c) 0.4964).Conclusion:Computer-based analysis of bone microarchitecture detected significant differences in osteoporosis progression among different LuTX groups, while microarchitecture assessment at a single time point does not. Trabecular reorganization can be measured quantitatively in HR-pQCT data, and reveals disease-specific patterns.

Topic: Medical Physics

P 60 In vitro study of biological compatibility of different surface coating

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The orthodontic therapy is based on applications of forces of diverse magnitudes for tooth movement, and for each point of force application a point of anchoring is required. The success of orthodontic anchor is closely related by osseointegration as well health of boundaries soft-tissues, which in turn are crucially dependent on the properties of Ti surface. The aim of this study is to compare different kinds of titanium surface coating in terms of biocompatibility and possible use as orthodontic anchor devices. MG-63 osteoblastic cells were grown on four different surfaces: group 1 Ti6Al4V with TiN coating, group 2 Ti6Al4V with Teflon® coating, group 3 SLA® (Straumann) commercial surface, and group 4 Ti6Al4V without surface treatment. Cells grown on plastic served as a control. Cell proliferation/viability was measured after 2 and 7 days of culture, expression of osteogenesis-associated genes was measured after 7 days of culture. After 2 days, cell viability was found to be lowest in cells grown on teflon compared to other groups (teflon vs. control, $p<0.05$; teflon vs. SLA, $p<0.05$). In addition, cell viability in Ti6Al4V group was significantly lower than in control group ($p<0.05$). After 7 days, the cell viability in teflon group was significantly higher than that in TiN and Ti6Al4V groups ($p<0.05$). Real-time PCR analysis showed significantly higher mRNA expression levels of ALP and osteocalcin in cells cultured on the teflon-coated discs compared with SLA and Ti6Al4V ($p<0.05$). The expression of osteoprotegerin was significantly higher on the Teflon coated discs than on Ti6Al4V ($p<0.05$), whereas no differences in the expression of RANKL was observed. Summarizing, our in vitro study showed that the teflon-coated discs present no cytotoxicity effect on the viability and proliferation of osteoblastic like cells (MG63), promote the expression of genes that are related to the osseointegration, and seem to increase OPG/RANKL ratio.

Topic: Regeneration of Bones and Joints

P 61 The novel IL-1 family member IL-33 is produced during human pregnancy and controls function of primary trophoblasts in vitro

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Cytokine-mediated processes are critically required for a successful outcome of pregnancy. Here we describe the role of Interleukin-33, a novel IL-1 family member, in the human placenta. In a first step, we assessed the expression pattern of IL-33 and its receptor ST2L at the feto-maternal interface and secondly, we analysed the effects of IL-33 in primary trophoblast model systems. Flow cytometry was used to analyse ST2L expression in primary first trimester cytotrophoblasts (CTBs) and extravillous trophoblasts (EVTs). Additionally, immunostainings for ST2L and IL-33 were performed in first trimester placenta and second trimester decidua. BrdU incorporation assays were carried out to study the effects of IL-33 on trophoblast proliferation in the absence or presence of its decoy receptor, sST2. Moreover, the invasive and migratory capacity of primary trophoblasts and villous explants was evaluated in the absence or presence of IL-33. Finally, supernatants of villous explants were subjected to Western blot analysis to assess IL-33-dependent effects on secretion of invasion-associated proteases. Descriptive analyses revealed a prominent ST2L expression in CTBs and EVTs. Interestingly, IL-33 was detected in Hofbauer cells in the placental villus, as well as in decidual fibroblasts and endothelium of spiral arteries. We found that IL-33 significantly increased proliferation of primary trophoblasts and CTBs in villous explants. Moreover, IL-33 enhanced invasion of EVTs and triggered trophoblast outgrowth in villous explants, which was accompanied by MMP-2 and MMP-9 secretion. Hofbauer cells as well as decidual fibroblasts represent putative sources of IL-33, which activates ST2L-expressing CTBs and EVTs. Functional assays revealed that IL-33 promotes trophoblast proliferation and invasion of primary trophoblasts and villous explants. Taken together, we suggest IL-33 as an important regulator of trophoblast proliferation and motility.

Topic: Molecular Signal Transduction

P 62 Gene expression and bone architecture in men with osteoporotic hip fractures

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Osteoporosis is extremely frequent in post-menopausal women; nevertheless, osteoporosis in men is a severe, frequent and often underestimated disease. In a previous study we found evidence of osteoblast dysfunction in middle aged men with idiopathic osteoporosis. The aim of this study was to investigate gene expression and bone architecture in bone samples derived from elderly osteoporotic men with hip fractures in comparison to bone samples from age matched non-osteoporotic controls. Femoral heads and adjacent neck tissue were collected from 10 men with low-trauma hip fractures (mean age 81,9 ± 7,1) and consecutive surgical hip replacement. 14 bone samples of age matched patients undergoing hip replacement due to osteoarthritis served as controls. One half of the bone samples was subjected to RNA extraction, reverse transcription, and real-time polymerase chain reactions. The second half of the bone samples of each patient was analyzed by ex-vivo dual x-ray absorptiometry and by high resolution peripheral micro-computed tomography. We could show a significantly decreased runx2, osterix, sclerostin and osteocalcin expression in bone samples from hip fracture patients compared to controls. Areal bone mineral density was significantly lower in fracture samples. Comparing local bone microstructure the femoral head displayed significantly lower BV/TV and trabecular thickness in the fractured when compared to the osteoarthritic bone samples. Therefore, decreased local gene expression of runx2, osterix and osteocalcin in men with hip fractures strongly supports the concept of osteoblast dysfunction in male osteoporosis.

Topic: Regeneration of Bones and Joints

P 63 Local circuits in the intermediate CA1 hippocampus

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The connectivity between different brain areas defines what kind of information is processed and local circuits define how information is processed. Along the septo-temporal axis of the hippocampus the intermediate domain is in a unique position to integrate spatial, emotional and reward related information which can be directly transmitted to subcortical and prefrontal areas. In the dorsal CA1 (dCA1) hippocampus, local circuits are characterized by a diversity of GABAergic interneurons with each interneuron type contributing distinctly to the network organisation and activity. To investigate the intermediate CA1 (iCA1) local circuits we used the juxtacellular technique to record iCA1 interneurons and pyramidal cells in urethane anaesthetised rats. We identified different parvalbumin expressing iCA1 interneuron types and analysed their dendritic distribution, axonal targets and neurochemical expression. In comparison to the dCA1, our anatomical and immunohistochemical analysis shows many similarities but also differences in the investigated interneuron types of the iCA1. The analysis of iCA1 interneuron firing patterns revealed the distinct phase coupling to local field potential (LFP) theta oscillations and activation or inhibition during sharp wave ripple events. Simultaneous LFP recordings in the dorsal CA1 stratum pyramidale allowed us to determine the structure of spike timing across the dCA1 and iCA1 during theta oscillations. Our data suggests that the sequence of interneuron type and pyramidal spike timing is the same, although possibly more compressed, in the iCA1 but is shifted in time together with a shift in the iCA1 LFP theta. Theta oscillations along the septo-temporal axis might reflect a general principle of how interneuron activity is organised in time in the CA1 hippocampus. Natural variability or changing inputs and the need of different local circuit computations might account for the differences between the dCA1 and iCA1 local circuits.

Topic: Neuroscience

P 64 IFN gamma inducible GTPase guanylate binding protein 1 negatively regulates T cell receptor activation at a very early stage

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Once a T cell is activated, it is important to tightly regulate proliferation and differentiation in order to prevent overshooting immune reactions. GTPases are predestined for this purpose, because they act as important switches in many signaling events in the cell. Although the two major families of GTPases – small G-proteins and heterotrimeric G-proteins – are subjects of intensive studies, only little is known about a third major family: the IFN inducible GTPases. Silencing of one family member called human guanylate binding protein (GBP) 1 in primary T cells as well as in Jurkat T cells leads to a higher Ca^{2+} flux with subsequently higher activation of the IL-2 promoter and higher IL-2 secretion. Detailed analyses of intracellular Ca^{2+}-flux in GBP-1 silenced Jurkat T cells with the help of thapsigargin, a specific inhibitor for sarco/endoplasmic reticulum calcium ATPase shows that the higher activation of GBP-1 silenced Jurkat cells is dependent on early T cell receptor (TCR) signalling events. Further investigation of the early TCR signalosome with the help of phosphorylation specific antibodies revealed higher phosphorylation at protein specific activation sites of important signalling molecules like Zap70, LAT and PLC-gamma. These results demonstrate that GBP-1 interferes early in the TCR signalling cascade. With the help of mass spectrometry we identified so far unknown binding partners of GBP-1, playing a role in T cell activation.

Topic: Immunology

P 65 Regulation of Scavenger Receptor Class B, type I by mTOR in vascular endothelial cells

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Deregulation of lipid metabolism is causally involved in type 2 diabetes mellitus (T2D), a mayor source of morbidity in the western world. The mechanistic target of rapamycin (mTOR)was shown to play a role in lipid metabolism as well as in the modulation of insulin signalling. In this project we investigate the role of the mTOR pathway in the regulation of the high density lipoprotein (HDL) receptor Scavenger Receptor Class B, type I (SR-BI) in human umbilical vein endothelial cells (HUVECs). Rapamycin, which is in clinical use as an immunosupressor, was utilized as a pharmacological inhibitor of the mTOR pathway. Inhibition of mTOR by treatment with 20 nmol/l rapamycin for 24 hours resulted in a reduction of SR-BI mRNA by 60%, translating into a 75% decrease of SR-BI protein. Inhibition of mTOR also resulted in decreased mRNA levels of FASN (fatty acid synthase), LDL (low density lipoprotein) receptor and HMGCR (3-hydroxy-3-methylglutaryl-CoA reductase), suggesting reduced activity of both SREBP-1 and SREBP-2. Furthermore, PPAR gamma expression was reduced upon rapamycin treatment. Whether the down-regulation of these transcription factors is causally involved in SR-BI regulation by mTOR, is currently investigated by promoter studies. Taken together we suggest, that the modulation of mTOR, for instance by pharmacological inhibition with rapamycin or by induction with insulin might be a pathophysiological mechanism influencing endothelial SR-BI levels and thus HDL transport through the endothelium.

Topic: Vascular Biology

P 66 Functional and physical interactions between P2Y receptors and ion channels

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Neuronal P2Y receptors, i.e. nucleotide-sensitive G protein coupled receptors (GPCRs), are known to control various voltage-gated ion channels, in particular Kv7 K^+ and Cav2.2 Ca^{2+} channels. The differential modulation of these ion channels via GPCRs was shown to rely on the presence or absence of scaffolding proteins such as AKAP79/150 (Zhang et al, J Neurosci 31, 7199, 2011) and NHERF-2 (Filippov et al, J. Neurosci 30, 11068, 2010). Since scaffold proteins are believed to bring GPCRs and ion channels in close proximity to guarantee efficient G protein-mediated modulation, this project evaluates whether a tight contact between P2Y receptors and ion channels is a prerequisite for their functional interaction. P2Y1 or P2Y12 receptors with fluorescent tags (CFP or YFP) were expressed together with fluorescently labeled Kv7.2/7.3 or Cav2.2 channels in tsA 201 cells and the channel modulation by nucleotides was determined by measuring the according currents. Activation of P2Y1, but not of P2Y12, receptors by ADP inhibited the K^+ currents in a concentration dependent manner by up to 20.5 ± 1.9%. Conversely, activation of both, P2Y1 and P2Y12 receptors reduced the Ca^{2+} currents by up to 60.1 ± 7.4% and 76.3 ± 4.2%, respectively. To evaluate the behavior of the receptors and channels in the membrane, fluorescence recovery after photobleaching (FRAP) was determined by confocal laser microscopy. In initial experiments, fractions of 40 to 60% of the channels and receptors were shown to be mobile with time constants of recovery between 0.01 and 0.02 s-1. Future experiments will reveal whether receptors/channels can influence each other's mobility and whether they are in close proximity as determined by fluorescence resonance energy transfer (FRET).

Topic: Cell Communication in Health and Disease

P 67 Reducing damages of the lung following lower limb ischemic-reperfusion injury by postcondition

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Introduction: Operation on the infrarenal aorta or the major vessels of the lower limb could cause ischemic-reperfusion (I/R) injury in local tissues and in remote organs (e.g. in the lung) subsequently. Locally released metabolites, mediator molecules, oxygen-free radicals can cause – via activated polymorfonuclear leucocytes – damage in the lung. Objectives: Our aim was to reduce damages in the lung, after lower limb I/R with postconditioning. Materials and methods: Male Wistar rats (n=90) underwent 180 minutes bilateral lower limb ischemia. Animals were divided into three groups: Sham (n=10), Control (I/R, n=10), Postconditioned (PostC, n=10). In postconditioned group, postconditioning was used after ischemia. Then groups were divided according to reperfusion time: 3h, 24h and 72h groups. After sampling, serum total antioxidant level, TNF-□; and IL-6 levels, histological changes, Wet/Dry (W/D) ratio, myeloperoxidase (MPO) activity and Hsp72 level were investigated. Results: Histological changes were pronounced in the 4h and 24h I/R group, but PostC group has better results. Total antioxidant level is significantly higher in the 4h PostC group than in the I/R group. TNF-□; level is significantly higher ($p<0.01$) in the 4h I/R group (44.9±8.6pg/ml) than in the PostC group (22.9±4.9 pg/ml). IL-6 levels are increased both in the 4h I/R and PostC groups, but without significant difference (251.0±51.1vs.317.4±37.7 pg/ml). Compared to the I/R group, lung MPO activity is increased to a lesser degree in the PostC group in all time-points (18.23vs.15.18; 13.63vs.14.63;30.43vs.21.60). Hsp72 level in the 4h PostC group is higher than in the I/R group (1.3 vs. 1.0), without significant difference. W/D ratio in PostC groups is lower in all time-points, moreover in the 4h PostC group this difference is significant (60.15%vs.63.20%;65.58%vs.68.28%;68.40%vs.72.08%). Conclusion: Postconditioning can reduce damages of the lung after lower limb ischemic-reperfusion injury.

Topic: Other

P 68 Non-responsiveness to certain routine vaccines involves different regulatory immune cell populations and IL10 production

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Non-responders (NR) lack a sufficient protective immune response after vaccination, yet mechanisms remain widely unknown. Thus we performed a study with NR to tick-borne encephalitis (TBE) or Hepatitis B and compared their immune responsiveness after TBE and Influenza vaccination at humoral and cellular level. In TBE-NR TBE titers remained low but vaccinees mounted protective Influenza titers. Hepatitis B–NR reacted with adequate titers to both vaccines as did controls, i.e. TBE high-responders. Cellular immune response (cytokines) correlated with ab titers in TBE non/low- and high-responders. This was not the case in Hepatitis B-NR, where cellular immune responses to TBE and Influenza were down-regulated despite protective ab titers. Low IFNg and IL2 production was however accompanied by high IL10 levels. In order to evaluate which cell population accounts for IL10 production, co-staining of IL10 and FOXP3 in CD4 T-cells and analysis of FOXP3- Tr1 cells, tolerogenic DCs (DC10), immature transitional B-cells/B-regs (CD19/24/38+) and B10 cells (CD19/24/27+) was done in selected donors. B-regs were highest in Hepatitis B-NR and remained so post booster. Similarly, in TBE-NR this population significantly increased post booster from lower pre-booster numbers. In contrast, TBE high-responders had medium to high numbers of B-regs pre-booster which clearly decreased after vaccination. Both NR groups showed increased FOXP3+ T-regs post booster and in selected donors increased IL10 production of these cells was observed, normal controls however had decreased T-regs after vaccination. B10 sub-populations varied greatly in all 3 groups and remained unchanged. Tr1 cells were not found in selected donors of all groups and neither was the Tr1 inducing DC10 population present in a conclusive manner. Our current data indicate that NR is associated with a significant presence of regulatory cells, of which IL10 producing B-regs and T-regs seem to play an important role.

Topic: Immunology

P 69 Probing the first crystal structure of a voltage-gated Na channel

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In voltage-gated Na channels the S6 transmembrane segment of domain IV (DI-VS6) is part of the lining of the inner part of the pore. It is of pivotal importance for inactivation gating. We recently showed that amino acid I1581 of DI-VS6 (rNav 1.4 amino acid numbering) is extraordinarily sensitive to both local and distal mutations suggesting a unique role in coupling of voltage-sensor movements to conformational changes in the pore. To date the only structural information relevant to voltage-gated Na channels can be derived from the recently crystallized bacterial channel NavAb. In this structure the amino acid homologous to I1581 faces the lipid phase and is in close spacial relationship to the voltage sensing apparatus. If this arrangement holds true for the eukaryotic Na channel then site 1581 should not be exposed to bulk solution. We tested this hypothesis by replacing I1581 by a titrable histidine. In wild-type channels changing the pH of the bulk solution form 7.4 to 8.2 had no effect on the voltage-dependence of fast inactivation. However, in I1581H the same change in pH resulted in a 9.51 mV ($p < 0.05$) hyperpolarizing shift of the voltage-dependence of fast inactivation. This suggests that site 1581 is at least partially exposed to the bulk solution and not completely embedded in the lipid phase.
Funding support: Austrian Science Fund P210006-B11, W1232-B11

Topic: Molecular Mechanisms of Cell Biology

P 70 The ambiguous role of TREM-2 in Escherichia coli peritonitis

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Backgound: The triggering receptor expressed on myeloid cells (TREM)-2, is a type-1 transmembrane protein, expressed on macrophages and other myeloid cells. Impaired TREM-2 signaling in humans causes a rare disease (Nasu Hakola disease) characterized by bone cysts and presenile dementia. Functionally, TREM-2 is an important mediator in immunity, as it negatively regulates TLR-mediated responses by macrophages and dendritic cells. Based on these facts and reports that identified TREM-2 as a phagocytic receptor for apoptotic cells and bacteria, we hypothesized that TREM-2 importantly contributes to the host defense against bacterial infections, such as E. coli peritonitis. Observations: In vitro studies confirmed the role of TREM-2 as a negative regulator. TREM-2 deficiency in peritoneal macrophages enhanced, while TREM-2 overexpression decreased cytokine productions in response to E. coli or LPS. Surprisingly, 6h after induction of E. coli peritonitis, we did not perceive increased cytokine levels in TREM-2 deficient mice compared to wild type animals. In contrast, TREM-2 deficiency prevented systemic inflammation, as indicated by lower IL-6 serum levels. Despite the fact that TREM-2 deficient mice produced lower levels of IL-6, IL-1beta and KC, we noticed an increased cell influx into the peritoneal cavity, which was associated with substantially enhanced levels of the chemoattractant for inflammatory monocytes, MCP-1. At later stages of the disease, TREM-2 deficient mice continued to exhibit elevated cell numbers in their peritoneal cavity together with higher bacterial counts, indicating impaired clearance of bacteria and possibly apoptotic cells. Conclusion: In this study we show that TREM-2 effects differ in vitro and in vivo. The impact of TREM-2 deficiency in vivo seems to be more complex and its role cannot be reduced to its function as a negative regulator of TLR signaling.

Topic: Cell Communication in Health and Disease

P 71 Epitope grafting between Bet v 1 and its homologue Api g 1 produces chimeric proteins with different lysosomal stability

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Background: We have shown that the major birch pollen allergen Bet v 1.0101 and its homologue Api g 1.0101 differed in their ability to polarise the allergen-specific immune response. In order to identify surface regions responsible for this behaviour, we produced four chimeras of Bet v 1.0101 and Api g 1.0101. In each of the chimeras roughly one fourth of the surface area of Api g 1.0101 were replaced by corresponding residues of Bet v 1.0101. It is known that resistance toward lysosomal degradation enhances immunogenicity. Our aim was to test our chimeras for lysosomal stability. Methods: Surface residues forming the P-loop (Api-Bet-1), the region opposite of the P-loop (Api-Bet-2), the area surrounding the C-terminus (Api-Bet-3), or the C-terminal alpha helix (Api Bet 4) of Bet v 1.0101 were grafted onto Api g 1.0101. Secondary structures were checked by CD-spectroscopy. For the degradome assay, Bet v 1.0101, Api g 1.0101 and the chimeras were digested with mircosomal/endo-/lysosomal enzymes. Reactions were stopped by heat denaturation followed by SDS-PAGE analysis and mass spectrometry. Results: All chimeras adopted secondary structures equivalent to Api g 1.0101. Degradome assays showed that one of the four chimeras (Api-Bet-2) had a remarkably higher lysosomal stability as compared to Bet v 1.0101 and Api g 1.0101. A 50% degradation was observed for Bet v 1.0101 after 9 hours, for Api g 1.0101 after 12 hours but for Api-Bet-2 after 96 hours. In contrast, almost total degradation was observed for Api-Bet-1 and -3 after 3 hours and for Api-Bet-4 after one hour. Conclusion: Alteration in protein structure when constructing allergen chimeras can result in unexpected increase or decrease of the overall protein stability. We produced a chimera whose stability was remarkably greater than the both starting molecules. This change may result in a shift of the immune response polarisation as compared to the wildtype allergens.

Topic: Immunology

P 72 Centerpoint Replotting And Its Effects On Central Retinal Thickness In Four Prevalent SD-OCT Devices

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P: In clinical and scientific practice central retinal thickness of CM and CP is frequently used as a quantative parameter obtained by SD-OCT. However this value might be dependent on correct plotting of the CP. An incorrect output of CMT/CPT values could lead to wrong clinical decisions (e.g. PRN). The reliability of this parameter is therefore of major importance. In this study the influence of CP plotting on CMT and CPT was investigated. M: 29 pathologic eyes were scanned on 4 commonly used SD-OCTs (SPEC, CIRR, TOP2, NID3) on the same day in random order. In a blinded fashion 2 experienced SD-OCT grading supervisors manually marked the true CP in all image stacks. All segmentation errors that could potentially confound thickness measurements were corrected manually. The distance between the manually set CP and the device-CP as well as the resulting differences in CMT and CPT were automatically calculated by the software and compared between the devices. R: The agreement of CP plotting between the two graders was excellent. The mean distance between the two plottings was 71,63 (SPEC: 58,83, CIRR: 90,92, TOP2: 78,9, NID3: 56,88). The mean distance between the true CP and the device-CP was 290,85 (SPEC: 190,5, CIRR: 248,28, TOP2: 530,9, NID3: 193,71). This inaccuracy of CP plotting resulted in mean thickness errors of 19,76 (CMT) and 37,44 (CPT). Thickness deviation was significantly different between the four devices (CMT/CPT; SPEC: 17,38/41,55, CIRR: 16,93/31,93, TOP2: 34,1/55,76, NID3: 10,66/20,52). The max deviations were (CMT/CPT) for SPEC 177/479, CIRR 137/173, TOP2 141/197, NID3 48/96. (All data in micrometers) C: In all tested devices inaccuracy of CP plotting was frequent and resulted in clinically relevant CMT and CPT deviations. However there is substantial inter-device variability in plotting performance. Manual replotting of the device-CP is necessary to control for thickness deviations that might confound clinical decisions.

Topic: Medical Physics

P 73 Facilitation of synaptic strength in the spinal cord induced by prolonged opioid exposure

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The use of opioids to treat moderate to severe pain might be limited by tolerance and opioid-induced hyperalgesia (OIH). We have reported recently that synaptic strength at C-fiber synapses in the superficial spinal dorsal horn is potentiated upon abrupt withdrawal from an acute systemic opioid administration [Science 325 (2009) 207]. In the present study, we show that prolonged administration of the ultra-short acting μ-opioid receptor agonist remifentanil also facilitates synaptic strength. This may contribute to the development of apparent opioid tolerance and OIH. In deeply anesthetized male adult rats C-fiber evoked field potentials were recorded in laminae I/II of the spinal cord dorsal horn after electrical stimulation of the ipsilateral sciatic nerve. Remifentanil was administered i.v. as a bolus injection followed by an infusion of up to 8 hours. The opioid receptor antagonist naloxone and the microglia inhibitor minocycline were administered i.v., the non-competitive N-methyl-D-aspartate (NMDA) – receptor antagonist D-AP5 and the glial cell inhibitor fluorocitrate were added to the artificial cerebrospinal fluid continuously circulating over the recording site. Remifentanil acutely depressed synaptic transmission at C-fiber synapses in the superficial laminae of the spinal cord dorsal horn to 41 ± 9% of control. This depression slowly turned into facilitation of synaptic strength to 188 ± 34% of control at spinal C-fiber synapses at 8 hours ($n = 8$, $p < 0.007$). This facilitation was blocked by naloxone. Glial inhibition by fluorocitrate fully prevented the increase in synaptic strength. In contrast to other known forms of facilitation of synaptic strength in the spinal cord, the blockade of NMDA receptors did not block synaptic facilitation during ongoing opioid administration. We are currently studying the signaling pathways underlying the facilitation of synaptic strength at nociceptive C-fiber synapses induced by prolonged systemic remifentanil treatment.

Topic: Neuroscience

P 74 Mass Spectrometrical Identification of Hippocampal NMDA Receptor Subunits NR1, NR2A-D and Five Novel Phosphorylation Sites on NR2A and NR2B

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The NMDA receptor (NMDA-R) is a key element in neural transmission and mediating a vast variety of physiological and pathological processes in the nervous system. It is well-known that phosphorylation is required for functioning of the NMDA-R, and we therefore decided to study this post-translational modification in subunits NR1 and NR2A-D. Immunoprecipitation with an antibody against NR1 was carried out from rat hippocampi and SDS-PAGEs were run. Bands were punched, destained, and digested with trypsin and chymotrypsin and peptides were identified by nano-LC-ESI-MS/MS using an ion trap (HCT). Proteins were identified using specific software. Phosphorylations were verified by phosphatase treatment and reanalysis by mass spectrometry. The NMDA-R subunits NR1 and 2A-D were identified. On NR2A, a novel phosphorylation site was observed at S511, and on NR2B, four novel phosphorylation sites were revealed at S886, S917, S1303, and S1323 by mass spectrometry and verified by phosphatase treatment with mass spectrometrical reanalysis. A series of NMDA-R phosphorylations have been reported and these serve different functions as receptor activation, localization, and protein-protein interactions. Herein, findings of novel phosphorylation sites are extending knowledge on chemical characterization of the NMDA-R and warrant studying function of site-specific receptor phosphorylation in health and disease.

Topic: Neuroscience

P 75 Protective effect of biliverdin and biliverdin reductase against bile acid-induced toxicity in liver cells

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BACKGROUND: The accumulation in hepatocytes of bile acids, such as deoxycholic acid (DCA), induces oxidative stress, which may result in cell injury. Under these circumstances, several antioxidant mechanisms, such as biliverdin reductase • (BVR•)-mediated bilirrubin (BR)/biliverdin (BV) cycle may play a protective role. A mutation (c.214C>A, p.Ser44X) in BVR• gene (BLVRA) has been recently described. Homozygous patients for this mutation suffer from episodes of green jaundice during cholestasis. AIM: To investigate whether, in these individuals, hepatocytes are less protected against potential bile acid-induced toxicity. METHODS/RESULTS: To express BVR• in mammalian cells and Xenopus laevis oocytes, the open reading frame of BVRA was cloned in appropriate plasmids. The c.214C>A mutation was reproduced using site-directed mutagenesis. Upon expression, mutated BVR• (mtBVR•) was analyzed by WB and immunofluorescence and its enzymatic activity was determined by HPLC-MS/MS. The results indicated that mtBVR• was a truncated protein with no ability to transform BV into BR. Using three human liver cell lines, cell viability and ROS generation were measured by flow cytometry using propidium iodide and dichlorofluorescein-diacetate, respectively. Differences in the expression levels of BVR••(HepG2>PLC/PRF/5>Huh-7), as determined by RT-QPCR and WB, and ROS generation (Huh-7>PLC/PRF/5>HepG2) were found. Treatment with increasing concentrations of $K_2Cr_2O_7$ (used here as a positive control of toxicity) or DCA resulted in enhanced ROS production and cell death. Both effects were inhibited, in a dose-dependent manner, by administration of BV. The expression of BVR•• and heme oxygenase-1 was not affected by the treatment with BV but was decreased by $K_2Cr_2O_7$ and up-regulated in response to DCA. CONCLUSION: These results suggest that BV may have a protective effect against bile acid accumulation, which is dependent on BVR• activity. This mechanism of defense could be important in liver diseases accompanied by cholestasis but is absent in individuals bearing homozygous inactivating mutations in BVRA.

Topic: Other

P 76 Imaging of Endogenous mRNA Variants in Living Plant Cells

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Nonsense-mediated decay (NMD) is a post-transcriptional surveillance mechanism that targets aberrant mRNAs for degradation. Removal of these transcripts is important to prevent the translation of potentially harmful truncated proteins. There seem to be several signals which act to trigger NMD such as the presence of a premature termination codon (PTC), an upstream open reading frame or a large distance between the termination codon and the poly(A) tail. A PTC needs to be positioned at least 50 nt upstream from the next exon junction complex in order to act as substrate for NMD. Previous work from Simpson et al. (2008) established an RT-PCR panel to identify and quantify alternative splicing in multiple genes of plants simultaneously. We used this technique to examine alternative splicing variants in NMD mutants including upf1-5 and upf3-1. The data shows that some transcripts containing signals for NMD are resistant to degradation. What keeps these transcripts from being translated? One possibility is that these particular splicing variants are retained within the nucleus. In 2011, Marquez-Ortiz et al. showed that at least 55 % of all Arabidopsis thaliana genes possess alternative transcripts, but unlike in animals, the most abundant splicing event in plants is the retention of introns (IR). We therefore focused on PTC-positive alternative transcripts with IR events, which are not sensitive to NMD. We want to investigate whether certain splice variants of the same gene localize to different compartments within the cell and/or nucleus. In particular, we would like to determine the abundance of mRNA variants of RS2Z33 (a plant specific SR protein) and other genes of interest in the cytoplasmic and nuclear fraction of the cells. Our approach combines a standard cell fractionation protocol with in vivo imaging of RNA molecules via hybridization-sensitive probes, termed Molecular Beacons (MB).

Topic: RNA-Biology

P 77 The role of Stat3 in murine Natural Killer Cells

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Natural Killer (NK) cells are classified as lymphocytes belonging to the innate immune system, but recent findings grant them multiple adaptive immune features. Generally, they play a major role in eliminating virus- or pathogen-infected as well as transformed cells. Their development and effector functions are tightly controlled by the Jak/Stat signaling pathway. Therefore, deregulations in this pathway are associated with developmental alterations and malfunctions in NK cells. In this study, we are investigating the role of Stat3 in NK cell development, survival and effector functions. To do so, we crossed Stat3fl/fl mice to Ncr1Cretg mice to specifically delete Stat3 in the NKp46+ NK cell compartment. We could show that loss of Stat3 does not influence the presence of NK cells in the periphery, but transcription factors involved in NK cell development like T-bet and Eomes feature an altered expression. In line with this, the maturation pattern and the expression of distinct surface markers and receptors are changed. Importantly, NK cells display a severe reduction in the expression of the cytokines IFN-□, TNF-□ and GM-CSF after IL-12 and PMA/Ionomycin stimulation in the absence of Stat3. Accordingly, we could detect higher viral titers in salivary glands after sublethal infection with murine cytomegalovirus (MCMV). Additionally, in vitro cytotoxicity against low MHC class I expressing tumor targets is impaired in NK cells lacking Stat3. In summary we conclude that Stat3 has an important role in the regulation of NK cell effector functions which is consequential with respect to the use of Stat3 inhibitors in NK cell surveyed diseases and in order to design optimal therapeutic treatments.

Topic: Molecular Signal Transduction

P 78 NeuROn - Neuropsychological Rehabilitation Online

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In pediatric neuro-oncology, cognitive impairments, such as challenges in attention and memory, are recognized as long-term side effects of brain tumors and/or treatment-related factors. Neuropsychological online-intervention for rehabilitation (NOIR) in this field still is at its very early stage of development; however, initial evidence shows a pressing need. The aim of this study is to provide an evidence- based, empirically-validated NOIR for adolescents with brain tumors. The focus will be on memory, learning and related functional outcomes. Drawing on international collaboration, an internet platform will be created, offering a rehabilitation program designed as a game called 'NeuROn' (Neuropsychological Rehabilitation Online). For empirical validation, a parallel-group randomized control trial will be conducted. Extension to the internet will significantly increase the accessibility to NOIR and allow for a greater potential of rehabilitation in patients.

Topic: Clinical Neuroscience

P 79 Abstract Withdrawn

P 80 Serum levels of Dkk-1 in patients with normal and impaired fracture healing

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Fracture healing is a complex process, which is induced by many activating and inhibiting mediators. Many factors for delayed fracture healing are well documented in literature. However, further studies are necessary for better understanding of decreased bone formation in non unions with regard to cell biology. The Wnt-ß-catenin signal pathway plays a major in the role in osteogenesis. Dicckopf-1 is a negative regulator of this pathway and inhibits bone formation by preventing osteoblastic differentiation. Many animal experimental studies could prove the potent effect of Dickkopf-1 neutralizing antibodies on bone development in general as well as on fracture healing. But no data exist on systematically quantified Dkk-1 levels during fracture healing in humans. This study should determine the activity of Dicckopf-1 in each phases of fracture healing. Furthermore the data should detect a possible nexus between Dkk-1 activity and delayed fracture healing.

Topic: Regeneration of Bones and Joints

P 81 The Role of Natural IgM Antibodies with Specificity for Malondialdehyde-adducts in Atherosclerosis

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Introduction: Atherosclerosis is a chronic inflammatory disease of the vascular wall. Inflammation, which involves both the innate and adaptive immunity, significantly modulates the disease process. Innate B1 cells that are the major source of IgM natural antibodies (NAbs) have been shown to be atheroprotective. Recently, we discovered that more than 30% of all NAbs are directed against various oxidation-specific epitopes (OSEs). It was further found that >10% of all B1 cell-derived IgM antibodies specifically bound malondialdehyde (MDA)-adducts. The major objective of my thesis is to characterize natural IgM responses to MDA-adducts and define their function in atherosclerosis. Methods/Results: Monoclonal IgM antibodies specific for OSEs were cloned from the spleens of atherosclerotic mice. Out of 195 hybridomas with specificity for OSEs, 52% bound to MDA-type adducts. The fine binding specificity of seven hybridomas was assessed by competition ELISA and all clones were found to have high specificity for MDA-type adducts. Sequencing of the variable regions of the hybridomas identified the VH gene usage and denominated >90% germ-line gene usage. Summary/Outlook: We identified a panel of MDA-specific NAbs in atherosclerotic mice. Following the identification of dominant MDA-specific NAbs, we will functionally analyze their role by selectively silencing single clones or clone-families by shRNA knock-down in the bone-marrow of atherosclerosis-prone mice. It is hypothesized that MDA-specific IgM NAbs protect from atherosclerosis and that they are to a large part responsible for the atheroprotective effect of serum IgM.

Topic: Inflammation and Immunity

P 82 An Essay Concerning the Molecular Prediction of Disease Outcome in Melanoma Patients

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Purpose: Melanoma is the most lethal form of skin cancer. Current prognostic clinical and morphological parameters are insufficient to accurately predict the outcome of individual patients. Several studies have described gene expression signatures to predict survival or metastasis of primary melanoma patients, however the reproducibility among these studies is disappointingly low. Experimental Design: We followed extended REMARK/Gould Rothberg criteria to identify gene sets predictive for disease outcome in patients with primary cutaneous melanoma. For class comparison, gene expression data from 116 patients with clinical stage I/II and 72 with III/IV primary melanoma were used. Significance analysis of microarrays identified the top 50 differentially expressed genes. In an independent data set from a second cohort of 31 primary melanoma patients, these genes were analyzed by multivariate Cox regression analysis and leave-one-out cross validation for association with disease outcome. Results: In a multivariate Cox regression analysis, expression of the genes Duffy blood group chemokine receptor and ribosomal protein S27-like gave the best predictive value ($P < 0.01$). A multivariate Cox proportional hazards model revealed these genes as an independent predictor for disease outcome, which significantly added ($P < 0.01$) to the predictive value of the most important morphological indicator, Breslow depth. Conclusion: Combination of molecular with morphological information may enable an improved assessment of disease outcome in primary melanoma patients. A strength of the gene expression set is the small number of genes, which should allow easy reevaluation in independent data sets and adequately designed clinical trials.

Topic: Dermatology

P 83 IgE cross-reactivity between Bet v 1 and the mung bean proteins cytokinin-specific binding protein and Vig r 1 in patients with birch pollen-associated allergy to mung bean sprouts

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Mung beans (Vigna radiata) contain two Bet v 1-related proteins: Vig r 1, a member of the PR-10 subfamily, and cytokinin-specific binding protein (CSBP), a protein with low sequence identity (31%) to Bet v 1. We aimed to compare Vig r 1 and CSBP to Bet v 1 regarding biochemical and immunological properties. Percent surface identity between Bet v 1, CSBP and Vig r 1 was calculated based on structural alignments using an algorithm considering backbone conformations and identities of aligned residues. The allergens were expressed in Escherichia coli and purified by chromatographic methods. Binding and cross-reactivity of IgE from Bet v 1-sensitized patients' sera to rCSBP, rVig r 1.0101 and rBet v 1.0101 were examined by ELISA, ELISA inhibition and RBL assays. Structural comparison of the three proteins revealed that 29% of the solvent-accessible surface area of CSBP was identical to Bet v 1, while Vig r 1 and Bet v 1 shared 50% surface area. In addition, two surface patches conserved between Bet v 1 and CSBP were identified as potential cross-reactive epitopes. 76% and 33% of Bet v 1-sensitized birch pollen allergic patients' sera (n=33) showed IgE binding to Vig r 1 and CSBP, respectively. Of 18 Bet v 1-sensitized patients, who reported reactions or had positive prick-to-prick tests to mung bean sprouts, 78% showed IgE binding to Vig r 1 and 50% to CSBP. Bet v 1 totally inhibited IgE binding to CSBP and Vig r 1. CSBP showed inhibitory activity on IgE binding to Vig r 1 and vice versa. Detection of Bet v 1-related proteins in mung bean extracts by an immunoblot using an anti-Bet v 1 antibody revealed a 17 kDa band in sprouts but not in dried beans. This study, supported by grants P-22559-B11 (CR) and SFB-F01802 (HB) from the Austrian Science Fund, demonstrates IgE cross-reactivity between Bet v 1, Vig r 1 and CSBP, despite their low sequence identity. Additionally to Vig r 1 CSBP might contribute to allergic reactions in mung bean sprouts.

Topic: Immunology

P 84 O-GlcNAc modification of STAT5a is essential for its transforming properties

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According to the Austrian Institute for Statistics hematological neoplasms are the reason for approximately 7% of all new cancer diagnoses in Austria every year. They can be divided into the three classes of leukemias, lymphomas and myelomas. The Signal Transducer and Activator of Transcription (STAT) 5 transcription factors are known to play a role in the differentiation and proliferation of all hematopoietic cell types and there is evidence that they play a crucial role in the formation of all three major classes of hematologic neoplasms. Persistent activation of STAT5 is essential for the leukemic function of chromosomal translocations like BCR/ABL or point mutants like JAK2V617F. Although there are no known activating mutations in STAT5 proteins in cancer, the single point mutation Ser710 to Phe of STAT5a (=cS5) renders it prolonged active once induced by cytokine or growth factor action. Ectopic expression of cS5 in bone marrow cells leads to multi-lineage leukemia in bone marrow transplant experiments into lethally irradiated recipient mice. To elucidate interacting molecular players required for STAT5-induced leukemic transformation, we introduced an additional mutation into cS5. We mutated Thr92 of STAT5a to abolish O-linked N-acetylglucosamine (O-GlcNAc) modification of STAT5, which is a prerequisite for binding of the transcriptional co-activator CBP to DNA bound STAT5a. Genetic complementation assays in primary STAT5δNδN T-cells or wild type bone marrow transductions with STAT5 variants followed by transplants revealed that this mutation maintains biological activity of STAT5a, but surprisingly, it completely abolished cS5-induced leukemogenesis. Our data suggest that full transcriptional activity of oncogenic STAT5a requires O-GlcNAc modification at Threonine 92.

Topic: Molecular Signal Transduction

P 85 Oxidative Damage to Basal Ganglia in Multiple Sclerosis

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The mechanisms of tissue injury in multiple sclerosis are only partly understood. Our previous data show that oxidative damage (Haider et al, Brain, 2011) through NADPH-oxidase mediated respiratory burst is involved (Fischer et al, Brain, 2012). Iron, which is stored within the CNS in oligodendrocytes, primary targets of MS lesion formation, shows marked increase in the Basal Ganglia (Spatz, 1922). Basal ganglia atrophy is one of the earliest, probably preclinical, events in MS that are detected with MRI-imaging (Filippi, Lancet Neurology, 2012) and is significantly correlated with mental deficits (Batista, J Neurol, 2012). We have analyzed parafin embedded autopsy material from 38 MS patients and 11 controls by histochemistry and immunocytochemistry with antibodies detecting oxidized epitopes and microglia activation. We confirmed the high iron content in the Basal Ganglia of MS patients and controls. Demyelinating lesions in the Basal Ganglia were small, in median affecting 5% of Basal Ganglia area and were found in 18 of 32 patients. Lesions in the Basal Ganglia were mainly inactive, demyelinated plaques and microglia activity depended upon plaque activity. As in other demyelinated MS lesions acute neuronal injury exceeded that of controls. Normally appearing gray matter in Basal Ganglia from MS patients showed more microglia activity compared to controls, as determined by densitometry and neuronal and axonal damage in normally appearing gray matter of Basal Ganglia was significantly increased compared to that measured in controls. Our study shows that besides demyelinating lesions, there is a diffuse neurodegenerative process in MS patient's Basal Ganglia, which appears to be the substrate of currently unexplained radiological and clinical events of this disease.

Topic: Neuroscience

P 86 Notch signaling plays a role in human placental development: regulation of cell column proliferation and trophoblast invasion

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Objective: Notch signaling is a highly conserved pathway controlling proliferation, cell death and differentiation. Here, we investigated for the first time localization of all Notch receptors (Notch1-4) and their ligands (Jagged1 and 2, Dll1, 3, 4). Moreover, the role of Notch signalling in cell column proliferation and trophoblast invasion was analyzed. Methods: Expression patterns of proteins were analyzed in 1st trimester placentae using immunofluorescence. Notch activity was evaluated in the trophoblast cell line SGHPL-5 using a luciferase reporter containing wildtype or mutant RBPJÎº binding sites; constitutive activation of the pathway was achieved by overexpression of the Notch-1 intracellular domain (NICD). Invasion and migration were studied in transwell assays of SGHPL-5 cells and villous explant cultures on collagen-I, respectively. Proliferation was measured by BrdU labelling. Cross-talk to Wingless (Wnt) signalling was analyzed by using a canonical Wnt reporter. Experiments were performed in the absence or presence of the Notch inhibitor DAPT.Results: Immunofluorescence indicated predominant expression of Notch 1-3 in cell columns, whereas non-proliferating, extravillous trophoblasts specifically expressed Dll3. RBPJÎº reporter assays revealed basal Notch activity in SGHPL-5 cells which was enhanced upon overexpression of NICD. Activation of the pathway through NICD also suppressed Wnt signalling. Inhibition of Notch signalling increased invasion and migration of the trophoblasts, but also enhanced proliferation in cell columns. Conclusion: Notch signalling could play a major role in controlling cell column proliferation and differentiation. In proliferative trophoblasts Notch signalling may negatively affect Wnt signalling since the canonical Wnt pathway is active in invasive trophoblasts but not in cell columns. However, complex expression patterns of Notch receptors and ligands in the placenta suggest trophoblast subtype-specific roles of the pathway.

Topic: Molecular Signal Transduction

P 87 Characteristics, treatment modalities, clinical outcome and evaluation of new treatment techniques in bony avulsion of the FDP

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Introduction: A bony avulsion of the flexor digitorum profundus tendon (FDP), or „Jersey Finger", is a rare flexor tendon injury. Our primary objective was to state the characteristics, evaluate treatment options and to present long term clinical outcome. New suture anchor techniques were tested for pull out strength and loading capacity. Methods: 22 Patients with a bony avulsion of the FDP were evaluated. We analyzed treatment modalities, trauma mechanism, as well as functional outcome. Patients were separated into 2 Groups: Group1: conservative treatment of the FDP bony avulsion. Group2: operative treatment of the FDP bony avulsion. Patients underwent functional examination testing the range of motion, tensile strength and pinch strength. Outcome questionnaires supported the clinical result. Two different suture anchor implants were identified and we are conducting a comperative study on 30 human cadaver distal phalangae. Data of pull out strength and loading capacity, as well as dislocation tendency and failure mechanism is being collected. Results so far: Injury at the ring finger was prevalent. In 13 cases an ORIF was performed to reattach the bony avulsion. Screws and pullout button were used. These patients showed a slight extension or flexion deficit at the end of the therapy. Nine patients were treated conservatively for 3-4 weeks at a dislocation up to 1 mm of the bone fragment. These patients presented a good functional outcome despite a static treatment. Discussion: Our clinical study shows that both treatment methods lead to a satisfying result, although the operated patients had a slightly longer functional recovery. At the moment we are testing two suture anchor implants and comparing their tensile strength and loading capacity by cyclic testing. We expect to gain data about the stability of the anchors and the suture material preventing further dislocation after operative treatment and so, ensuring enough strength for dynamic postoperative treatment.

Topic: Regeneration of Bones and Joints

P 88 Microbial analysis in saliva – a new way to determine periodontopathic bacteria

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Objectives: The determination of bacteria associated with periodontal disease is a common method to facilitate the decision for a possible antibiotic therapy additionally to conservative periodontal treatment. Currently this is mostly performed by inserting paper points in the periodontal pocket after supragingival plaque removal, drainage and collection of a pooled sample. The aim of this study was to investigate whether the microbial analysis of saliva samples is correlating with the much more intricate paper point method. Material and Methods: Seventy-five patients with chronic and aggressive periodontitis participated in saliva and subgingival plaque sampling before conservative periodontal therapy. For the stimulated saliva collection, the "Saliva Collection System®" (Greiner Bio-One, Kremsmuenster, Austria) was used. Twenty-one different bacteria were extracted from the samples and determined by PCR "ParoCheck®"(Lambda, Rainbach, Austria) and a correlation analysis between the two methods was performed for each bacterial species. Results: The semi-quantitative analysis of Aggregatibacter actinomycetemcomitans (A.a.), Porphyromonas gingivalis (P.g.), Tannerella forsythia (T.f.) and Treponema denticola (T.d.) as well as 14 other bacterial species showed a significant correlation between the saliva and subgingival plaque samples (Spearman's rho: 0.237-0.797; $p < 0.05$). Yet, the red complex (P.g., T.d., T.f.) was detected more frequently in subgingival plaque samples compared to the saliva samples. Conclusion: The identification of periodontopathic bacteria in saliva is largely equivalent to the already established subgingival sample collection. Salivary diagnostics could be used for the screening of periodontal disease by combining the determination of bacteria and periodontal disease-associated biomarkers. Nevertheless, the accurate microbial analysis of single sites is still only possible by paper point sampling in the periodontal pocket.

Topic: Regeneration of Bones and Joints

P 89 Evaluation of the mechanism of action of a novel anti cancer compound

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Background: A novel quinoxalinhydrazide derivative, originally developed as an HIV-1 integrase inhibitor, was identified as a promising drug candidate for the treatment of cancer due to its strong cytotoxic activity. Preliminary studies that showed similarity to substances inhibiting topoisomerases and the compound's structure suggest that it targets DNA binding or processing enzymes, but the main mode of action remains unknown to date. The project aims at elucidating the compound's mechanism of action. Observations: The compound was included in an anti-cancer activity screen by the National Cancer Institue (NCI-60 Screen) where it was tested against 60 different human tumor cell lines. This screen revealed very strong anti-cancer activity in tumor cell lines of all cancer types with a mean IC50 of 200 nM. Based on these data a computer analysis of drug activity profiles (NCI COMPARE) was performed in order to detect compounds with a similar cytotoxicity profile in the NCI-60 screen. Most of the hits resulting from this analysis are compounds that have a mode of action related to DNA damage induction and are interacting with or inhibiting topoisomerases. Together with the similarity to Camptothecin and Mitoxantrone (topoisomerase I and II inhibitors, respectively) that was suggested by preliminary gene expression studies, this points towards a DNA damage-inducing mode of action of the investigational compound. Indeed, in immunofluorescence stainings gamma-H2AX was found to be elevated upon treatment, an observation further underlined by elevated p-ATM protein expression levels. Conclusion: Concluding, first impressions of the mechanisms of action of this potent drug candidate could already be gathered, suggesting that it is a potentially new DNA damage-inducing agent. However, the exact nature of DNA damage and its mode of action still need to be investigated in more detail.

Topic: Malignant Diseases

P 90 Expression Level of the Receptor for Advanced Glycated End Products (RAGE) in the Syncytiotrophoblast correlates with the Severity of Per-Eclampsia as demonstarted by a novel Method for automated in-situ Quantifications of Proteins

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OBJECTIVES: The receptor for advanced glycated end-products (RAGE) contributes to tissue damage in many inflammatory diseases. Ligand-RAGE interaction activates a cell response that promotes inflammation. Pre-eclampsia (PE) is characterized by systemic inflammation. Available data suggest up-regulation of placental RAGE during PE and consequently involvement of the ligand-RAGE axis in the promotion of inflammation in PE. However, human-based approaches to quantify placental protein-expression remain semi-quantitative and prone to inter-observer differences. Tissue-Cytometry combines microscopic analysis of tissues with fast and reproducible computation of tissue-associated parameters. Automated image segmentation is essential for computer-based tissue evaluation, but often operates by cell identification via their nucleus preventing analysis of multinuclear or a-nuclear tissue elements, e.g. erythrocytes. To enable automated in-situ analysis of placental RAGE expression in health and PE, we developed and validated algorithms able to segment the multinuclear syncytiotrophoblast (STB) in the complex shaped placental villi. METHODS: Paraffin-sections of chorionic tissue from PE (n=14) and control (n=13) placentas were labeled with target-specific primary and fluorescent secondary antibodies. A composite image consisting of 81 frames/placenta was acquired and digitalized with a software-driven motorized epifluorescence microscope (TissueGnostics GmbH). Automated identification of STB area by cytokeratin-7-expression or of auto-fluorescent erythrocytes by their shape was done combining classical digital image-processing and pattern recognition approaches with machine-learning techniques. RESULTS: The developed algorithms identified STB areas as well as erythrocytes in-situ as good as human experts. RAGE expression was quantified after in-silico subtraction of background fluorescence caused by erythrocytes. A positive correlation between RAGE-expression in the STB and severity of PE was demonstrated showing 2.5-fold increase of RAGE in severe cases of PE. CONCLUSION: We established a novel approach for fast and reproducible automated analysis of immunofluorescent-labeled placental chorionic tissue, and demonstrated correlation of RAGE expression levels with severity of PE.

Topic: Molecular Mechanisms of Cell Biology

P 91 Follistatin as an inducer of lymph vessel formation in melanoma

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Activin A proteins are homodimers of inhibin βA subunits and potently inhibit blood vessel angiogenesis in part via induction of p21. Follistatin serves as an antagonist, it binds activin with high affinity and neutralizes most but not all of its biological actions. To investigate the role of follistatin in lymph vessel angiogenesis in melanoma, we selected human A375 melanoma cells which express high levels of the inhibin βA subunit. We stably over-expressed control vectors or follistatin and injected them intradermally into SCID mice. The arising primary tumours were excised at a size of 400mm3 and animals were monitored for metastasis to sentinel lymph nodes (SLN). Blood and lymph vessel formation was quantified by immunohistochemistry and real time PCR. We found growth rates of primary tumors to be equal in controls and follistatin over-expressing melanoma, as well as percentages of Ki67 positive cells. In contrast, mean times to metastasis to SLN were reduced by follistatin and this inversely correlated with significantly increased numbers of lymph vessels in primary melanoma. In conclusion, our results point to a novel role of follistatin as an inducer of tumor lymph angiogenesis.

Topic: Vascular Biology

P 92 Hippocampal levels and activity of the glutamate transporter 1 (GLT-1) and sodium/potassium transporting ATPase subunit alpha-3 (AT1A3) are paralleling memory training in the multiple T-Maze in the C57BL/6J mouse

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The glutamate transporter 1 (GLT-1) is essential for glutamate uptake in the brain and associated with various psychiatric and neurological disorders. The sodium potassium transporting ATPase subunit alpha 3 (AT1A3) has been proved to be linked to memory mechanisms in rodents. But no studies on regulation of native GLT-1 and ATPase complexes by memory performance in a land maze have not been shown so far. C57BL/6J mice were used for the spatial memory training experiments and trained mice were compared to untrained (yoked) animals. Total enriched membrane fraction was prepared from mouse hippocampi. Membrane proteins were separated by blue native PAGE with subsequent Western blotting against GLT-1 and AT1A3. Moreover, membrane protein complexes containing GLT-1 and AT1A3 were identified by mass spectrometry. Animals learned the MTM task and complexes containing GLT-1/AT1A3 were clearly detected on BN-PAGE Western blotting, respectively. GLT-1/AT1A3 complexes levels were significantly higher in the trained group and antibody specificity was verified by immunoblotting on multidimensional gels. ATPase activity was higher in trained mice. GLT-1 and AT1A3 were unambiguously identified by mass spectrometry combined with multi-enzyme digestion and novel posttranslational modifications were observed. It is revealed that increased membrane protein complex levels of GLT-1 and AT1A3 are paralleling and are linked to spatial memory training. We provide evidence that signal termination, represented by the excitatory amino acid transporter GLT-1 complexes, is involved in spatial memory mechanisms. It was shown that increased AT1A3 protein levels for the dimer as well as AT1A3 activity represented by the monomer and the dimer were paralleling memory training in the MTM. This may be relevant for understanding the role of the catalytic hydrolysis of ATP coupled with the exchange of sodium/potassium ions across the plasma membrane that generates the electrochemical gradient of sodium/potassium ions.

Topic: Neuroscience

P 93 Characterization of the MHC-helices' tertiary structure via differential geometric parameters

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Major histocompatibility complexes (MHC) are heterodimeric cell surface receptors present in most vertebrates. Although their overall structure, consisting of two alpha-helices and eight antiparallel beta-strands, is essential identical for all MHC structures, subtle alterations in the spatial arrangement of certain amino acids in this overall shape are observable in x-ray structures. These arrangements can be caused by differences in the primary amino acid sequence (MHC alleles), by different peptides presented by the MHC, or by other receptors binding to the MHC. We have previously shown how the MHC alpha-helices can be described in an structured and reliable way by fitting curves through their C-alpha coordinates in a local coordinate system (Hischenhuber et al., 2012). This method enabled us to compare MHC alpha-helices between different MHC alleles as well structural alteration over time for the same complex. In the present study we move one step forward and present several differential geometric parameters for these curves to obtain a deeper understanding of the MHC alpha-helices. These parameters include: (1) The local curvature, which is a measurement describing the deviation of a curve from a straight line. For a better description of the curve progression, we calculated additionally the total curvature over specific ranges of the curves. (2) The local torsion describes the deviation of a curve from a plane developing. Due to the same reasons as above described, we calculated the total torsion over specific ranges. (3) Furthermore we calculated the area of the ruled surface spanned by the two curves of the alpha-helices. We additionally intend to calculate the surface curvature. These parameters will be the basis for further studies, where we analyze the influence of different peptides, bound inside the MHC binding groove, and further compare different MHC-types in a structured way.

Topic: Medical Informatics, Biostatistics and Complex Systems

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P 94 Gene amplification but not epigenetic alterations cause aberrant vitamin D 24-hydroxylase expression in colon cancer

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Background: Colorectal cancer is the third most common cancer in the world. Low vitamin D status is associated with increased risk of colorectal cancer. The most active vitamin D metabolite, calcitriol, is catabolized by CYP24A1 (1,25-dihydroxyvitamin D3-24-hydroxylase). This enzyme is overexpressed in colorectal cancer and likely reduces the anti-tumorigenic functions of calcitriol. Aim: We examined whether genomic rearrangements and promoter hypomethylation are causes behind the overexpression of CYP24A1 in colorectal cancer. Methods: We determined CYP24A1 genomic copy number (N=127) and mRNA expression of vitamin D pathway genes and proliferation markers (N=69) in colorectal tumors and respective adjacent mucosa by quantitative real-time PCR. The methylation status of the promoter was assessed by bisulfite sequencing (region +494 to -609, N=20). Results: We detected gene amplification of CYP24A1 in 60% of colorectal cancer patients. A high copy number gain of CYP24A1 correlated with increased mRNA expression (Spearman Correlation Coefficient (SCC) 0.620, $p<0.032$). Further, we found a correlation between CYP24A1 expression and proliferation marker MCM2 (SCC 0.341, $p<0.009$) which strongly increased in patients with high copy number gains (SCC 0.857, $p<0.001$). We detected no differences in the CYP24A1 promoter methylation status between tumors and their respective adjacent mucosa. Conclusion: Gene amplification but not promoter hypomethylation is a mechanism behind the overexpression of CYP24A1 in colorectal cancer. The high expression of the calcitriol catabolizing enzyme CYP24A1 likely limits the tissue exposure to the anti-proliferative effects of calcitriol and thereby provides a growth advantage for the tumor.

Topic: Tumorbiology - Oncology

P 95 Adipokines in pediatric and adult obesity - TOBI Kids

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Prevalence of pediatric obesity is rising alarmingly. It is associated with a chronic low-grade inflammation ending up in type 2 diabetes mellitus. The aim of the study was to evaluate differences in metabolic and inflammatory alterations between obese kids and obese adults. We recruited 71 non-diabetic obese young with BMI exceeding the 97th percentile for age and sex, aged from 10 to 18 years. The adult cohort contained 55 patients, aged from 20 to 65 years with BMI greater 40 kg/m². Anthropometric parameters were evaluated and measures of insulin resistance were obtained by oGTT and using HOMA-IR. Serum and plasma samples were taken. Anthropometric data of the study populations (mean±STD): Weight was 100.1 (26.6) in kids and 134.2 (24.3) kg in adults. Metabolic and inflammatory data: the mean glucose levels were 82.4 (9.7) (kids) and 93.5 (9.9) mg/dl (adults), insulin in kids 24.4 (24.2), in adults 22.5 (18.2) U/ml, HOMA-IR 5.3 (6.4) (kids) and 5.3 (4.6) (adults) and the hs-CRP was 0.52 (0.6) (kids) and 1.0 (0.9) mg/dl (adults). Mean levels of adipokines: adiponectin 6.9 (3.1) (kids) and 8.6 (3.2) g/ml (adults), leptin 50.3 (26.9) (kids) 67.8 (22) ng/ml (adults), IL-6 5.4 (9.9) (kids) 5.8 (3.2) pg/ml (adults) and MCP-1 306.3 (125.6) (kids), 185.5 (49.2) pg/ml (adults). We found significant differences between kids and adults in all analyzed adipokines: adiponectin ($p=0.001$), MCP-1 ($p\leq0.001$), IL-6 ($p\leq0.001$) and leptin ($p=0.001$). MCP-1 was the only adipokine which was significantly higher in the pediatric cohort than in the adult.Obesity in pediatric patients is associated with alarming signs of low-grade inflammation and insulin resistance. The comparison between pediatric and adult obesity showed significantly higher MCP-1 levels which may be a predictive factor in pediatric obesity for state of insulin resistance.Support: European Community 7th Framework Programme (FP7/2007-2013), grant no. 201608 (TOBI - Targeting OBesity-driven Inflammation) and the FWF DK-CCHD (W1209-B09; all to T.M.S.).

Topic: Cell Communication in Health and Disease

P 96 Mechanistic analysis of costimulation blockade-resistant rejection of donor bone marrow triggered by donor T cells

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Background: Donor T cells have pleiotropic effects in allogeneic bone marrow transplantation (BMT). Co-transplanting high doses of donor T cells with donor BM causes rejection of donor BM despite costimulation-blockade. In the present study we investigate the molecular mechanisms responsible for this seemingly paradoxical phenomenon. Methods: Recipients (C57BL/6) were treated with 3Gy TBI and received approximately $20x10^6$ unseparated Balb/c BMC and costimulation-blockers (anti-CD154mAb, CTLA4Ig). 30x106 CD4 T cells (MACS isolation) from Balb/c, CB6F1 (Balb/cxB6), irradiated Balb/c or C3H donor were co-transplanted. Groups either received anti-IL-6, anti-IFN-y, anti-LFA1mAb or rapamycin. Multilineage chimerism was followed by flow cytometry and cytokine release was analyzed. Results: Co-transplantation of $30x10^6$ CD4 T cells but not CD8 T cells triggered rapid BM rejection of donor BM under costimulation-blockade within one week in an otherwise successful protocol (0/13 vs 17/20 chimeras, $p<0.001$). The levels of IL-6, IFN-y, IL-17A ($p<0.05$) and TGF-ß were found to be higher in mice treated with additional donor T cells. The neutralization of IL-6, but not of IFN-y abrogate the detrimental effect of donor T cells (5/7 vs 0/5 chimeras; $p<0.05$). The injection of CB6F1 or irradiated Balb/c CD4 T cells induced chimerism (5/6 and 4/5 vs. 0/4 chimeras with Balb/c T cells; $p<0.05$) whereas C3H CD4 T cells led to BM rejection (0/5 vs 9/9 chimeras BMT, $p<0,001$). The additional treatment with rapamycin or anti-LFA1 overcame the negative effect of donor T cell injection (5/5 and 6/6 vs 0/4 chimeras; $p<0.01$). Conclusion: The abrogation of BM engraftment through co-transplantation of donor CD4 T cells depends on IL6, requires proliferative capacity of injected donor T cells and needs to recognize the recipient as allogeneic. Neutralisation of IL-6, rapamycin and anti-LFA1 overcome the effect of co-transplanted donor CD4 T cells and offer potential targets for therapeutic intervention in costimulation blockade-resistant rejection.

Topic: Inflammation and Immunity

P 97 The impact of recipient age on the outcome of bone marrow transplantation-based tolerance induction

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Background: In skin and heart graft models immune senescence substantially alters alloreactivity. In young murine recipients tolerance induction through mixed chimerism holds promise for clinical translation. Therefore we investigated the influence of recipient age on the outcome of allogeneic bone marrow transplantation (BMT) for the purpose of mixed chimerism and tolerance induction. Methods: Young (2mts; 20g) and old (12mts; 25g) recipients (C57BL/6) were treated with either 3 or 1Gy TBI and received un-separated Balb/c BM cells adjusted to the body weight ($1x10^3$/kg, i.e. young:$20x10^6$/mouse; old:$25x10^6$/mouse) and co-stimulation blockade consisting of anti-CD154mAb and CTLA4Ig. Lymphocyte subsets and cytokine production were compared between young and old naïve mice and multilineage chimerism was followed by flow cytometry. Results: Old naïve mice contained significantly higher frequencies of CD4 and CD8 memory T cells (CD44highCD62Llow; $p<0.001$), early activated CD4 T cells (CD4+CD69+; $p<0.001$), less CD4 ($p<0.05$) and CD8 (p=n.s.) T cells and comparable amounts of regulatory T cells (Tregs; CD4+CD25+, p=n.s). After polyclonal T cell stimulation, older CD4 T cells released significantly more IFN-gamma ($p<0.05$), IL-6 ($p<0.05$) and as well IL-2, IL-10 and TNF-alpha. Old recipients became chimeric with frequencies comparable to young recipients following an irradiation with 3Gy TBI and co-stimulation blockade (26/29 vs 20/30 chimeras, week1 and 15/17 vs 17/18, week6). Notably, old recipients became even chimeric with reduced of TBI of 1Gy, whereas, in sharp contrast, none of the young recipients became chimeric under those conditions (4/8 vs 0/8 chimeras in young recipient, $p<0.05$, week3). Conclusion: BM engraftment and chimerism was successfully induced in aged recipients even with a lower dose of irradiation. Those results support the clinical relevance of the chimerism strategy for tolerance induction for a wide group of potential patients.

Topic: Inflammation and Immunity

P 98 Computertomography-based evaluation of volumetric changes after sinus floor augmentation

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Background: Maxillary sinus floor augmentation through a lateral approach is the most frequently used method to increase bone height in the posterior maxilla to allow for implant placement. However, substantial resorption of autologous bone grafts occurs during healing. Addition of Bio-Oss® granules may reduce graft resorption, thus improving graft stability. Aim: The aim of the present study is to assess volumetric changes of different ratios of grafting material for maxillary sinus floor augmentation using computed tomographies. Materials & Methods: Postoperative computed tomographies were available from 31 sinus floor augmentations (25 patients). Computed tomographies of the maxillary sinuses were obtained postoperatively, after 14days and 5months, respectively. Different ratios of Bio-Oss® to autologous bone were used for grafting through a lateral approach. Volumetric changes of the grafts were evaluated using the Definiens Developper XD software. Results: Mean graft volume after 14 days and 5 months was 1,7cm3 (±0,8cm3) and 1,5ccm (±0,8ccm), respectively. Based on volumetric measurements of the grafts mean shrinkage was 16%. The volumetric reduction was significantly influenced by the ratio of Bio-Oss® and autologous bone ($rs=-0.52$, $p<0.001$). No influence of age, gender and Bio-Oss® particel size was observed. Conclusion: Within the limits of the study, the results indicate a significant reduction of graft volume after 5 months of healing. Higher percentages of Bio-Oss® resulted in reduced graft shrinkage. However, further studies are needed to assess the optimized ratio of Bio-Oss® and autogenous bone to achieve long-term graft as well as implant stability.

Topic: Regeneration of Bones and Joints

P 99 Establishment of a new ischemic excision model

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To test therapeutic schemes improving blood perfusion in a preclinical setting, we wanted to develop a delayed wound healing model caused by insufficient perfusion of the wound area. To induce ischemia, an abdominal flap (8x8cm) was harvested. After ligation of an unilateral caudal epigastric bundle the flap was sutured back and wounds (ø1.5cm) were created in the ischemic and non-ischemic side of the flap. Wounds were analysed by a planimetric software (Lucia G1) and expressed as a percentage of the total postoperative wound surface area. Laser Doppler imaging was performed to confirm ischemia in the corresponding area. Histological and immunohistochemical staining were performed to follow new vessel formation. Animals were divided into three groups (n = 6-8) according to the treatment of the excisions: Group VEGF (100ng human VEGF-165 in 1mL fibrin sealant), group fibrin treated (1mL fibrin sealant) and group sham (control group). Laser Doppler imaging revealed enhanced perfusion over 7 days in all groups, with an insufficient perfusion in the ischemic area. After 7 days all wound areas decreased compared to day 0. Wound healing in vital areas occurred faster than in ischemic areas, with up to 50% smaller wounds (sham group). VEGF treated wound closure was slower than in fibrin or sham group. Histology and immunohistochemistry need to be analyzed to follow new vessel formation and reveal differences in wound contraction and reepithelialization.
Study was supported by EC-1014483 Angioscaff (FP7-NMP-2007)

Topic: Regeneration of Bones and Joints

P 100 Bacterial Ghosts as Delivery Systems for Foreign Proteins

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Using immunogenic structures of pathogens as part of (recombinant) subunit vaccines has become a popular method in vaccination, as it reduces the risks and side effects commonly associated with vaccines derived from inactivated or attenuated live microorganisms. While subunit vaccines allow for specific and selective immune responses, many antigens need to be supplied together with adjuvants to induce protective immunity in a host. The Bacterial Ghosts platform technology constitutes a novel approach in vaccine design; the recombinant expression of antigens is combined with the formation of Bacterial Ghosts (BGs) that function as adjuvant. Due to the controlled expression of gene E from bacteriophage φX174, a transmembrane tunnel structure is formed, sealing the periplasm and lysing the bacteria; the cytoplasm and DNA are expelled during the lysis process. The resultant empty cell envelopes are referred to as BGs and preserve the structural and morphological determinants of their living counterparts. Antigens are retained in BGs by anchoring them in either the Inner or Outer Membrane or exporting them into the sealed periplasmic space (PPS). In this work, two highly immunogenic outer membrane proteins from Chlamydia trachomatis, MOMP and PorB, were chosen as model antigens for incorporation in the PPS of non-pathogenic Escherichia coli strains. After small and large scale production of recombinant BGs and downstream processing, quality control including colony forming unit determination, flow cytometry analysis, determination of residual DNA by qPCR as well as immunoblotting against lysis protein E and recombinant proteins was performed. Quantification of chlamydial model antigens in BGs showed that the recombinant proteins were retained after E-lysis and constituted between 20% and 30% of the resultant BGs' dry mass. These data support and encourage the use of recombinant BGs as safe and effective delivery vehicles for antigens within an adjuvant environment.

Topic: Other

P 101 Topical Application of Glucocorticoids for Hearing Preservation in an Animal Model of Cochlear Implantation

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Glucocorticoids (GC) are used for the treatment of a wide variety of inner ear diseases. Limited effectiveness of systemic GC-therapy and severe side effects of long term GC-treatment make topical drug delivery to the inner ear favorable to systemic therapy. The application of thermoreversible hydrogels has emerged as a promising approach to improve topical drug administration. Dexamethasone, a highly potent GC, has beneficial effects in the setting of various conditions affecting the inner ear, including cochlear implant electrode insertion trauma. Another GC, triamcinolone-acetonide, is used successfully in surgery-protocols aiming at preservation of inner ear function (e.g. residual hearing preserving cochlear implantation), but only limited experimental data on this GC is available in the literature. In particular, triamcinolone-acetonide has never been applied in a thermoreversible poloxamer 407 hydrogel with the aim of prolonged drug delivery and a direct comparison of dexamethasone with triamcinolone-acetonide for otoprotective effects, using such a hydrogel for drug delivery, has not been performed up to date. We therefore have established a guinea-pig model for cochlear implantation at our department, which is used to compare the otoprotective effects of these drugs. After implantation of a cochlear implant electrode into the basal turn of the cochlea, 50 µl of GC-containing hydrogel, hydrogel only or NaCl are applied to the round window niche. Hearing is measured pre- and postoperatively as well as after 3, 7, 14, 21 and 28 days, using compound action potentials of the cochlear nerve and auditory brainstem responses. The model as well as preliminary data comparing hearing preservation in the different groups will be presented.

Topic: Molecular Signal Transduction

P 102 Does the MTHFR 677C>T influence genetic imprinting and FVIII activity in hemophilia A carriers?

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Background: In hemophilia A carriers the unaffected FVIII allele is assumed to be correctly transcribed leading to adequate factor VIII (FVIII:C) activity levels. Occasionally, carriers of a mutated FVIII are reported to exhibit a hemophilia A phenotype which may be due to inactivation (XCI) of the unaffected X-chromosome. The inactivation of one X chromosome in each female cell is achieved by hypermethylation. In this process the methylene tetrahydrofolate reductase (MTHFR) plays an important role. The MTHFR concentration is affected by the MTHFR 677C>T mutation, and it is thus possible that the MTHFR genotype influences XCI as well as FVIII levels. Patients and methods: We evaluated XCI in 55 proven carriers of hemophilia A and in 103 unrelated healthy women by analysis of the HUMARA locus. FVIII:C was determined by a one-stage clotting assay. MTHFR genotyping was performed by allele-specific PCR. Results and Conclusion: Comparing the methylation pattern of both X-chromosomes (X1:X2) we obtained a median ratio of 2.16 in carriers and 2.17 in controls. XCI did not correlate with FVIII:C in both groups (p=0.854 carriers, p=0.362 controls). The MTHFR genotype did not statistically significantly influence XCI. Among controls MTHFR 677CC females had an X1:X2 ratio of 2.23 while 677TT individuals had a ratio of 2.55 (p=0.288). Hemophilia carriers with MTHFR 677CC had a median X1:X2 ratio of 2.10, MTHFR 677TT carriers had a ratio of 1.93 (p=0.323). Surprisingly, we found an association of the MTHFR genotype with FVIII:C in hemophilia carriers. 677CC carriers had FVIII:C levels of 97%, in the presence of 677TT FVIII:C was 65%. Due to the small number of cases the difference was not statistically significant (p=0.153). We did not see an influence of the MTHFR genotype on FVIII:C levels in controls (p=0.592). The observation of an association between the MTHFR genotype and FVIII levels in hemophilia A carriers needs to be confirmed in larger cohorts.

Topic: Molecular Biology in Medicine

P 103 High Dietary Vitamin D Reduces the Load of Chemically-Induced Colorectal Tumors in Mice

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Colorectal cancer (CRC) is one of the leading causes of cancer morbidity and mortality in industrialized countries. The most active metabolite of vitamin D3, 1,25-dihydroxyvitamin D3 (1,25-D3), has anti-tumorigenic effects and is considered protective against CRC. High tissue levels of 1,25-D3 in organs prone to sporadic cancer are assumed to counteract the development of incipient neoplasms. The aim of this study is to investigate the effect of high vitamin D diet on colorectal tumor development in vivo. We fed C57BL/6J mice with five different vitamin D concentrations ranging from 100 to 5000 IU/kg diet to determine the sufficient concentration preventing the development of chemically induced Aberrant Crypt Foci (ACF), a precursor of CRC. To induce ACF we injected mice once with 10 mg/kg azoxymethane intraperitoneally, followed by three cycles of Dextran Sodium Sulfate Salt (DSS) in the drinking water.The colon of mice fed with a low vitamin D diet showed more and further progressed dysplasia compared with mice fed with a diet containing higher vitamin D concentrations (Spearman Correlation Coefficient (SCC) -0.579, p=0,002). Expression of the vitamin D activating enzyme 25-hydroxyvitamin D3 1α-hydroxylase (CYP27B1) in the kidney decreased with rising vitamin D concentration in the diet (SCC -0.452, p=0.016), whereas the vitamin D degrading enzyme 1,25-dihydroxyvitamin D3 24-hydroxylase (CYP24A1) increased (SCC 0.518, p=0.005). The expression of vitamin D receptor (VDR) did not change (SCC 0.041, p=0,016). Our data show that high vitamin D concentrations in the diet are able to reduce the development of chemically induced ACF.

Topic: Malignant Diseases

P 104 Alternative splicing of EGFR ECD in human melanoma

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Introduction. Eighty percent of the known genes have one or more splice variants, which are different from each other both in structure and in function. In molecular biological researches, the presence of splice variants are almost never taken into consideration, neither at protein nor at nucleic acid level. The epidermal growth factor receptor (EGFR) is activated by multiple ligands including EGF, TGF-α, HB-FGF, which all activate its downstream signaling pathways (MAPK, Akt). The dimerisation of the receptor is controlled by the extracellular domain (ECD), therefore its spatial structure has crucial role in this signaling process, and the mutated forms of the ECD are regarded oncogenic. Methods. 7 set of primers were designed to cover the coding sequence of the extracellular domain of the EGFR protein using Primer Premier Program. RNA was isolated from seven different melanoma cell lines, and following reverse transcription PCR reaction was performed with different combinations of our primers. PCR products were sequenated, and the acquired nucleotide sequence was analyzed and compared to the „wild type" sequence in the NCBI Blast database. Results. Our study group have previously described that in human melanomas besides the well-known EGFR VIII splice-variant, there are three new variants: the EGFR 2,3, EGFR 4 and the EGFR 13,14. The variants validated by PCR technique have significance only in that case if functional proteins are translated from them. Conclusion. Alternative splicing may result in dramatically altered isoforms of the original protein therefore it may also significantly change its function as well. In order to prove the significance of these altered isoforms, we have designed customized antibodies to search for functional proteins derived from the splice-variant sequences. In the near future we plan to map the expression profile of different EGFR isoforms during the progression of human melanoma both in cell cultures and clinical samples.

Topic: Tumorbiology - Oncology

P 105 A genome-scale collection of gene deletion mutants in C. glabrata

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Within the last decade, there has been a steady increase of invasive fungal infections in immunocompromised patients. The most common of these infections are caused by Candida spp., which has a high mortality in its disseminated form. C. glabrata (C.g.) now is the second most frequent cause and accounts for 15 – 20 % of all cases of Candidiasis. Importantly, C.g. is inherently tolerant to azole antifungals when compared to most other Candida spp. This is of clinical importance because of the wide use of azole therapy in fungal infections. Moreover, C.g. is unable to form true hyphae or secrete proteases, which are considered important virulence factors of C.a. These differences, as well as the high tolerance to conventional fungal therapies, leave the nature of virulence factors largely unknown. To identify candidate virulence and drug resistance factors, we initiated the construction of a genome-scale collection of C.g. deletion mutants. This collection enables studies on the molecular functions of genes implicated in virulence and drug resistance, as well as those modulating signaling pathways and stress response. Hence, we use a reverse genetic approach to generate a bar-coded C.g. gene deletion collection, which is subsequently analyzed in vitro for their sensitivity to different environmental stress conditions including heat, pH and osmotic stress, as well as a variety of other conditions including antifungal drug susceptibility. The collection now comprises more than 700 deletion strains. We shall present a comprehensive data set on the phenotypic profiling of the deletion collection in comparison to data from clinical patients isolates. Interestingly, a number of C.g. clinical isolates display high-level resistance to azoles (Fluconazole, Voriconazole and Posaonazole) as well as echinocandins (Caspofungin). Based on the phenotypic data from the deletion collection, we have now started to generate deletion mutants in clinical isolates to delineate the contributions of specific genes to clinical drug resistance phenotypes.

Topic: Molecular Mechanisms of Cell Biology

P 106 Ultramicroscopy (UM): 3D reconstructions of vascular networks in mice using lectin-staining

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Ultramicroscopy (UM) allows 3D-visualisation of a large volume of microscopic structures with micrometer resolution (Dodt et al., 2007). In neurobiological studies it is often essential to know the interconnections of the neuronal network to the vascular system. Here we present a microscopy method, whereby the architecture of the blood vessel system of entire organs can be visualized. By combining light sheet based UM with lectin-labelling, 3D reconstructions of vascular structures can be generated. Lectins are proteins that bind to sugar complexes, which are attached to proteins and lipids. We employed an approach using fluorescent conjugated lectins during the transcardial perfusion of mice to contrast the endothelium building up the vascular system (Jährling et al., 2009). We present 3D-reconstructions of the vascularisation topology of entire organs such as murine brains and spinal cords generated by ultramicroscopy. This novel approach allows one to visualize even the fine vascular branches and thus has the potential to open up new vistas in neurobiology and histology, specially in the field of tumor pathology. In future we want to characterize the vascular network of glioblastoma in mice. These complex networks of the vessel system might show abnormalities in the architecture in glioblastoma region. References Dodt et al., 2007, Nat. Methods 4:331-6 Jährling et al., 2009, Organogenesis 5:50-54 This study was supported by the SFB 361 and the Hertie Foundation.

Topic: Neuroscience

P 107 Genetic variability of the human GLA gene in a healthy Austrian population

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The human GLA gene encodes the enzyme alpha-galactosidase A, which is deficient in Fabry disease (FD) patients. So far more than 600 pathogenic mutations have been identified in the GLA gene and numerous coding and non-coding single nucleotide variants or structural variants have been reported. A systematic characterization of these variants, as well as population specific data of healthy individuals is missing in the current literature. In order to obtain population specific data, 100 healthy Austrians (52 females and 48 males) were screened for mutations among the entire GLA gene (coding and non-coding regions). The GLA gene was amplified in 16 overlapping fragments using polymerase chain reaction followed by bi-directional sequence analysis. Conventional cloning was applied for the identification of a certain heterozygous insertion within intron 1. The healthy study population exhibited 39 GLA variants (35 single nucleotide variants, 4 structural variants) of which 14 were novel. Fifty-four subjects (19 females and 35 males) were GLA wild type (no mutation). Two females showing 17 different variants represented the most polymorphic genotype. Based on 152 analyzed chromosomes, the minor allele frequencies ranged between 0.7 and 16.4%. The observed genotype distributions did not significantly deviate from the Hardy-Weinberg equilibrium ($p>0.001$). All 39 variants were selected for pair-wise correlation analysis, which demonstrated several variants in strong linkage disequilibrium. One haplotype block between the GLA loci g.4572 and g.10115 was predicted. The results of this study provide novel information about the genetic variability within the GLA gene in a healthy population. These information are of utmost importance for mutation screening among patients with suspected FD.

Topic: Endocrinology and Metabolism

P 108 Fine specificity of the human antibody response after tick-borne encephalitis virus infection and vaccination

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Tick-borne encephalitis virus (TBEV) is a major human pathogenic flavivirus and closely related to yellow fever, dengue, Japanese encephalitis and West Nile viruses. The surface of these viruses is tightly covered by the envelope protein E which is the major target of neutralizing as well as protective antibodies and consists of three structural domains: DI, DII and DIII. The fine specificity of polyclonal serum antibodies induced by natural TBEV infection or vaccination (with a highly purified formalin-inactivated whole virus vaccine) and the contribution of different antibody subsets to virus neutralization are unknown. In order to assess the relevance of individual variation of immunodominance, we analyzed serum samples obtained from 38 TBE patients and 43 TBE vaccinees in immunoassays with whole virus, recombinant soluble E, isolated DIII and the heterologous sE from West Nile virus as antigens. In general, extensive individual differences were observed with respect to the antibody fine specificities, both after infection and vaccination. Overall, post-vaccination sera had higher titers against all antigens tested and significant differences were found in the fine-specificities of antibodies induced by vaccination compared to natural infection. In relation to sE, the proportion of DIII antibodies was found to be higher in post-infection sera, whereas more broadly flavivirus cross-reactive antibodies were induced by vaccination. Our results suggest that the dominance of certain antigenic sites within the E protein is not only subject to strong individual variation, but also differs when the virus is presented to the immune system in the course of replication or as an inactivated particle in the vaccine.

Topic: Immunology

P 109 Blockade of cellular adhesion mediated by osteopontin and its protease-cleaved forms by monoclonal antibodies

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Osteopontin (OPN) is a widely expressed matrix protein and inflammatory cytokine involved in immune cell migration. OPN has been shown to be a key molecule in obesity-associated adipose tissue inflammation and associated disorders such as insulin resistance that leads to type 2 diabetes. The OPN-dependent recruitment of monocytes and macrophages into the adipose tissue of obese individuals may be mediated by its highly conserved RGD integrin binding site, a second cryptic SVVYGLR integrin binding site, which is exposed after proteolytic cleavage by matrix metalloproteases and thrombin, as well as other unknown regions. The aim of this study is to in vitro investigate the role of distinct OPN regions in adhesion of monocytes and macrophages mediated by OPN and protease-processed forms thereof. First, we developed a reliable high throughput fluorescence labelling-based adhesion assay using HEK293 cells as a reference cell line. Thereby we investigated binding properties of cells to OPN. Particularly the cleaved forms of OPN enhanced adhesion of cells. Strikingly, protease-cleaved OPN-induced adhesion was reduced by a monoclonal antibody designed in our lab. The results of this study will provide information on epitopes of OPN that can be targeted to prevent macrophage recruitment to sites of chronic inflammation.

Topic: Endocrinology and Metabolism

P 110 Renin-angiotensin-aldosterone system blockers in diabetic nephropathy: the role of epithelial to mesenchymal transition

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Introduction: In diabetic nephropathy (DN) the renin-angiotensin-aldosterone system (RAAS) is activated. The elevated renal angiotensin II induces the epithelial to mesenchymal transition (EMT), which is a key element of renal fibrotic transformation. Here we investigated the development of EMT in diabetes and after various RAAS inhibitor treatments Methods: After 5 weeks of streptozotocin (65mg/bwkg ip.) induced diabetes male Wistar rats were treated for 2 weeks with ACE inhibitor enalapril, ARB losartan or aldosterone-antagonists spironolactone or eplerenone. Untreated diabetic and healthy animals served as controls (n=6/group). Mesangial matrix expansion, vascular hyalinisation and intersititial fibrosis were analyzed on PAS and Masson stained kidney sections. Renal αSMA protein level and localization were examined byWestern blot and immunofluorescent staining. Results: Diabetes induced significant mesangial matrix expansion, vascular hyalinisation and interstitial fibrosis, which all were ameliorated by the various RAAS blockers. In parallel the increased renal αSMA level in diabetes was lowered by RAAS inhibitor treatments. While in controls αSMA was only visible around the vessels, in diabetes intraepithelial and glomerular signal was also detectable. Different RAAS blockers minimized αSMA staining in these structures. Conclusion: EMT could play a role in the development of DN. Inhibition of this process could serve as a new therapeutic target of RAAS blockers.

Topic: Molecular Biology in Medicine

P 111 The GPCR - associated sorting protein 1 regulates rimonabant – induced downregulation of GPR55

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The G protein-coupled receptor 55 (GPR55) has recently been suggested to be responsible for those cannabinoid responses that could not be attributed to either the cannabinoid 1 (CB1) or cannabinoid 2 (CB2) receptor. Several potent GPR55 agonists were identified, such as lysophosphatidylinositol (LPI) and several synthetic cannabinoids: One of these is Rimonabant (SR141716A), an antagonist at the CB1 receptor, which showed clinical promise, but approval was revoked due to adverse events. Generally, the activity of GPCRs is coordinated by receptor signaling, receptor desensitization and receptor resensitization. One regulatory mechanism to guarantee appropriate GPCR expression levels in physiological conditions is that of downregulating GPCRs via the G protein-coupled receptor-associated sorting protein 1 (GASP-1), thus leading to an attenuation of cellular signaling events. GASP-1 was originally found to target δ opioid receptors to lysosomes and, hence, to the degradative pathway. It was shown that GASP-1 is a key determinant in the development of analgesic tolerance to cannabinoids via its role in facilitating downregulation of the CB1 receptor. By a variety of approaches, we demonstrated that Rimonabant promotes downregulation of GPR55 via the GPCR-associated-sorting-protein-1 (GASP-1) in vitro and in vivo. We show that GPR55 interacts with GASP-1 in vitro and that disrupting the GPR55-GASP-1 interaction prevents post-endocytic receptor degradation, and thereby allows receptor recycling. Together, these data implicate GASP-1 as an important regulator of Rimonabant-mediated downregulation of GPR55. This work provides tangible evidence that GPR55 is degraded after prolonged agonist stimulation and this mechanism is regulated by the G protein-coupled receptor-associated sorting protein 1.

Topic: Molecular Biology in Medicine

P 112 Scopolamine Administration Modulates Muscarinic, Nicotinic and NMDA Receptor Systems

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Studies on the effect of scopolamine on memory are abundant but so far only regulation of the muscarinic receptor (M1) has been reported. We hypothesized that levels of other cholinergic brain receptors as the nicotinic receptors and the N-methyl-D-aspartate (NMDA) receptor, known to be involved in memory formation, would be modified by scopolamine administration.C57BL/6J mice were used for the experiments and divided into four groups. Two groups were given scopolamine 1 mg/kg i.p. (the first group was trained and the second group untrained) in the multiple T-maze (MTM), a paradigm for evaluation of spatial memory. Likewise, vehicle-treated mice were trained or untrained thus serving as controls. Hippocampal levels of M1, nicotinic receptor alpha 4 (Nic4) and 7 (Nic7) and subunit NR1containing complexes were determined by immunoblotting on blue native gel electrophoresis.Vehicle-treated trained mice learned the task and showed memory retrieval on day 8, while scopolamine-treatment led to significant impairment of performance in the MTM. At the day of retrieval, hippocampal levels for M1, Nic7 and NR1 were higher in the scopolamine treated groups than in vehicle-treated groups.The concerted action, i.e. the pattern of four brain receptor complexes regulated by the anticholinergic compound scopolamine, is shown. Insight into probable action mechanisms of scopolamine at the brain receptor complex level in the hippocampus is provided. Scopolamine treatment is a standard approach to test cognitive enhancers and other psychoactive compounds in pharmacological studies and therefore knowledge on mechanisms is of pivotal interest.

Topic: Neuroscience

P 113 The effect of weight loss and metformin treatment on glycotoxic intermediates in type 2 diabetes

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Background: Hyperglycaemia increases the formation of intracellular reactive oxigen species and glycotoxic intermediates. Methylglyoxal (MG) is a high potent glycatic agent that is throught to contribute to late diabetic complications either as a toxic agent or as a precursor for advanced glycation end products. MG is formed from the degradation of triosephosphate intermediates and detoxified by the glyoxalase enzyme system. We studied the effects of weight loss and metformin combined treatment on the production and detoxification of MG and on the generation of an AGE product carboxy-methyllysine (CML). Materials and methods: In a monocentric pilot study 9 type 2 diabetic individuals were recruited. The patients were educated for a low energy diet and treated with metformin (2000 mg/day) for 6 month. Glycaemic control was determined by glucose and HbA1C. Plasma MG was detected by high-performance liquid chromatography. The glyoxalase system was measured by enzyme assays in peripherial blood mononuclear cells. Plasma CML concentration was determined by ELISA. Results: The mean weight loss in the 6 month treatment period was 6 ± 3 kg (P=0,002). Serious side effect was not reported. A positive correlation between the plasma glucose levels and the plasma MG concentration was shown (P=0,045, r=0,307). Plasma MG and CML levels were significantly decreased by the combined therapy (P= 0,038 and P=0,006). Conversely glyoxalase-I and glyoxalase-II activities were significantly elevated in peripherial blood mononuclear cells (p=0,026 and P= 0,03). Conclusion: Metformin and weight loss diet combined therapy might beneficial influence on the glycotoxic intermediate-methylglyoxal-metabolism in type 2 diabetes.

Topic: Endocrinology and Metabolism

P 114 NPHS2 p.V290M mutation in adult-onset steroid-resistant nephrotic syndrome – should it be screened for?

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Steroid-resistant nephrotic syndrome (SRNS) represents a common cause of ESRD both in adult- and in childhood. In its genetic form, the most frequently mutated gene is NPHS2. Current approach for mutational screening recommend the sequencing of all NPHS2 exons only in childhood-onset SRNS and the screening of the p.R229Q variant in adolescents and adults. Thirty five Hungarian children presenting with SRNS or nephrotic range proteinuria with an onset before the age of 14 years were screened for NPHS2 mutations by traditional sequencing of the coding exons and splicing junctions. Eleven patients (31%) were found to carry NPHS2 mutations. In four of them only one heterozygous mutation was identified. Three patients carried the p.V290M mutation on at least one of the two alleles. Patients with severe mutations and one patient with the p.V290M mutation progressed to ESRD before the age of 10 years. In contrast, two patients carrying the p.V290M mutation presented with an extremely mild clinical course: both of them were diagnosed with nephrotic-range proteinuria at the age of 9.7 and 14 years. The 18-year-old man carrying the p.V290M mutation in homozygous state has never developed edema so far and his regularly checked albumin level dropped below 30g/l only once. The second patient, a 31-year-old woman, compound heterozygous for p.V290M and p.R138Q developed first edema at the age of 27.5 years and her serum albumin level first dropped below 30g/l at the age of 24.3 years. Thus, if their proteinuria had not been detected by screening programs, these two patients would not have been diagnosed with NPHS2 mutations by the currently proposed mutation-screening. In conclusion, we propose that not only the NPHS2 p.R229Q variant, but also the p.V290M mutation should be screened in Central and Eastern European patients with an onset of nephrotic syndrome below the age of 30 years.

Topic: Other

P 115 Alzheimer's disease risk gene-product Lymphocyte-specific Protein Tyrosine Kinase regulates neuritic outgrowth, long-term synaptic strengthening and in vivo hippocampus-dependent spatial learning and memory

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The Lymphocyte-specific Protein Tyrosine Kinase (Lck), which belongs to the Src kinase-family, is abundantly expressed in neurons of the hippocampus, a structure critical for learning and memory. Recent evidence has demonstrated significant down regulation of Lck in Alzheimer's disease, additionally suggesting an involvement of Lck in the function of memory. However, the neuronal role of Lck and its involvement in learning and memory continues virtually unexplored. We combined in vitro electrophysiology, confocal microscopy and molecular biological techniques together with in vivo behavioral studies in order to examine the role of Lck in the mouse hippocampus. Specific pharmacological inhibition and genetic silencing unveiled a requirement of Lck for the progression of neuritic processes. In loss-of-function electrophysiological experiments, Lck inhibition selectively impaired long-term forms of NMDA-mediated hippocampal synaptic plasticity without affecting AMPA-mediated synaptic transmission when functional pre-established synaptic networks where analyzed. Specific Lck inhibition in vivo further significantly impaired hippocampus-dependent long-term spatial learning and memory. These data constitute the first functional characterization of Lck, a proposed risk gene for Alzheimer's disease, describing its importance as a critical regulator of both, neuronal morphology and long-term memory storage.

Topic: Neuroscience

P 116 Anti-tumour effects of combination resveratrol and celecoxib in vitro and in vivo

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Resveratrol is a naturally occurring polyphenol found in some plants such as red grapes or cranberries. It has been shown to possess anti-proliferative and proapoptotic effects in a plenty of cancer cell lines. A relative high dose of resveratrol has a suppressive effect on the growth of cancer cells; however, lower concentrations can stimulate the cell growth. Celecoxib, a selective inhibitor of COX-2 activity, displays the anti-proliferative effects in vitro as well as in vivo. The combination of resveratrol and celecoxib led to the significant decrease in metabolic activity of MCF-7 cells after 24 h exposure as evaluated by the MTT assay. Combination of both agents increased the percentage of cells with dissipated MMP after 48 h exposure in compare to control group, without any changes in the proportion of Annexin V positive cells. However, long-term administration of resveratrol and celecoxib in a chemically induced mammary carcinogenesis in female Sprague-Dawley rats resulted in the significantly reduced tumour incidence, prolonged latency period and significantly decreased tumour frequency and tumour volume. In conclusion, while resveratrol and celecoxib showed only weak proapoptotic activity in MCF-7 breast cancer cell line in vitro, the effect of them together was found to be very effective in vivo. Resveratrol administration in combination with other anticarcinogenic agents may improve the ability to suppress tumorigenesis by targeting different pathways. The combined chemoprevention allows lower doses to be effective as well as reduces agent toxicity. This work was supported by the Slovak Research and Development Agency under the Contract No. VVCE-0001-07 and by Pfizer Luxembourg SARL, Slovakia.

Topic: Tumorbiology - Oncology

P 117 Implication of interleukin – 1 beta in the formation of neuromyelitis optica – like lesions in the rat brain

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Neuromyelitis optica (NMO) is an inflammatory, demyelinating disorder of the central nervous system (CNS), affecting preferentially optic nerve and spinal cord. It is characterized by immune cell infiltrates, complement protein deposition and loss of aquaporin 4 (AQP4) water channel of astrocytes in the affected areas. Besides the presence of neuromyelitis optica-specific immunoglobulins (NMO-IgG) in the serum, an additional factor is needed to trigger the onset of the disease. Our earlier observations suggest that inflammation-inducing CNS antigen-specific T cells can disrupt the integrity of the blood-brain barrier (BBB) and allow the entry of NMO-IgG and complement into the CNS parenchyma. Here, we show that the cytokine interleukin-1 beta (IL-1β), when injected intracerebraly, is also able to "open" the BBB and lead to accumulation of NMO-IgG, complement and granulocytes in the CNS compartment. This then leads to a loss astrocytic AQP4 protein around blood vessels of the brain. Since the role of granulocytes is critical in the pathogenesis of NMO, we also tested whether endothelial cells of cerebral blood vessels are able to produce granulocyte-recruiting mediators upon stimulation with IL-1β. In comparison to other pro-inflammatory cytokines, IL-1β was the most potent inducer of G-CSF, Cxcl1 and Cxcl2 gene expression. Taken together, our findings might have identified the pathological driver for the evolution of rapidly growing, longitudinally extensive lesions in NMO patients.

Topic: Neuroscience

P 118 Characterising the interaction between the COPII component SEC24C and the human serotonin transporter

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The serotonin transporter (SERT) belongs to the SLC6 family of neurotransmitter transporters, which mediate reuptake of previously released neurotransmitters from the synapse. Mutation of C-terminus residues RI607–608 to alanine results in intracellular retention of SERT [1]. We subsequently showed that SERT depends on the COPII component SEC24C for its ER export and proposed RI607–608 as a putative interaction site on SERT for SEC24 proteins [2]. The aim of our current study is to characterise the nature of ER export of monoamine transporters. Using siRNAs to knock down SEC24 isoforms A–D in HeLa cells, we screened a series of double and truncation mutants generated along the C-terminus of SERT. HeLa cells were transfected with Sec24 siRNAs and, after 48 h, with YFP-tagged transporter plasmids. Functional effects of SEC24A–D knockdowns were determined by substrate uptake assays. Export of the IK(609,610) AA-SERT mutant was not sensitive to knockdown of Sec24C. Remarkably, the closely related transporters for dopamine (DAT) and noradrenaline (NET), rely on Sec24D, and not C, for their ER export [2]. Accordingly, we replaced K610 by a tyrosine residue (Y) to switch the SERT export motif to a NET/DAT motif. The resulting K610Y-SERT mutant was more sensitive to the knockdown of SEC24D than of SEC24C. These observations predicted that SLC6 family members with a K-residue at the pertinent position ought to be clients of Sec24C. This prediction was verified by examining mGAT4. The data imply that residue K610 and the equivalent residues in other transporters specify which SEC24 paralogue is recruited for ER export. These export signals work independently because a concatemer of SERT and GAT-1 is affected by depletion of both SEC24C and SEC24D. 1. El-Kasaby A et al, JBC 2010,285:39201-39210 2. Sucic S et al, JBC 2011, 286:16482-16490

Topic: Cell Communication in Health and Disease

P 119 Evaluation of health-related and legal interventions regarding allegedly delinquent and convicted opioid addicts in Austria

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The main goal was to investigate the implementation and current status of quasi-compulsory treatment (QCT) in Austria. In a cross-sectional design, 96 opioid dependent individuals receiving health-related measures (HRMs) were assessed by means of structured interviews. Objective data of 228 imprisoned individuals were gathered through the official prison registry and 19 juridical, health care and political experts were qualitatively interviewed. HRMs were offered more often for addicted individuals with narcotics possession and/or trade whereas imprisonment was ordered more frequently when concomitant property or violent crimes had been committed ($p<0.001$). The population receiving HRMs was highly burdened. Women more often reported serious anxiety ($p=0.030$) and prescribed medication for psychiatric problems ($p=0.045$), but presented violent behavior less often than men ($p=0.003$). Individuals in inpatient therapy were significantly more strained and in higher need of drug treatment and legal counseling (all $p<0.001$), reported higher counts of previous convictions ($p<0.001$) and intravenous drug use (IDU; $p<0.001$), had a longer duration of opioid use ($p=0.024$) and lower lifetime prevalence of alcohol ($p<0.001$), cocaine ($p=0.016$) and amphetamine use ($p=0.005$). IDU was associated with higher rates of hepatitis infection ($p<0.001$) and need for drug treatment ($p=0.006$). Higher age was related to more severe self-assessments of treatment need ($p<0.001$) and burden ($p<0.001$), external rating of the medical status ($p=0.011$) and a less severe external rating of drug problems ($p=0.025$). The evidence suggests that the decision to apply QCT relies on the primacy of narcotics- related crimes and that the specific treatment modality depends on the severity of the addiction and related problems. The results emphasize the need for QCT to treat this clinically highly burdened population by means of a gender- and age-sensitive approach instead of ordering imprisonment.

Topic: Mental Health and Behavioral Medicine

P 120 Macrophage-Colony Stimulating Factor (M-CSF) and Transforming Growth Factor-beta 1 (TGF- ß1) - Predictive Serum Markers for Fracture Healing?

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Introduction Among numerous cytokines that are involved in fracture healing, M-CSF as well as TGF-ß1 play a unique role. The aim of this study was to evaluate the usefulness of M-CSF and TGF-ß1 as biomarkers of human fracture healing. Material and Methods Serum samples of 113 patients with long bone fractures were collected over a period of 6 months following a standardized time schedule. TGF-ß1 as well as M-CSF serum concentrations were analyzed using ELISA. Patients were assigned to 2 groups: first group contained 103 patients with physiological healing. Second group contained 10 patients with impaired healing. Results Highly elevated M-CSF serum concentrations were found in patients with physiological fracture healing over the entire observation period. Further, significant differences in the M-CSF serum concentration between patients with normal and impaired fracture healing were observed. TGF-ß1 serum concentrations increased during the early healing period and were significantly higher in patients with physiological healing compared to controls ($P = 0.04$). Thereafter, it decreased continuously between weeks 2 and 8 and fell again after week 8. TGF-ß1 serum concentrations in patients with physiological healing were significantly higher at week 24 compared to controls ($P = 0.05$). Since serum concentrations regarding the predictive character for non-union of M-CSF and TGF-ß1 lack clearly defined cut-off values, an eligible cut-off level at the 0.25 quartile was selected. At 6 weeks after trauma M-CSF had a sensitivity of 68 % and a specificity of 84 %. TGF-ß1 had a sensitivity of 100% and a specificity of 61% 6 weeks after trauma. Conclusion TGF-ß1 serum concentrations were beneficial to predict impaired fracture healing in our study with high sensitivity and specificity. However, due to the small size measurement of serum TGF-ß1 concentration can not yet be recommended in daily practice. Future studies are required to clarify the role of TGF-ß1 in fracture healing.

Topic: Regeneration of Bones and Joints

P 121 Pharmacological characterization of circular plant peptides with oxytocin-like activity

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Cyclotides are a large class of plant peptides defined by a head-to-tail cyclized backbone and three conserved disulfide bonds in a knotted arrangement. These unique structural features confer them with remarkable stability and due to a range of bioactivities they are extensively investigated as templates in drug discovery.Based on the use of Oldenlandia affinis in traditional African medicine for its uterotonic principle we investigated crude plant extracts and semi-pure cyclotide fractions for the ability to induce uterine contractions using a collagen-gel contractility model. Pharmacological analysis of the effects led to the identification of the oxytocin receptor, a representative of the G-protein coupled receptor (GPCR) family, as a molecular target for cyclotides. Mass spectrometry-based sequence analysis of ‚active' fractions revealed cyclotides with high similarity to the human oxytocin (h-OT) peptide that exhibited weak binding to the human oxytocin receptor. We further analyzed synthetic cyclotide-derived small OT-like peptides and grafted the h-OT sequence into the stable cyclotide frame. These peptides showed increased binding and activation as compared to native cyclotides.These findings may open new avenues for the discovery of GPCR ligands from natural peptide sources. GPCRs are promising drug targets and ~50% of currently used drugs act via binding to these receptors. Natural combinatorial peptide libraries are likely to play an important role in identifying novel GPCR ligands. Particularly plant cyclotides cover a large chemical space based on their high sequence diversity. Together with their range of bioactivities and unique stable structure suggests that cyclotides are of current and future interest for drug discovery and development.
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Topic: Molecular Signal Transduction

P 122 Protective effect of a single radiation dose applied before transient ischemia in rat's hippocampal neurons

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The Ischemic stroke is the third leading cause of death and disability in the world. However, at present no effective neuroprotective drugs are available to treat acute stroke. For this reason, scientists have oriented their attention to defining the brain's own evolutionarily conserved endogenous neuroprotective mechanisms, manifested in ischemic tolerance (IT). Ischemic pre- or postconditioning (pre-C, post-C) is actually the most effective approach to prevent or reverse neurodegeneration. IT can be induced by exposing animals to a broad range of endogenous and exogenous stimuli that are not necessarily hypoxic or ischemic. In this study we used ionizing radiation (IR) as a stressor to achieve the protection against ischemic brain injury and the promotion of neuronal survival in adult male Wistar rats. Doses of 10, 20, 30 or 50 Gy of gamma irradiation were used as pre-C 2 days before lethal ischemia, induced by four-vessel carotids occlusion for 8 min. Staining with Fluoro Jade B, as a marker of neurodegeneration, was used for visualization the changes of neuronal survival in selectively vulnerable CA1 area of hippocampus 7 days after ischemia with and without pre-C. Our findings show protective effects of pre-C in pyramidal cells. Ischemia alone caused the degeneration of 49,92% neurons in the CA1 region. Application of IR before ischemia resulted in remarkable survival of CA1 neurons. The effect of pre-C was approximately proportional to the radiation dose. Behavioral changes in animals were also examined: ischemia significantly reduced the ability of spatial memory in the Morris water maze; pre-C with IR did not significantly enhance the spatial memory after ischemia (except the dose of 50 Gy, which was effective).

Topic: Neuroscience

P 123 Train the Brain – Neural responses as a measure of therapeutic success in patients with anosmia

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Purpose: Anosmia, i.e. the complete loss of the sense of smell, is a prevalent neurological disorder with drastic impacts on nutritional health and safety. Up to 5 % of normal population suffers from total loss of olfactory perception. Currently, there is no commonly accepted therapeutic approach to regain olfactory abilities in anosmic persons. Recently, a study found that olfactory functional learning may recover olfactory function in anosmics. The neuronal basis of this innovative method is, however, unknown. Materials and methods: The main goal of the study is the encoding and understanding of olfactory processing in anosmic patients. Furthermore, different experimental strategies are used to investigate olfactory perceptual learning and involved differences of olfactory function. By means of functional magnetic resonance imaging (fMRI) the neuronal encoding of odors in the olfactory cortex in anosmic patients and healthy controls will be compared. Moreover the neuronal basis of olfactory perceptual learning in anosmics will be examined. Therefore, cortical activity induced by chemosensory stimulation is compared before and after the completion of the olfactory training. An additional aim is the investigation of trigeminal processing in anosmics compared to healthy controls by using functional magnetic resonance spectroscopy (fMRS). Results and conclusion: The results of this study will contribute to new insights of chemosensory perception in anosmic patients and how these processes can be modified by olfactory training. Based on these findings new therapy approaches can be developed. Furthermore, a better understanding of the olfactory system will help to register early changes of olfactory performance in neurodegenerative disorders, such as Alzheimer's disease or Parkinson's disease and may enhance therapeutic outcomes.

Topic: Clinical Neurosciences

P 124 A Normative database of the serotonergic system in healthy subjects using multi-tracer PET

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Purpose Apart from the serotonin (5-HT) transporter (5 HTT) at least 16 subtypes of 5 HT receptors have been identified exhibiting a broad variability in anatomical distribution. These signaling proteins underlie dynamic up- or downregulation in relation to the brain's functionality. Establishing a quantitative topological map of 5 HT receptor subtypes in healthy subjects is therefore a substantial step towards normative in vivo values leading to a better assessment of brain functionality. Methods The major inhibitory (5 HT1A and 5 HT1B) receptors, the major excitatory (5 HT2A) receptor, and the 5 HTT were quantified using PET and the highly selective radioligands [carbonyl-11C]WAY100635, [18F] altanserin, [11C]P943 and [11C]DASB, respectively, in 83 healthy subjects (age=27.7±6.8, 55% males). A standard template in MNI stereotactic space served for region of interest (ROI) delineation. Means and standard deviations for each region are presented. Results Highest 5 HT1A values were found in the temporal pole (5.22 ± 1.03) and parahippocampal gyrus (5.98 ± 1.19). 5 HT2A binding was more homogenous with highest binding potentials in the middle temporal gyrus (1.99 ± 0.46), the angular gyrus (1.93 ± 0.47) and the calcarine fissure (1.97 ± 0.39). 5 HT1B displayed balanced binding and peaked in the calcarine fissure (1.40 ± 0.30), pallidum (1.42 ± 0.67) and nucleus accumbens (1.49 ± 0.41). 5 HTT was highest in subcortical regions with peak values in the dorsal raphe nucleus (4.80 ± 0.97), midbrain (3.28 ± 0.50), and thalamus (2.18 ± 0.27). Conclusion This database might improve the interpretability of PET studies observing alterations of the serotonergic system in neuropsychiatric disorders such as depression, anxiety disorders or schizophrenia. Furthermore, binding values in interventional PET studies can be referenced to this large multi-receptor database, which might be crucial in studies investigating e.g. pharmacological modifications at one of these serotonergic structures.

Topic: Clinical Neurosciences

P 125 alpha-Catulin down-regulates E-cadherin and promotes Melanoma Progression and Invasion

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Metastasis is associated with poor prognosis for melanoma responsible for about 90% of skin cancer-related mortality. In order to metastasize, melanoma cells must escape keratinocyte control, invade across the basement membrane and survive in the dermis by resisting apoptosis before they can intravasate into the circulation. Alpha-Catulin (CTNNAL1) is a cytoplasmic molecule that integrates the crosstalk between NF-kappaB and Rho signalling pathways, binds to beta-catenin and increases the level of both Alpha-catenin and beta-catenin and has therefore potential effects on inflammation, apoptosis, and cytoskeletal reorganization. Here, we show that Alpha-catulin is highly expressed in melanoma cells. Expression of Alpha-catulin promoted melanoma progression, and occurred concomitantly with the downregulation of E-cadherin and the upregulation of expression of mesenchymal genes such as N-cadherin, Snail/Slug and the matrix metalloproteinases 2 and 9. Knockdown of Alpha-catulin promoted adhesion to, and inhibited migration away from keratinocytes in an E-cadherin-dependent manner, and decreased the transmigration through a keratinocyte monolayer, as well as in Transwell assays using collagens, laminin and fibronectin coating. Moreover, knockdown promoted homotypic spheroid formation and concomitantly increased E-cadherin expression along with down-regulation of transcription factors implicated in its repression (Snail/Slug, Twist, ZEB). Consistent with the molecular changes, Alpha-catulin provoked invasion of melanoma cells in a 3D culture assay by upregulation of the matrix metalloproteinases 2 and 9, activation of Rho and prevention of anoikis. As such, Alpha-catulin may represent a key driver of the metastatic process, implicating potential for therapeutic interference.

Topic: Tumorbiology - Oncology

P 126 Inhibition of pro-inflamamtory effects in human cardiac myocytes and endothelial cells by levosimendan

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PURPOSE Levosimendan is a positive inotropic drug for the treatment of acute decompensated heart failure (HF). Clinical trials showed that levosimendan was particularly effective in HF due to infarction. In animal models levosimendan could reduce infarct size. Our aim was to examine whether levosimendan has anti-inflammatory effects on human adult cardiac myocytes (HACM) and human umbilical vein endothelial cells (HUVEC).METHODS HACM and HUVECs were treated with tumor necrosis factor-α (TNF-α) (2000U/ml) or interleukin-1 (IL-1b) (200U/ml) and pre-treated with or without levosimendan (10 µM). IL-6 and IL-8 expression by HACM and expression of E-selectin, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) by HUVECs were measured by specific ELISA, rt-PCR and flow cytometry at different time points. HUVECs treated with IL-1b+/- levosimendan for 4h were incubated with polymorphonuclear cells (PMN) for 5 and 30 minutes to examine cell adhesion.RESULTS Treatment with TNF-α and IL-1b increased expression of IL-6 and IL-8 in HACM. Levosimendan decreased the effects of TNF-α on protein levels of IL-6 and IL-8 by 35% and 48% ($p<0.05$) and the IL-1b-induced protein levels of IL-6 and IL-8 by 50% ($p<0.05$) and 60% ($p<0.001$), respectively. This was confirmed by rt-PCR. Preincubation of HUVECs with levosimendan down regulated the expression of ICAM-1 by 60% ($p<0.01$), E-Selectin by 65% ($p<0,05$) and VCAM-1 by 30% (n.s.), as measured by flow cytometry and confirmed by rt-PCR. Cell adhesion experiments showed a markedly decreased number of PMNs bound to HUVECs when pretreated with levosimendan ($p<0.01$).CONCLUSIONS Levosimendan down-regulates inflammatory mediators in cardiac myocytes and the expression of adhesion molecules in endothelial cells and decreases adhesion of PMNs in vitro. This could explain, at least in part, the beneficial effects of levosimendan after myocardial infarction due to a decrease of ischemia reperfusion injury.

Topic: Vascular Biology

P 127 Rapid ROS generation by the preclinical anticancer ruthenium compound KP1339 predicts cancer cell sensitivity

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KP1339 trans-(tetrachlorobis(1H-indazole)ruthenate (III)) is a ruthenium-based compound with promising anti-cancer activity in early clinical trials. During an in vitro screening several cancer cell lines with pronounced and drug-specific hyper-sensitivity to KP1339 were discovered. The aim of this study was to comparatively analyze gene expression responses to short-term KP1339 exposure in dependence on drug sensitivity to gain deeper insights into the mechanisms determining KP1339 response. To this end, total mRNA was isolated from two hyper-sensitive and comparably resistant tumor cell lines of different cancer origin after KP1339 treatment (3h and 6h). Whole genome gene expression analysis and data mining for differently expressed genes revealed that KP1339 treatment led to the induction of genes involved in cellular redox balance predominantly in the hyper-sensitive cell models. Notably, several genes such as thioredoxin 2 (TXN2) and glutathione peroxidase (GPX1) were induced upon KP1339 treatment only in sensitive cell lines. In addition, mRNAs which encode for the ROS detoxifying enzymes thioredoxin reductase 1 (TXNRD1) and xanthine dehydrogenase (XDH) were down-regulated in hyper-sensitive and up-regulated in comparably resistant cell lines. In accordance to this data, DCF-DA staining revealed stronger ROS generation in sensitive cancer cells in response to the ruthenium drug. In addition, KP1339 treatment induced a pronounced activation of SAP/JNK pathway in sensitive cell lines which was proven by the known radical scavengers ascorbic acid and N-Acetyl cysteine. Taken together our data reveal that KP1339 hyper-sensitivity might be associated with enhanced vulnerability to cellular redox disturbance, a characteristic of multiple cancer types.

Topic: Malignant Diseases

P 128 Using statistical measures for automated comparison of in-beam PET data for ion beam therapy verification

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Positron emission tomography (PET) is considered to be the state of the art technique to monitor particle therapy in-vivo. Due to the different physical characteristics of delivered dose and measured activity, Monte Carlo simulations have to be performed to obtain a prediction of the β+-activity distributions. This simulation has to be compared to the measured PET images to evaluate beam-delivery. Until now the range assessment is performed by a group of experts via visual inspection. This procedure is time consuming and requires well trained personnel. In this study an approach is presented to support human decisions in an automated and objective way. The study was based on in-beam PET (ibPET) data sets recorded during the carbon ion treatments at GSI. For each data set simulated β+-activity distributions were altered to obtain range modifications of 4, 6 and 10 mm water equivalent path length (WEPL). Pearson's correlation coefficient (PCC) was calculated using both an unmodified simulation and an ibPET measurement as reference input. Based on the PCC, Receiver Operating Characteristics (ROC) curves were used as a quality factor of the results. Using ibPET measurements as reference images the PCC were significantly different regarding the unmodified and modified data sets for all modifications of -10 mm WEPL and 10 out of 12 for +10 mm WEPL. Two third of -6 and -4 mm modified data sets showed a significant difference. Considering an unmodified simulation as reference all ROC curves were superior. For 10 and 6 mm modifications a sensitivity and specificity of 90-100% was obtained. The usage of correlation coefficients to compare ibPET data is a promising method to automate the required comparison of measured and simulated ibPET images. The results are comparable to the outcomes gained via visual inspection. Furthermore, this approach can be easily implemented into a verification software that potentially facilitates the clinical workflow in ion beam centers.

Topic: Medical Physics

P 129 Detection of short-lived protein-protein interactions - Identification of protein phosphatase 2A (PP2A) substrates

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PP2A-CDC55 is involved in the regulation of mitosis and is suspected to be a master cell cycle regulator that integrates signals from different cellular cues. Even though genetic assays have suggested many substrates for PP2A, the verification of these substrates proves to be difficult since most PP2A-substrate-interactions are very short-lived and therefore hard to detect with current methods. Thus, our lab adopted and further developed a two-hybrid approach named M-TRACK, in which a protein-protein interaction is detected by a simultaneously occuring enzyme-substrate interaction between a histone lysine methyl transferase (HKMT) fused to the bait and the histone H3 N-terminus fused to the prey, which results in a specific and persistent methylation of the prey. The bait CDC55-fusion-protein rescued a cdc55Δ defect in the spindle assembly checkpoint and was incorporated into an active holoenzyme complex, indicating its functionality in vivo. Consistent with this, we detected in an M-TRACK assay a direct interaction between PP2A-CDC55 and NET1, a protein involved in the regulation of mitosis. The detection depended on CDC55 and the Cdk-targeted phosphosites in NET1. Our data provide strong evidence for M-TRACK's ability to detect in vivo the short-lived interaction between an enzyme and its specific substrate. Using the example of the CDC55-NET1 interaction we defined a first set of "M-TRACK rules" that have to be applied in order to identify PP2A substrates. We are currently applying these rules for the validation of putative PP2A substrates that were indicated by genetic screens and in SILAC experiments. Furthermore, we intend to make use of M-TRACK beyond the identification of substrates, as for example in the biogenesis of PP2A in which we use the persistent methylation-mark to determine the chronology of events.
Supported by Austrian Science Fund (FWF) doctoral program APW01220FW

Topic: Molecular Mechanisms of Cell Signaling at the MFPL

P 130 Pharmacochaperoning of the ER-retained A1-adenosine receptor

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The A1-adenosine receptor is a member of the rhodopsin-related subfamily of G protein-coupled receptors. Point-mutations in the conserved NPxxY (x)5,6F sequence at the junction of helix seven and the carboxyl terminus/helix eight disrupt surface targeting of the receptor and result in its intracellular retention [1]. This trafficking arrest can be overcome by addition of receptor ligands (pharmacochaperoning) that stabilize the receptor fold and thus promote surface expression. The mutants serve as a tool to explore a ramification of the retaliatory metabolite complex: hypoxia leads to intracellular accumulation of adenosine – by breakdown of ATP and by inhibition of adenosine kinase. Intracellular and extracellular adenosine levels are in equilibrium because of the action of the equilibrative nucleoside transporters. Extracellular adenosine dampens cellular metabolism by acting on inhibitory A1-adenosine receptors and thus counteracts the impact of hypoxia. If adenosine also pharmacochaperoned A1-adenosine receptors during hypoxia, it would enhance its effectiveness as a protective agent. Accordingly, we created cell lines that stably expressed A1-receptor-Y288A with either a C-terminal yellow fluorescent protein (YFP) or an N-terminal FLAG-epitope fused to a streptactin peptide (SF-TAP). These cell lines were incubated with a combination of inhibitors to test whether manipulations of intracellular adenosine levels increased the accumulation of the receptor. The A1-antagonist DPCPX (dipropyl-cyclopentyl-xanthine) was used as an internal control. Inhibition of adenosine deaminase, of adenosine kinase or of the equilibrative nucleoside transporters did per se not suffice to rescue the receptor. However, combined addition of these inhibitors was as effective as DPCPX in pharmacochaperoning the receptor.
References: 1. Malaga-Dieguez L et al. (2010) Pharmacochaperoning of the A1 adenosine receptor is contingent on the endoplasmatic reticulum. Mol Pharmacol 77:940-952

Topic: Cell Communication in Health and Disease

P 131 Phagocytosis of mesothelial cells in peritoneal dialysis

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Background: Peritoneal dialysis (PD) is a common used, safe and cost-effective way to treat renal failure. The two predominant cell populations in the peritoneal cavity are free-floating macrophages (PMØ) and resident mesothelial cells (MC) lining the peritoneum. They are harmed by PD fluids (PDF) resulting in decreased phagocytosis of PMØ, increasing risk for peritonitis and inflammation and detachment of MC, which may lead to peritoneal membrane failure. For the first time, we focus on the interplay between both populations. We hypothesize that detached MC are phagocytosed by PMØ, and that this process is significantly involved in regulation of the peritoneal inflammatory status during PD. Methods: To assess phagocytosis of MC by PMØ in-vivo, cells from human peritoneal effluents were isolated by centrifugation and stained for cytokeratin (ck), a MC-specific marker, and CD45, a leukocyte marker. Stained cells were analyzed using flow cytometry and fluorescent microscopy. Additionally an in-vitro model was established using an immortalized human mesothelial cell line (MeT-5A) and immortalized macrophage-like cell line (U937). A pH-sensitive fluorescent dye (pHrodo) was used to label MC to distinguish between attached and phagocytosed cells. Results: Analysis of human peritoneal effluent showed that a large number of cells are CD45-positive leukocytes. A ck-positive MC population was also detected. Additionally CD45-ck-doublepositive cells could be detected by flow cytometry. These cells proved to be PMØ with phagocytosed MC when analyzed by fluorescent microscopy. The successfully established in-vitro model showed similar results. MeT-5A are phagocytosed to a significant extent by U937. Conclusion: These data show that MC are phagocytosed in-vivo during PD. The successful establishment of an in-vitro model now enables us to further analyze this process under controlled conditions. It will be part of future experiments to find out the role of this phagocytosis process in the regulation of peritoneal inflammation.

Topic: POeT - Programme for Organfailure, -replacement and Transplantation

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P 132 Response to the extracorporeal photophersis in patients with bronchiolitis obliterans syndrome according to the new National Institutes of Health (NIH) consensus criteria after allo-HSCT: prospective study

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Background: Bronchiolitis obliterans syndrome (BOS) following allogeneic hematopoietic cell transplantation (HCT) is considered a manifestation of chronic graft-versus-host disease (cGVHD) with poor prognosis and 5-year survival rates are around 20%. Survival and treatment of patients with BOS after HCT has not improved over the last 20 years. Evidence regarding the efficacy of various immunosuppressive (IS) therapies in BOS is still sparse. Extracorporeal photopheresis (ECP) is one of the very few treatment options with proven benefit for patients with BOS. Aim: The purpose of the study was to prospectively compare the efficacy of adjunct ECP with other IS treatment modalities in BOS patients newly diagnosed and evaluated uniformly according to the NIH consensus project criteria. Patients and methods: Forty-six patients with a median age of 41 years developed BOS at median of 336 days after HCT. At onset of therapy BOS was mild in 80% and moderate in 9 patients. The median FEV1 at onset was 65%. Signs of air trapping in HR-CT scans were present in 70% of patients and BO was proven histologically in 21 patients. Response was assessed according to complete, partial, stable or progressive. The overall survival (OS) was analysed after a median follow-up of 35 months after HCT. Results: When assessed after 1 year, 17 patients of 63% responded to ECP and 12 patients of 19 (63%) responded to other IS therapies.Eight patients given adjunct ECP as first-line therapy of BOS achieved a response (CR n=3, PR n=5) compared to 12 patients given first-line therapy with CNI and corticosteroids alone. OS at 3 years was 86% for responders. Patients with progressive BOS after ECP had worse OS (0.09) compared to CR, PR (0.008) and stable disease. Conclusion: The data shows clinical effectiveness in ECP treatment of BOS and in OS. The earlier evaluation of progressive BOS type and intensification of IS treatment must be considered.

Topic: Immunology

P 133 Firing patterns of identified prefrontal interneurons in freely-moving rats

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The prefrontal cortex plays a major role in several cognitive processes including working memory formation, temporal organization of events and decision making (Siapas et al., 2005; Jones and Wilson, 2005; Benchenane et al., 2010). On the other hand, GABAergic interneurons provide the temporal framework for information processing in the cerebral cortex. But how do distinct types of interneuron, having different postsynaptic targets and molecular expression profiles of signaling molecules, shape the organisation of prefrontal activity at the network level during native behavior? We recorded the firing activity of juxtacellularly labelled interneurons in the prelimbic cortex of freely-moving rats. The preliminary analysis of an identified parvalbumin positive basket cell shows an increase of firing during periods of head movement acceleration enclosed by periods of slow wave activity and quiet wakefulness. Furthermore, these periods with head movement accelerations are accompanied by increased amplitudes of gamma oscillations. We hypothesise that distinct types of GABAergic interneuron in the medial prefrontal cortex uniquely contribute to the temporal coding during different behavioural states.
This work was supported by the CCHD Program of the FWF.

Topic: Cell Communication in Health and Disease

P 134 GLI1 as a novel marker for pituitary adenoma stem cells

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During the last decades, a new model of cancer development based on the identification of tumor-initiating "cancer stem cells" (CSC) has evolved. The small population of CSC shares common features with adult stem cells and is thought to be the driving force in tumor initiation, maintenance and relapse. Recent data suggest that the CSC model could also be of relevance for the pathogenesis of benign tumors. Only few marker genes could be associated with putative pituitary tumor stem cells yet but it is crucial to ascertain the tumor stem cell phenotype in order to identify tumor stem cells. In the present study, we want to further evaluate the phenotype of potential pituitary adenoma stem cells. We studied expression levels of the embryonic, neural and glioma associated stem cell markers SOX2, NANOG, NESTIN, CD133 and GLI1 in 24 human pituitary adenomas (hormone-expressing and non-functioning, respectively) by qRT-PCR and immunofluorescence staining. In addition, volumetric tumor measurements were performed using OsiriX imaging software. The expression extent of NANOG, SOX2, NESTIN and CD133 show a significant correlation ($p<0,001$). GLI1 is an important regulator of the Hedgehog signalling pathway which is physiologically active during embryonic development and was shown to be reactivated in various types of cancer. In our study, GLI1 showed a significant correlation with the other investigated markers in relapsing tumors ($p<0,001$). On the other hand, tumor size correlates with the expression extent of GLI1 in non-relapsing tumors only ($p=0,038$). Since the first Hedgehog signalling inhibitor Vismodegib was approved earlier in 2012, this could be a promising treatment option for pituitary adenomas overexpressing GLI1.

Topic: Endocrinology and Metabolism

P 135 Establishment of a fluorescent proliferation assay for fungal cells suitable for flow cytometry analysis

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Candida glabrata (C.g.) is the most common opportunistic yeast pathogen in humans after Candida albicans. In a mouse model of systemic infection by C. g., the fungal cells disseminate in all organs without causing a strong inflammatory response. In vitro, C.g. co-cultured with macrophages can persist and proliferate for days within host cells by preventing phagosome maturation. The molecular mechanism of C.g. immune escape remains unclear. To investigate the host factors promoting C.g. persistence and proliferation, we aimed to establish a multi-label assay allowing monitoring by flow cytometry and microscopy C.g. division, persistence or death of phagocytosed and unphagocytosed fungal cells. For this purpose we tested two different fluorescent dyes, eFluor® 670 and CFDA-SE, both established as proliferation dyes for mammalian cells. Contrary to mammalian cells, the fungal plasma membrane is protected by a rigid carbohydrate-protein structure -the fungal cell wall. Upon cell division, most of the fungal cytoplasm is evenly divided between the mother and the daughter cell. By contrast most of the cell wall is not transmitted but synthesized de-novo. Only CFDA-SE efficiently crossed the fungal cell wall and stained intracellular proteins. The proliferation staining was combined with a live/dead stain to simultaneously quantify fungal killing by innate immune cells. Finally, a fungal specific stain that does not cross mammalian cells plasma membrane, Calcofluor white, was used to distinguish phagocytosed from not phagocytosed C.g. Using this 3-color assay, we observed that C.g. is able to proliferate and persist in innate immune cells such as bone marrow-derived macrophages or dentritic cells for at least up to 24h. Thus "partitioning" dyes are suitable tools to monitor fungal cell divisions. Using this assay, the host factors determining the ability of C.g. to divide or be eliminated in different cell culture models will be investigated.

Topic: Inflammation and Immunity

P 136 Effect of different therapy options on immunoglobulin levels in patients suffering from acute, acute-on-chronic, or chronic liver failure

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Introduction: Hypergammaglobulinemia is commonly found in patients suffering from chronic hepatic failure (CHF), whereas in patients with acute liver failure (ALF) normal immunoglobulin values can be found. There are sparse data available about the effect of therapy options on antibody levels in patients with liver failure. The aim of this study was to determine serum immunoglobulin concentrations in patients suffering from CHF, ALF, or acute-on-chronic liver failure (ACLF) and evaluate the impact of liver transplantation (LTX) or MARS treatment on antibody levels. Materials and methods: We followed ten patients with ALF, twelve with ACLF and 18 with CHF. Eight patients with ALF and eight with ACLF underwent MARS therapy, whereas the rest received LTX. 13 healthy volunteers served as controls. Serum immunoglobulin levels were assessed by ELISA technique. Results: In patients with CHF median serum levels of IgA, IgG and IgM were increased in comparison with ALF or healthy individuals. IgA and IgG concentrations were also significant elevated compared to ACLF. Furthermore a decrease in immunoglobulin levels after LTX was detected in patients with CHF, whereas in patients with ALF or ACLF who underwent transplantation no comparable effect was observed. MARS treatment had no apparent effect on the immunoglobulin profile in patients with ALF or ACLF. Conclusion: We provide evidence that LTX reverses hypergammaglobulinemia in patients suffering from CHF, which could be traced back to a reconstituted hepatic antibody clearance, whereas MARS treatment does not affect immunoglobulin levels.

Topic: Vascular Biology

P 137 Evaluation of IMRT and VMAT treatment plan quality delivered with and without flattening filter using Pareto optimal fronts

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Purpose: To evaluate treatment plan quality of IMRT and VMAT plans for high energy photon beams delivered with (FF) and without (FFF) flattening filter. Methods: 9-field IMRT and 360° single arc VMAT plans, based on 10 MV beams with and without flattening filter, were created using Monaco (Elekta/CMS, v.2.04, USA) for three different patients (prescribed PTV dose 78 Gy). This treatment planning systems was commissioned for FF and FFF beams provided by an Elekta linac. The Pareto optimal fronts were created by calculating different treatment plans with varying rectum constraints and evaluating the PTV volume receiving less than 95% of the prescribed dose (V<95%) and the volume of the rectum receiving 70Gy or more (V70Gy). Treatment plan efficiency was evaluated by recording number of monitor units (MUs) and by measuring the delivery time (T) using an Elekta Precise linear accelerator in FF and FFF mode. Result: The POFs of the rectum for both IMRT and VMAT in FFF mode were similar or even superior to the FF- modalities. For two of the three patients the POFs of IMRT and VMAT revealed a systematic difference of about 2% in target coverage. The POFs of the remaining patient showed virtually no difference for all four modalities. The delivery time of IMRT FFF decreased by about 23% compared to IMRT FF. In contrast to the IMRT techniques, delivery time increased by 20% when using VMAT FFF compared to VMAT FF. Conclusion: The evaluation of the POFs confirms that a flattening filter is not necessary for static and rotational IMRT treatments. Similar studies are needed for other IMRT indications, like head-and-neck cancer, to draw final conclusions. Sequencing algorithms for FFF-beams need to be optimized and further improvement of the mechanical MLC properties are desirable for VMAT in FFF mode.

Topic: Medical Physics

P 138 Reduced somatostin production in colorectal cancer with uncontrolled cell proliferation, as compared to controlle cell growth in young and adult normal 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 and 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 (≤0,05). 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.

Topic: Tumorbiology - Oncology

P 139 Lysosomal Membrane Protein 2 (LAMP-2), a potential new receptor involved in antigen presentation in Dendritic Cell

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LAMP-2 has been extensively used as a marker of lysosomes and late endosomes and more recently as been shown to help lysosomal integrity and antigen presentation. LAMP-2 is also express on the cell surface of most cell type and is the target of autoantibodies in over 90% of patients with ANCA associated vasculitis (1) but its function on plasma membrane LAMP-2 was never studied. Due to the involvement of LAMP-2 in autoimmunity and in antigen presentation, human Monocytes Derived Denditic cells (MDDC) are the best candidate in order to clarify membrane LAMP-2 function. Results Isolation of human Monocytes by CD14 beads from PBMCs. LAMP-2 is present on DCs both intracellulary and on the cell surface. After DCs activation, there is no decrease in the amount of surface LAMP-2. Incubation with anti-LAMP-2 antibody causes the uptake of the Abs. The uptake is specific and receptor mediated through LAMP-2 because it's not influenced by presence of Fc block and is significantly higher then other IgG1 Abs that have basal uptake. Finally the uptake is abrogated in DCs isolated from Danon disease patient, a genetic disorder causing deletion of LAMP-2 protein. The interaction with the Abs, change the intracellular localization of LAMP-2. Confocal images show that LAMP-2 transit into the HLA-DM positive compartment 1h after the internalization of the Abs, this co-localization does not occur in unstimulated cell and disappears after 3h. In conclusion, LAMP-2 behave as a specific receptor for internalization of extracellular molecules as demonstrated by the uptake of anti-LAMP-2 Abs, the interaction with ligand is followed by transit into MIIC compartment rising the question whether it function as an antigen capturing receptor. (1) (Nature Medicine 14, 1088 - 1096 (2008)

Topic: Immunology

P 140 Transdifferentiating alpha cells into insulin producing beta cells

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The endocrine function of pancreas is mainly carried by α, β, σ, and PP cells which secret glucagon, insulin, somatostatin and pancreatic polypeptide hormones. Type I diabetes is characterized by selective loss of insulin-producing β cells. Beside current insulin therapies, the transformation of mature α to β cells is a potential treatment. Recent studies showed either downregulation of Arx or upregulation of Pax4 can induce α-β cell transformation. Pharmaceutical regulation of Pax4 or Arx is a potential way to replenish β cells from α cell. Based on this knowledge, we would like to establish a high-throughput screen in ELISA format on our PLACEBO platform and hope to find novel Pax4 activators or Arx inhibitors which can transform α cell into insulin-producing cells. Furthermore, the transcriptome and chromatin binding patterns of both Pax4 and Arx, which have not been reported yet, will be analyzed based on chromatin immunoprecipitation followed by next generation sequencing. Even more interesting, three significant hits were identified on RNAi screen for pancreatic-related genes. Further study is undergoing to investigate the function of these genes.

Topic: Endocrinology and Metabolism

P 141 Role of mTOR during Differentiation of Human Amniotic Fluid Stem Cells to Schwann Cells

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Schwann cells play an essential role in the conduction of nerve impulses along the axon. Additionally they support and protect peripheral neurons and are therefore also crucially involved in the regeneration of nerve lesions. Demyelinating diseases are frequently caused by genetic mutations effecting Schwann cell survival and these diseases could potentially be treated by cell based regenerative approaches. Here we investigate signaling pathways instrumental to drive human amniotic fluid stem (hAFS) cells into the Schwann cell differentiation phenotype. We reprogram hAFS cells by treatment with retinoic acid in serum free media followed by incubation in media containing 10% FBS, forskolin, platelet-derived growth factor-AA and heregulin for 14 days. We observe inhibition of Schwann cell marker expression(S100b, Glial Fibrillary Acidic Protein, Nerve Growth Factor Receptor p75) upon treatment with rapamycin, a potent mTORC1 inhibitor. On the contrary, treatment with the pan AKT selective small molecule inhibitor MK2206, does not result in altered Schwann cell marker expression. We conclude that during initial steps of hAFS cell differentiation to Schwann-like cells mTORC1 provides essential signaling functions. Hence for regeneration purposes, the yield in differentiating cells can potentially be maximized by activation of mTORC1 (e.g.: by Insulin or amino acid stimulation) during initial steps of hAFS cell to Schwann cell differentiation.

Topic: Malignant Diseases

P 142 Targeting cancer epigenetic vulnerabilities using small molecules inhibitors

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In the last ten years a crucial role for epigenetics in cancer has emerged. Aberrant DNA methylation and histone post-translational modifications have both been described in different tumors. Epigenetic changes, in contrast to genetic mutations, are reversible and this has fostered the development of epigenetic drugs: the first small molecules targeting chromatin modifying proteins have been recently approved for clinical application as anti-cancer drugs (e.g. the HDAC inhibitor Vorinostat and DNMT inhibitor 5-azacytidine). Nevertheless, the role of most chromatin proteins in cancer development remains poorly characterized if not unknown. Therefore, we have initiated an epigenome-wide investigation of the role of chromatin proteins in cancer. We use functional genomics approaches in combination with next generation sequencing to identify new chromatin proteins affecting the proliferation rate of cancer cells. We screened our epigenome-focused shRNA library on a panel of breast cancer isogenic cell lines and monitored changes of cellular fitness as a consequence of the knockdown of a specific chromatin protein. At the same time, some chromatin proteins have already been shown to play a crucial role in cancer development. An interesting example is given by PHD domains, the "readers" of histone methylation. Translocations fusing the PHD domain of JARID1A or PHF23 with the nuclear pore complex protein NUP98 have been reported in acute myeloid leukemia (AML) patients. The interaction between the PHD domain of these fusion proteins and the methylated histones has been shown to be crucial for tumorigenesis. There are currently no compounds available to interfere with the interaction between methylated lysines and PHD domains. Therefore, using the PLACEBO platform in our lab, we are currently looking for small molecules that could inhibit this interaction or target new epigenetic vulnerabilities of cancer.

Topic: Molecular Signal Transduction

P 143 Proteomic analysis of cell populations in artificial peritoneal dialysis effluents

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Peritoneal dialysis (PD) is a safe and cost-efficient renal replacement therapy but PD-fluid (PDF) has cytotoxic effects on the cells within the peritoneal cavity. Proteomics is an attractive method to analyse the expression of proteins as main effectors of cellular mechanisms on a global level. Due to high concentrations of serum proteins in PD-effluents it is not possible to analyse the expression profiles of cellular proteins. The overall aim is to reveal the cellular protein pattern after depletion of dominant plasma proteins in PD-effluents. An artificial PD-effluent was composed by mixing unused PDF with human plasma and protein extracts from a human monocyte cell line under control or heat stress conditions. The protein concentration of the different fractions was equalized by adaption of a depletion strategy to reduce the dynamic range of highly abundant plasma proteins. The artificial effluent was analysed by 2D-DIGE before and after the depletion step. Differentially abundant proteins were identified by mass spectrometry (MS) and western blotting. Within the artificial effluent the high abundant serum proteins cover the signals of the differential regulated cellular proteins on the 2D gel. Without the serum background, 2D gels allow the discrimination between protein patterns of cells under control and heat stress conditions. After the depletion, the cellular protein fraction reaches the detection limit and the expression profiles can be examined. Accordingly, heat shock proteins, which were differentially abundant in the cell extracts and serve as the gold standard of the cellular stress response, could be identified by MS and Western blotting. The presented study shows that the used depletion method enables the analysis of low abundant plasma proteins as well as of proteins of cellular origin in artificial PD-effluents. The established workflow will now be applied to well documented clinical material.

Topic: POeT - Programme for Organfailure, -replacement and Transplantation

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P 144 Heme Oxygenase-1 specifically modulates pro- and anti-adipogenic molecules to inhibit white adipose differentiation

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Adipocyte differentiation is a highly regulated process controlled by a complex signalling network. Mounting evidence suggests an essential role for Heme Oxygenase-1 (HO-1) in adipocyte generation and function. However, the underlying mechanisms are currently unknown. Here, we aimed to investigate a potential mechanism by which HO-1 regulates adipogenesis. Construction of transgenic mice was generated with the Cre-loxP system ultimately ablating HO-1 in adipocytes in vivo. Transgenic cell lines were made for studying constitutive stable HO-1 knock-down or overexpression in 3T3-L1 preadipocytes. Adipocyte differentiation studies were performed on isolated stromal vascular fractions from transgenic mice (containing preadipocytes) and 3T3-L1 cells. HO-1 is expressed in 3T3-L1 preadipocytes and declines during initial differentiation. Overexpression of HO-1 in 3T3-L1 cells prevents adipogenesis, whereas shRNAmir-mediated reduction of HO-1, promotes differentiation, as shown by increased lipid accumulation and elevated expression of adipogenic transcription factors PPARγ, CEBPα, KLF-15 and adipocyte marker genes aP2 and adiponectin. Our findings with 3T3-L1 cells were substantiated with HO-1 deleted preadipocytes isolated from HO-1loxP/loxP mice: Adeno-Cre virus mediated excision of HO-1 in vitro, followed by differentiation resulted in enhanced accumulation of neutral lipids, as compared to HO-1loxP/loxP preadipocytes infected with Adeno-GFP virus. GeneChip comparisons of control- and HO-1 overexpressing 3T3-L1 cells induced for differentiation over a time course, revealed several genes modulated by HO-1 including transcription factors, signalling proteins, as well as unknown genes. Some of these genes are now being investigated in functional assays as potential mediators of the anti-adipogenic effects of HO-1. Collectively, our data demonstrate an inhibitory function of HO-1 in adipocyte differentiation via specific modulation of pro- and anti-adipogenic molecules.

Topic: Cell Communication in Health and Disease

P 145 Understanding the role of Polo-like kinase in Trypanosoma brucei through chemical genetics

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Trypanosoma brucei (T. brucei) is a unicellular flagellated parasite that causes African trypanosomiasis in humans and livestock. In T.brucei, cell division occurs by binary fission and is a highly coordinated process in time and space. Polo-like kinases (PLKs) are known to regulate different cell cycle stages in eukaryotes. We are using a chemical genetics approach to selectively inhibit the single PLK homolog in T.brucei (TbPLK) in order to study how TbPLK regulates the cell cycle and organelle biogenesis in this organism. The approach involves introducing a functionally silent mutation in the enzyme's catalytic pocket. A conserved hydrophobic amino acid is substituted for a smaller residue; this enlarges the catalytic pocket and opens a space that can be occupied by an inhibitor designed to contain a bulky substituent. The modified inhibitor then uniquely inhibits the mutant enzyme while all other non-engineered kinases are unaffected. This method, in contrast to RNAi, allows us to inactivate and reactivate TbPLK quickly and specifically. Our data shows that the mutant kinase is sensitive to the modified inhibitor in vitro and in vivo. Acute TbPLK inhibition results in cell growth arrest within the first cell cycle and leads to cells with abnormal DNA content. TbPLK inactivation causes defects in the replication of several cytoskeletal structures. The bilobe, an organelle of unclear function, and the Flagellar Attachment Zone (FAZ), which keeps the flagellum adhered to the cell body, present severe defects in their assembly. TbPLK localizes to these structures throughout the cell cycle but, upon inactivation, it is not able to migrate to the FAZ anymore. By coupling a synchronization method based on centrifugal elutriation and the previously mentioned chemical genetics strategy, we have been able to further investigate the order of events of PLK-mediated regulation of cytoskeletal inheritance in T. brucei.

Topic: Molecular Mechanisms of Cell Signaling at the MFPL

P 146 A calcium channel-like selectivity filter modulates an access pathway for local anesthetic drugs in voltage-gated Na channels

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Some blockers of voltage-gated Na^+ and Ca^{2+} channels are assumed to pass through the membrane and then bind to amino acids in the internal vestibule by access from the internal side of the membrane. However, in the heart isoform of the voltage-gated Na^+ channel, in L-type calcium channels and in T-type calcium channels an additional external access pathway (EAP) through the protein has been suggested. Furthermore, in voltage-gated Na^+ channels mutations at a specific site in the middle of the domain IV transmembrane segment 6 (site 1575 in rNaV1.4, 1760 in 1.2) open an EAP for QX-222, a permanently charged, hydrophilic lidocaine analogue . Homology models of the structure of the NaV 1.4 channel suggest that I1575 is in close proximity to K1237 of the selectivity filter. The mutation K1237E changes the selectivity filter of the voltage-gated Na^+ channel such that it resembles the selectivity filter of voltage-gated Ca^{2+} channels. This mutant is permeable for Ca^{2+} and for large organic cations suggesting a widening of the selecivity filter. If residues K1237 and I1575 are in close proximity, as suggested from homology modeling, then the widening of the selectivity filter by the mutation K1237E may obstruct the EAP created by mutations at site 1575. To test this hypothesis, we measured block by external QX-222 in wild-type, I1575C, and K1237E/I1575C channels. External QX-222 blocked currents in wild-type channels by 10.8 ± 2.6%. Block was significantly enhanced in I1575C (36.1 ± 4.5%; $P < 0.05$ vs. wild type) consistent with the reported opening of the EAP. The additional mutation K1237E reduced block to wild-type level (0.5 ± 1.9%). Our results support the hypothesis that QX accessibility pathway can be closed by the mutated selectivity filter residue, and show that domain III selectivity filter and domain IV upper S6 segment residue I1575 are in close proximity. Funding support: Austrian Science Fund P210006-B11, W1232-B11

Topic: Molecular Biology in Medicine

P 147 Characterization of the protein microenvironment of the folate-receptor beta

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Rheumatoid Arthritis (RA) is a chronic, systemic autoimmune disorder, in which activated synovial macrophages have been identified as a key mediator of disease progression. Macrophage numbers and levels of macrophage activation are correlating with the extent of joint inflammation and tissue degradation. As eliminating the entire population of macrophages leads to deficiencies in tissue repair and fighting infections, the ability to identify and influence the specific harmful subset of these macrophages is an important undertaking. A receptor for folic acid, folate receptor beta (FRβ), has been shown to be selectively elevated in activated macrophages present in the RA-affected synovia. With the exception of high affine binding of folic acid (vitamin B9), nothing is known about its processing, organization in the membrane and potential signal transduction capacity. In order to elucidate the function of FRβ on activated macrophages, we are aiming to characterize the molecular microenvironment of FRβ. For this, we have generated a FRβ-overexpressing monocytic cell line for use in immunoprecipitation and subsequent mass-spectrometric analysis. Proteins, found to co-precipitate with the introduced FRβ, are verified using immunoprecipitation of the endogenous receptor in human differentiated peripheral blood monocytes. The data generated in this project will help to develop drugs targeting the cells that drive chronic inflammation and broaden our view on the underlying principles of macrophage function.
The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement NMP4-LA-2009-228827 NANOFOL.

Topic: Immunology

P 148 Intracellular recognition machinery for Streptococcus pneumoniae

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Streptococcus pneumoniae are gram-positive bacteria that colonize the lung and lead to severe diseases like pneumonia, meningitis and sepsis. The invasive clinical strains of S. pneumoniae are encapsulated with a polysaccaride capsule that is recognized by the host innate immune system. Streptococcus pneumoniae are gram-positive bacteria that colonize the lung and lead to severe diseases like pneumonia, meningitis and sepsis. Several surface structures of S. pneumonia are recognized by the host innate immune system and trigger immune responses An important aspect in protecting mucosal barriers within the innate immune system is the type I Interferon (IFN) cascade. There is increasing evidence that not only viruses but also bacteria provoke type I IFN secretion, however the role of Type I IFN in the host response to bacterial infections remains elusive. Recently it has been demonstrated that Type I IFN signaling induced by pneumococcal DNA contributes to the clearance of S.pneumoniae. Not only bacterial DNA but also mRNA has been proposed to contribute to the induction of Type I IFN signaling. Thereby the detection of both microbial mRNA and DNA is suggested to occur via an yet-to-be identified receptor present in the cytosol of innate immune cells. Previous data from our lab demonstrate uptake and recognition of S.pneumoniae not only from the surface but also from the phagosomal compartment of alveolar macrophages. Based on these findings, we aim to localize S.pneumoniae-mediated IFN-ß induction to either the phagosome or the cytosol and identify the host sensors involved in Type I IFN signaling. Furthermore the physiological role of IFN-ß induction during pneumococcal pneumonia should be investigated in mouse in vivo experiments using wt as well as Interferon α/β-receptor knock-out (IFNAR-/-) mice.

Topic: Cell Communication in Health and Disease

P 149 Chemical Genetics to Uncover Breast Cancer Vulnerabilities

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Breast cancer is the most common cancer in women, causing more than 500 000 deaths per year. Despite some novel therapy strategies in recent years, it continues to be challenging to target "undruggable" cancer genes and to predict drug sensitivity and therapy outcome. Thus, alternative approaches are urgently needed. Here we present a large-scale systematic approach to investigate functional interactions between genes frequently mutated in breast cancer and a panel of drugs to reveal synthetic lethal (or resistant) relationships. First, we have completed a panel of 70 cell lines that differ only in a single defined genetic aberration (i.e. overexpression of an oncogene or knock-down of a tumor suppressor) and contain a genetic barcode that makes them tractable in pooled screens. Such, we will be able to quantitatively assess the cellular fitness in response to drug treatments in a multiplexed manner by means of a fluorescent hybridization assay. Second, a drug library of about 250 small molecules was assembled and screened against the 70 cell lines, yielding some 17.500 individual interactions. This library includes approved chemotherapeutics and experimental anti-cancer drugs, as well as a collection of drugs approved for treatment of other diseases and several tool compounds. After performing the screen, the resulting hits will be carefully verified to exclude false positives. They will then be validated in patient-derived breast cancer cell lines. A central part of the project is the extensive investigation and elucidation of the underlying molecular mechanism. If possible, the interactions will be additionally investigated using publicly available databases and patient samples to address the translational potential of selected drug-gene interactions. In summary, the proposed study may contribute to another step forward towards personalized medicine by revealing novel gene-drug interactions that potentially predict tumor sensitivity or resistance in patients.

Topic: Malignant Diseases

P 150 Type I Interferons Exacerbate Lethality during Candida-Induced Sepsis Through CCL2-Mediated Recruitment of Inflammatory Monocytes

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Invasive fungal infections by Candida albicans (Ca) are a frequent cause of lethal sepsis in intensive care unit patients. While a contribution of type I interferons (IFNs-I) in fungal sepsis remains unknown, these immuno-stimulatory cytokines mediate the lethal effects of endotoxemia and bacterial sepsis. Using a mouse model lacking a functional IFN-I receptor (Ifnar1-/-), we demonstrate a remarkable protection against invasive Ca infections. We delineate a mechanism whereby IFN-I signaling controls the recruitment of inflammatory monocytes to infected kidneys by driving CCL2 chemokine expression. Within kidneys, monocytes differentiate into inflammatory DCs but fail to mature in Ifnar1-/- mice, as demonstrated by the impaired upregulation of the key activation markers PDCA1 and iNOS. The increased activity of inflammatory monocytes/DCs results in hyper-inflammation and lethal kidney pathology. Accordingly, pharmacological suppression of inflammatory monocytes by treating mice with Pioglitazone, a synthetic agonist of the nuclear receptor peroxisome proliferator-activated receptor-γ; (PPAR-γ), strongly reduces renal immunopathology during fungal sepsis and improves mouse survival. Taken together, our data link the sepsis-promoting effects of IFNs-I to the activity of inflammatory monocytes/DCs with high host-destructing potency. Hence, our data suggest a therapeutic relevance of PPAR-γ agonists for microbial infectious diseases where inflammatory monocytes may contribute to fatal tissue damage.

Topic: Inflammation and Immunity

P 151 Neuronal firing coding of the prefrontal cortex and hippocampus during a rule switching task

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The prefrontal cortex is regarded as an executive area, and plays a pivotal role in cognitive functions like working memory, decision making and attention shift. An increased activity of the prefrontal cortex during a rule switching task has been shown in humans with MRI. Similarly, in rats, lesions in the prefrontal cortex lead to impairment in the ability to adapt to change rules. As the prefrontal cortex receives major input from the hippocampus, which is well known to be important for memory consolidation, learning and spatial navigation, the relationship between these two areas is important to understand neuronal processes involved in rule switching. Previous work has shown that coherence between prefrontal cortex and hippocampus plays an important role in the learning of different rules. Still, the neuronal mechanism and cell assemblies coding underlying rule switching is not known. To asses this, we use a rule switching task, specially designed for rats. Rule switching is present in the rat behaviour when the rat realises a change in the already learnt rule, due to the lack of reward. This will encourage the rat to modify its conduct, looking for more reward. We are using a plus maze and the rat learns to switch between four rules, involving two different strategies: allocentric (cue referenced) and egocentric (self referenced) spatial representations. During this task, we recorded local field potentials and multi-unit activity from prefrontal cortex and hippocampus, using tetrode recordings in freely moving rats performing the task. We specifically implanted tetrodes in the anterior cingulate and prelimbic areas in the prefrontal cortex and dorsal CA1 in the hippocampus. We observed differences in the firing rate of the cells not only in the prefrontal cortex and hippocampal region, but also between the anterior cingulate and the prelimbic areas during task performance.

Topic: Neuroscience

P 152 Role of ERG in endothelium and its cooperation with NF-κB.

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ERG (Ets-Related Gene) seems to be particularly important for endothelial cells as it is exclusively expressed in endothelium. It exists in about 9 different isoforms and is involved in regulation of some endothelial specific genes, cell differentiation, angiogenesis, homeostasis, as well as apoptosis. Like other ETS factors ERG can also be expressed in different cancer types and leukemia´s. There are several reports suggesting that ERG acts as an anti-inflammatory mediator in endothelial cells, where it reduces the activity of NF-kappa B in contrast to cancerous situations where it increases the activity of NF-kappa B. We are aiming to reveal the specific functions that can be attributed to ERG in endothelial cells and to explain why ERG is anti-inflammatory in endothelial cells and pro-inflammatory in cancer cells. We are also trying to clarify potential positive or negative cooperativities of ERG and NF-kappa B during inflammation and cancer development. To achieve these objectives the techniques we are applying are Lentiviral transduction, fluorescence resonance energy transfer (FRET) microscopy; histology; co-immunoprecipitation; reporter gene assays; oligo-array analyses; qPCR, Scratch assays, Tube formation assays, transgenic mouse models. Our initial findings suggest that ERG has important roles in migration of endothelial cells. Moreover ERG is protecting endothelium from inflammation by suppressing NF-κB and its targeting genes.

Topic: Vascular Biology

P 153 Targeting the HIF pathway to inhibit tumour angiogenesis: Focus on HIF-1B

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Reduced oxygen supply, also termed hypoxia, provides a strong environmental stress for cells. In solid tumors hypoxia is a consequence of excessive proliferation. The hypoxia-inducible factor (HIF) pathway allows cells to adapt to this condition, for instance by initiating angiogenesis. The main key players in this pathway are HIF-1alpha and HIF-1beta, which form a heterodimeric transcription factor. The alpha subunit is regulated in an oxygen-dependent manner, whereas the beta subunit is regarded as constitutively expressed. This study aims to identify redundant signalling pathways which are activated due to HIF inhibition in hypoxia and enable cells to initiate angiogenesis. Our experiments showed unexpected effects of HIF-1beta as well as a non-canonical relationship between HIF-1alpha and HIF-1beta in the human melanoma cell line 518A2. Time-course experiments carried out with a panel of different human melanoma cell lines which were treated with the hypoxia-mimetic cobalt chloride revealed that HIF-1beta was strongly inducible in 518A2 and A375 cells. Knockdown of HIF-1alpha by siRNA in 518A2 cells decreases HIF-1beta on protein level after stimulation by cobalt chloride or exposure to hypoxia. To test whether HIF-1beta was simply protected from degradation by binding to HIF-1alpha we overexpressed the alpha subunit in 518A2 cells. These time-course experiments showed a time delayed increase of HIF-1beta after treatment with cobalt chloride suggesting other mechanisms rather than protection from degradation. Further HIF-1beta expression was increased on mRNA level in 518A2 cells after stimulation with cobalt chloride as demonstrated by qRT-PCR. Taken together, these findings demonstrate that HIF-1beta might also be regulated in an oxygen and HIF-1alpha dependent manner in 518A2 human melanoma cells. The biological relevance of this effect is currently under investigation using a Hen´s Egg Test – Chorioallantoic-Membrane (Het-CAM) model for angiogenesis and invasion.

Topic: Malignant Diseases

P 154 Neutrophils and NETs at the culprit lesion site of ST-elevation acute coronary syndrome

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Background Mechanisms of acute plaque rupture and coronary occlusion in ST-elevation acute coronary syndrome (STE-ACS) are poorly understood. In contrast to common knowledge implicating intralesional macrophages in the pathogenesis of acute coronary vascular syndromes, we hypothesize that circulating innate immune cells mediate plaque rupture and thrombotic occlusion. In former studies, we reported the accumulation of neutrophils and related proteins at the culprit lesion site. Neutrophil extracellular traps (NETS) represent an efficient effector mechanism of activated neutrophils. We aimed to characterize neutrophils in the different circulatory compartments (systemic, coronary fluidic and solid aspiration material) of STE-ACS patients. Methods STE-ACS patients who underwent primary percutaneous coronary intervention at the Vienna General Hospital were consented (n=70). Culprit site blood was aspirated with a thrombectomy catheter and particulate thrombus material was separated. In parallel, blood was sampled from the femoral arterial sheath. Flow cytometry was employed to evaluate neutrophils accumulating at the plaque rupture site in the fluidic and solid compartment. These results were complemented by ELISA and immunofluorescence assays. Results Neutrophils derived from coronary thrombi are highly activated compared to systemic values (CD66b, CD11b) and form aggregates with platelets. Coronary thrombi display excessive amounts of NETs. Neutrophil-derived degradation products are predominant in coronary thrombi. MPO in coronary plasma is significantly increased. By contrast, neutrophils in fluidic compartment of culprit site aspirates show reduced expression of CD11b and form fewer aggregates. Conclusion We report the presence of activated NET-producing neutrophils at the culprit lesion site of STE-ACS patients. NETs act as an active scaffold for the nascent coronary thrombus.

Topic: Cardiovascular and Pulmonary Disease

P 155 Specific monocyte subsets are increased at the culprit lesion site of ST-elevation acute coronary syndrome patients

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Background ST-elevation acute coronary syndrome (STE-ACS) is the leading cause of death. Mechanisms of acute plaque rupture and coronary occlusion are poorly understood. In contrast to common knowledge implicating intralesional macrophages in the pathogenesis of acute coronary vascular syndromes, we hypothesize that circulating innate immune cells mediate plaque rupture and thrombotic occlusion. Monocytes are early inflammatory cells implicated in the pathogenesis of ACS. CD16+ monocytes represent an activated and proinflammatory subtype. In the present study, we examined the distribution and activation status of these subsets in STE-ACS. Methods STE-ACS patients who underwent primary percutaneous coronary intervention at the Vienna General Hospital were consented (n=40). Culprit site blood was aspirated with a thrombectomy catheter and particulate thrombus material was separated. In parallel, blood was sampled from the femoral arterial sheath. Flow cytometry was employed to classify monocytes by their CD14:CD16 ratio (CD14+CD16-, CD14+CD16+, CD14-CD16+) at the plaque rupture site, both in the fluidic and solid compartments. Results CD16+ monocytes were selectively increased in the fluidic compartment at the culprit lesion site of STE-ACS patients. They express a distinct pattern of adhesion and activation markers and aggregate with platelets. Conclusion CD16+ monocytes accumulate at the culprit lesion site in STE-ACS patients. We hypothesize that interaction with platelets induces CD16 expression initiating pro-inflammatory downstream effects, including inflammatory cell recruitment.

Topic: Cardiovascular and Pulmonary Disease

P 156 CD4CD28null T-cells are specifically enriched at the culprit lesion site of ST-elevation acute coronary syndrome patients

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Background Acute coronary syndrome (ACS) is the leading cause of death. Coronary plaque rupture and thrombotic occlusion are the underlying key events and are still not well understood. CD4CD28null T-cells are an aggressive T-cell subset with high proinflammatory and cytotoxic capacity. This subset is enriched in ACS patients and can predict cardiovascular events, implicating an impact in the pathogenesis of STE-ACS1,2. We aimed to investigate whether CD4CD28null cells are enriched at the culprit lesion site in acute myocardial infarction (AMI) patients. Methods STE-ACS patients who underwent primary percutaneous coronary intervention at the Vienna General Hospital were consented (n=70). Culprit site blood was aspirated with a thrombectomy catheter and particulate thrombus material was separated. In parallel, blood was sampled from the femoral arterial sheath. Flow cytometry was employed to evaluate CD4CD28null T-cells accumulating at the plaque rupture site. These results were complemented by ELISA. Results CD4CD28null T cells are increased at the culprit lesion site compared to periphery. Intracellular Perforin and Granzyme B were decreased in coronary CD28null cells. Plasma concentration of Granzyme B, but of Perforin, was reciprocal increased. Conclusion We could show a specific enrichment of CD4CD28null T-cells at the culprit lesion site in STE-ACS patients. Moreover, we demonstrate a local release of Granzyme B at the site of coronary plaque rupture, implicating a specific role of these cells in the pathogenesis of STE-ACS.

Topic: Cardiovascular and Pulmonary Disease

P 157 The ex vivo and in vivo antioxidant effect of cortisol

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Background: It is now accepted, that the free radical producing myeloperoxidase (MPO) plays a role in the pathogenesis of various diseases including atherosclerosis. It is also known that cortisol has antioxidant effect, which in this work we examined amongst both in vitro and in vivo circumstances to reveal a possible connection between MPO activity and the antioxidant effect. Materials and Methods: In the first phase of our study neutrophil granulocytes of healthy volunteers were incubated with cortisol or cortisol and myeloperoxidase inhibitor 4-aminobenzoic acid hydrazide (ABAH) or indomethacin. Superoxide anion production was determined by photometry. In addition to this measurement healthy volunteers were injected intravenously by 25 mg prednisolone and the mean peroxidase index (MPXI) was determined two hours after injection. In the second phase adult male rats were fed lipid rich diet and treated with cortisol in the drinking fluid. Total scavenger capacity (TSC) was measured before and after 4 weeks of treatment in blood samples using a chemiluminometric assay. Results: Neutrophils incubated with cortisol showed significantly reduced superoxide production compared to control. When ABAH and indomethacine were added, superoxide generation increased and the decreasing effect of cortisol was inhibited. MPXI values were significantly higher two hours after prednisolone injection than the control values. In the animal model cortisol treatment caused increased TSC. The control groups showed no significant changes in TSC. Conclusions: We conclude that cortisol administration can improve the antioxidant status amongst ex vivo and in vivo circumstances and this effect is related to MPO activity. In our work MPO activity decreased the free radical production which is not in line with our prior expectations.

Topic: Endocrinology and Metabolism

P 158 Prostate Cancer Stem Cells Demonstrate Decreased mTOR Activity and are Resistant to mTOR Inhibition in Hypoxia

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Prostate cancer (PCa) is the most prevalent malignancy in males in developed countries. Tumor-initiating cells (TICs), also known as cancer stem cells (CSCs), were recently identified and characterized in prostate cancer. We hypothesized that one of the frequently altered signaling pathways in PCa, the PI3K/AKT/mTOR pathway, plays a role in PCa stem cell maintenance. Moreover, hypoxia is known to be involved in CSC maintenance and to have a regulating effect on mTOR. In our study, we evaluated the effects of mTOR inhibitors in hypoxia on proliferation of human as well as murine CSCs in vitro. We isolated CSC like subpopulations from the androgen independent human PCa cell line DU145 using FACS according to their expression of the stem cell marker CD44. Further, we sorted the murine PCa cell line TRAMP-C1 based on the expression of the murine stem cell markers Sca-1 and CD49f. We used sphere formation assays to confirm the stem cell properties of the sorted subpopulations. Next, we treated the CSC and non-CSC populations with mTOR inhibitors under normoxic and hypoxic (3% O2) conditions in order to determine their cell viability at time points up to 72 hours. Our results suggest that CSC like PCa cells with elevated expression of stem cell markers are more resistant to mTOR inhibitors when compared to their non-CSC counterparts. Most interestingly, while the non-CSC population displays increased sensitivity to mTOR inhibitors under hypoxic conditions, the viability of CSCs remains unaffected. Immunoblotting reveals generally lower mTOR activity in PCa stem cells compared to non-CSCs. Hypoxia leads to a decrease in mTOR activity in both subpopulations, though this effect is more pronounced in CSC like subpopulations. At the same time, we do not observe alterations in signaling through other PI3K effectors, such as AKT. We propose that PCa stem cells might be resistant against inhibitors of mTOR and that hypoxia might contribute to mTOR resistance of prostate CSCs.

Topic: Tumorbiology - Oncology

P 159 Unraveling the alternative splicing landscape in Arabidopsis using RNA-seq

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Alternative splicing (AS) is a key regulatory mechanism involved in increasing transcriptome complexity, controlling important biological processes such as development and response to the environment. The study of AS in plants has been mainly addressed with cDNAs/ESTs libraries. To determine the impact of AS on Arabidopsis transcriptome, we used a normalized cDNA library from wild type plants and subjected it to paired-end Illumina sequencing. We obtained a deeply covered transcriptome that resulted in the detection of ~150,000 splice junctions. ~93% of the predicted splice junctions depict typical plant introns, including an eight fold increase in the repertory of U12 introns. ~61% of the intron-containing genes in Arabidopsis present evidence of AS under normal growth conditions, indicating a major role of AS in shaping the Arabidopsis transcriptome. We provide experimental validation of 540 transcripts in 256 genes using the high resolution RT-PCR panel. AS analysis showed that intron retention (IR) is the most common event (~40% of the events), nevertheless it is only present in ~23% of the transcripts and ~51% of the genes possess AS transcripts that do not involve IR. This result suggests that the impact of IR in generating transcript diversity in plants is overestimated. Analysis of the IR events allowed identifying a set of cryptic introns inside exonic coding sequences. Interestingly, about 600 of such introns are in frame implying their role in protein isoform generation. This work represents a valuable resource for future studies on gene regulation in plants.

Topic: RNA-Biology

P 160 A single nucleotide polymorphism (rs342293) on chromosome 7q22.3 is associated with mean platelet volume in patients with carotid artery disease

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Background. Mean platelet volume (MPV) and platelet count (PLT) are inversely correlated and highly heritable. Recently, in a genome-wide association study a single nucleotide polymorphism (SNP rs342293, C>G) on chromosome 7q22.3 was found associated with mean platelet volume (MPV) in healthy subjects. The region spans PIK3 (phosphatidylinositol 3 kinase), and is an important regulatory site for hematopoiesis. Increased MPV represents a strong predictor of post event outcome in coronary disease and myocardial infarction. We investigated whether MPV predicts outcome in asymptomatic patients with carotid artery disease and if SNP rs342293 is associated with MPV in these patients. Patients and Methods. MPV values were determined in 1033 patients, the genetic study was performed in 982 of 1283 patients of the ICARAS (Inflammation in Carotid Artery Study) cohort recruited between March 2002 and 2003. Carotid stenosis was confirmed by duplex sonography. All patients were prospectively followed for progression of carotid atherosclerosis and development of a first major adverse cardiovascular event (MACE), respectively. Genomic DNA isolated from archived blood samples was used for SNP analysis by Real-time PCR. Statistical analysis was performed by multivariate Cox regression. Results. 266 patients (26%) experienced a MACE during a median follow-up of 3.5 years. An MPV >11.6fl was significantly associated with MACE. By genotyping we identified 271 CC, 507 CG and 204 GG carriers (G-allele frequency 0.46). Carriers of CC had 10.83±0.98fl, of CG 11.1±0.9fl, and of GG 11.18±0.75fl MPV. The minor G-allele was statistically significantly associated with higher MPV values (P <8.84 x 10-5). No association of rs342293 C>G with adverse cardiovascular outcome was found. Conclusion. MPV represents a risk factor for adverse cardiovascular outcome in patients with carotid atherosclerosis. The rs342293 G- allele is associated with higher MPV in patients with carotid atherosclerosis.

Topic: Cardiovascular and Pulmonary Disease

P 161 Liquid chromatography tandem mass spectrometry-based method for the rapid determination of lysosomal enzyme activities for selective metabolic and newborn screening in dried blood spots

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INTRODUCTION: The interest in early detection strategies and newborn screening approaches for lysosomal storage disorders has increased because of new therapy options such as enzyme replacement, stem cell transplantation, and substrate reduction therapy became available over the last 10 years. Based on an enzyme reaction test from dried blood spots, combined with a prior developed online sample clean-up HPLC-MS/MS approach, we tested a short incubation protocol that reduced analysis time, and minimized complex and time consuming sample preparation. MATERIALS AND METHODS: Dried blood spots of nine known patients and 600 control patients were tested in parallel with the new short(3h)and with a reference method that used overnight incubation of 16 to 20h to evaluate the performance. Two dried blood spots in two separate 96-plates were used for this test. One spot was diluted with 60 µl buffer and aliquots of 10µl were used to perform separate incubations for ASM (acid sphingomyelinase), ABG (acid beta glucocerebrosidase), GAA (alpha glucosidase) and GLA (alpha galactosidase) in specific buffer systems. The second spot was directly incubated with 30µl of IDUA (alpha L iduronidase) specific buffer. RESULTS: We observed that the kinetic of all tested enzymes was linear over time. Thus, it was possible to quantify enzyme activity after 3h. The online sample clean-up approach detected all affected patients accurately, and it was possible to differentiate known patients from newborns with normal enzyme activities. DISCUSSION: We could demonstrate that the analysis of five lysosomal enzyme activities with a LC-MS/MS based approach would be possible in less than four hours of total analysis time including sample pre-treatment, incubation and MS analysis per sample. This would offer the possibility to use this protocol and technology for rapid selective metabolic testing, high risk population and newborn screening approaches.

Topic: Endocrinology and Metabolism

P 162 A multigene signature-based approach to uncover the contribution of sphingolipid machinery to the process of epithelial to mesenchymal transition

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Sphingolipids comprise a highly diverse class of bioeffector molecules playing important roles in crucial cellular processes. Strong line of evidence indicates involvement of both sphingolipid-modifying enzymes and lipid mediators in the process of tumorigenesis. The role in the epithelial to mesenchymal transition (EMT) was not highlighted thus far. In our study, the A549 cell-based EMT model was established to assess novel, sphingolipid-based mechanisms contributing to the process of tumor invasion. To trigger the EMT program, A549 cells, a human lung adenocarcinoma cell line, were stimulated with TGF-beta, TNF-alpha or their combination. Monitoring of the EMT process was performed by microscopic evaluation of cell morphology, FACS analysis, and gene expression profiling of EMT markers. Sphingolipid-related multigene signature (n=35) was designed to cover the interconnected network of sphingolipid metabolism and applied for the real-time PCR-based expression profiling. About forty percent of analyzed genes showed modulated expression at the mesenchymal stage. Among others this includes individual members of the sphingosine-1-phosphate (S1P) receptor family as well as members of the sphingosine kinase, the S1P phosphatase and the lipid phosphate phosphatase families. Furthermore, for ceramide (Cer) metabolizing enzymes, mRNA levels of Cer kinase, Cer synthase 1 and 4 were found to be downregulated. Alterations of the sphingolipid-associated genes were found to be trigger-specific and showed distinctive patterns for different types of EMT. Data driven, sphingolipid-associated EMT-related pathways were built up using bioinformatics approaches. The self-created multigene signature allowed to get insights into the alterations in the sphingolipid-associated cellular machinery during the EMT process and nominate novel sphingolipid-related EMT-associated key players.
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Topic: Immunology

P 163 Cardiomyogenic effects of Cardiogenol C on lineage-committed progenitor cells

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Cell transplantation into the injured heart is a new therapy after myocardial infarction. Its success, however, is impeded by the limited capacity of donor cells to differentiate into functional cardiomyocytes in the heart. A strategy to overcome this problem would be the induction of cardiomyogenic function in cells prior to transplantation. Recently, synthetic small molecules (SySMs) were identified, which exhibit a remarkable capacity to trans-differentiate cells into other cell types. For example, the diaminopyrimidine Cardiogenol C (CgC) induces cardiomyogenesis in embryonic stem cells. The aim of this study is to address the cardiomyogenic effects of CgC on lineage committed progenitor cells. This includes the characterization of functional cellular properties e.g. electrophysiological features of CgC-treated cells. We found that treatment with CgC significantly up-regulates cardiac marker expression not only in P19 mouse embryonic carcinoma cells, but also in lineage-committed C2C12 mouse skeletal myoblasts. Moreover, whole cell patch clamp recordings revealed more cardiac-like Na current properties in C2C12 cells after CgC-treatment. In line with this finding cardiac Na channel protein levels were enhanced in CgC treated cells. In addition, CgC also promoted the development of beating cardiomyocytes from A5 cardiovascular progenitor cells aggregated to form cardiac bodies. Finally, we observed cardiomyogenic activity of CgC also on human skeletal myoblasts. We conclude that CgC induces cardiomyogenic differentiation not only in embryonic stem cells, but also in already committed cell types. This work is supported by the AWS (Z090391).

Topic: Molecular Signal Transduction

P 164 Copper complexation of thiosemicarbazones enhances apoptosis in human colon carcinoma

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Because of their high proliferation rate tumor cells are characterised by sensitivity to iron deprivation. With the aim to target this iron dependence, several iron chelators have been developed for the treatment of cancer. Most promising among these are thiosemicarbazone (TSC)-based drugs. Triapine, the most prominent member of this family, is currently under clinical evaluation. In this study, we analysed the anticancer activity of several thiosemicarbazones and their copper complexes. For this purpose the impact of copper complexes (Cu-FTSC, Cu-APTSC and Cu-Triapine) and their metal-free ligands on cell viability, cell cycle distribution (PI-staining) and apoptosis (HÖPI and JC-1 staining) were investigated in human colon cancer (SW480) cell lines. With regard to the antiproliferative, all complexes were found to be slightly less effective than the respective ligands, with exception of Cu-FTSC, where an about threefold increased activity compared to metal-free FTSC was observed. Notably, all copper complexes acted much faster than the respective metal-free ligands. The apoptosis-inducing potential of the copper complexes strongly differed. In the case of Cu-Triapine, no increase in apoptotis was observed. In contrast, apoptosis induction by Cu-FTSC correlated with the results obtained in MTT assay, whereas Cu-APTSC and Cu-BPYTA, no induction of apoptosis was found at IC50 levels. However, at higher drug concentrations a profound increase in the number of apoptotic cells up to 100% was detected. In contrast, cell cycle distribution analysis revealed no significant differences between the copper complexes and their respective metal-free ligands. Taken together, our data reveal that although copper complexes and their respective ligands are active in the same concentration range, their underlying mode of action substantially differs. Due to their redox activity, copper complexes might be of interest for targeting the specific hypoxic milieu of the solid tumor.

Topic: Malignant Diseases

P 165 Chromatin- and transcription-assisted inactivation of interferon-activated transcription factors Stat1 and Stat2

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Interferons (IFNs) regulate immune responses by triggering the JAK/Stat signaling pathway that, depending on the type of IFN, induces activation (tyrosine phosphorylation) and nuclear accumulation of the transcription factors Stat1 and Stat2. Tyrosine dephosphorylation was shown to be of fundamental importance for nuclear export of Stats and deactivation of the pathway. Premature or delayed inactivation of Stat1 and Stat2 would result in insufficient or exaggerated IFN responses which may in turn lead to increased susceptibility to infections or deleterious IFN-mediated tissue damage, respectively. However, the control of timing of Stat1 inactivation is not well understood. Time course studies revealed that tyrosine dephosphorylation of Stat1 and Stat2 was impaired if their recruitment to IFN-regulated promoters was inhibited. Furthermore, by blocking transcription with two mechanistically different inhibitors we found that tyrosine dephosphorylation of Stat1 and Stat2 was dependent on active transcription. Since de-novo protein synthesis was dispensable for this process, we propose that the tyrosine phosphatase or an adaptor might be recruited to Stat target promoters by components of the general transcription machinery. The detailed mechanism is currently being investigated by us. Such a mechanism may restrict Stat inactivation to molecules that have been already engaged in transcription whereas molecules from the yet unused pool would still be available for gene regulation. This so far unique mechanism may serve as model how the removal of transcription factors from their promoters is controlled. The project is funded by the Austrian Science Fund (FWF) through the grants W1220-B09 (DP \'Molecular Mechanisms of Cell Signaling) and P 22806-B11.

Topic: Molecular Mechanisms of Cell Signaling at the MFPL

P 166 Towards subtype selective modulators of GABAA receptors

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GABAA receptors are the major inhibitory transmitter receptors in the central nervous system. They are ligand-gated chloride channels composed of five subunits that can belong to different subunit classes. In mammalians, there are genes for six alpha 1 (a1), three beta 3 (b3), three gamma 2 (g2), one delta (d), one epsilon (e), one theta (t), one pi (p), and 3 rho (r) subunits. The majority of GABAA receptors are composed of two a, two b, and one g subunits. Recent findings point to a1-containing GABAA receptors as the "sedative" and a2- and/or a3- containing receptors as the "anxiolytic" subtype (s). If subtypes are addressed selectively by ligands, such as MRK-409, side effects should be reduced. In addition to the published prototypes of functionally selective ligands, certain pyrazoloquinolines and imidazobenzodiazepines also display functional selectivity for a3. The aim of this thesis is to systematically investigate the ligand features that lead to this a3 preference. The methods which will be used for this work are two electrode voltage clamp electrophysiology in WT and mutated receptors, Cysteine scanning mutagenesis with steric hindrance via cysteine modification and quantitative structure activity modeling (QSAR). Ligand synthesis will be done by cooperation partners. Summarizing our data, from a series of 18 pyrazoloquinolines that possess nanomolar affinity to a1, 2 or 3-containing benzodiazepine sites, only three feature nanomolar stimulation in a3b3g2 receptors while the others are highly potent null modulators at the benzodiazepine binding site. Similar findings for imidazobenzodiazepines also exist in our lab. Thus, it can be concluded that multiple classes of benzodiazepine site ligands contain functionally a3 preferring and unselective ligands and deciphering the rules behind this phenomenon enables future rational ligand design.

Topic: Neuroscience

P 167 Transcriptome analysis of 3T3-L1 preadipocyte differentiation using RNA-seq reveals formerly unidentified differentially regulated genes

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Background: It is generally accepted that the regulation of adipogenesis prevents obesity. However, the mechanisms controlling adipogenesis have not been completely defined. Much of our knowledge of the molecular cascade regulating adipogenesis has come from transcriptome profiling data of adipocyte differentiation generated by DNA Microarray experiments. RNA sequencing is a recently developed approach for transcriptome profiling, where the RNA/cDNA species within a sample are sequenced, that does not suffer from the limitations described for Microarrays. Our goals were to use RNA-seq to identify novel regulators of adipogenesis throughout 3T3-L1 preadipocyte differentiation and to compare sequencing data to results obtained from Microarrays. Materials: Murine 3T3-L1 preadipocytes were differentiated into mature adipocytes. Total RNA was extracted on days 0, 2, 4 and 6 and processed for transcriptome profiling either with Affymetrix Gene Level 1.0 ST arrays or the TrueSeq paired-end mRNA protocol on a Illumina platform. Results: Overall, the RNA-Seq approach identified a larger number of ±3.0-fold differentially gene expression (DEG) in 3T3-L1 preadipocyte differentiation as compared with Gene 1.0 ST arrays on Days 2, 4 and 6 i.e. RNASeq: 2620, 4019 and 3972 genes versus Microarray: 443, 788 and 910. Plotting FC values of RNA-seq versus Microarray revealed a population of genes that exhibited FC values at or near zero by Microarray analysis but exhibiting a wide range of FC values by RNA-seq, highlighting the sensitivity of the RNA-seq approach. Conclusion: Transcriptome profiling of adipocyte differentiation has improved our understanding of the molecular mechanisms of adipogenesis. Although microarrays have been instrumental in this regard, it is clear that these tools detect an incomplete set of DEG. Therefore the RNA-seq approach can be used to supplement these prior technologies, which could help identify novel target genes involved in adipogenesis in disease.

Topic: Endocrinology and Metabolism

P 168 Induction of IL-35 in human T cells upon co-stimulation via CD43 and PD-1

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IL-35 is a member of IL-12 family, consisting of two subunits, EBV induced gene 3 (EBI3) and p35. IL-35 has been characterised as an inhibitory cytokine and is extensively studied in mouse models. Recently, our group has shown the induction of IL-35 producing human regulatory T cells by human rhinovirus treated DC (R-DC) via up-regulation of B7-H1 (CD274) and sialoadhesin (CD169). Thus, a combined set of co-stimulatory signals for T cells seems to be critical to induce IL-35. In this study we investigated the underlying molecular mechanism involved on T cell side for the induction of IL-35. T cells isolated from human peripheral blood, were stimulated with a panel of monoclonal antibodies (mAbs) against putative accessory T cell surface receptors. Induction and release of IL-35 was analyzed by qPCR, cytoplasmic staining and a newly established IL-35 ELISA. Among the various combination of mAbs tested, T cells stimulated with plate-bound anti-PD-1 mAb in combination with CD43 mAb and CD3 mAb - OKT3 was identified to most potently induce IL-35. Interestingly, T cell activation via CD3+CD28 or other prominent pathways, using such plate-bound mAbs,failed to do so. T cells activated via CD3+CD43+PD-1 showed a diminished proliferative response compared to the cells stimulated via CD3+CD43. However, the T cell signature cytokine profile (IL-2, IL-4, IL-10, IL-17, IL-22, IFNγ) remained unaltered upon stimulation via CD3+CD43+PD-1, compared to other activation protocols. Thus,stimulation of human T cells via CD3+CD43+PD-1 seems to be a specific pathway to induce IL-35 production, that is not accompanied by a re-organisation in the T cell signature cytokine profile, and a novel pathway for the induction of immune-inhibitory T cells.

Topic: Inflammation and Immunity

P 169 Pediatric epilepsy surgery – predictors of (un)favourable outcome

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Among the neurological diseases in childhood, epilepsy has one of the highest frequencies. Due to recent progress in diagnostics and treatment, over 60% of newly diagnosed patients may enter remission during treatment with antiepileptic drugs. However, as many as 30-40% of patients remain difficult to treat or are drug-resistant, and hence it is important to gain a deeper understanding into their condition so as to ameliorate it. Such patients are usually referred to a tertiary centre for epilepsy-surgery evaluation. Following surgery, about 67% of the patients are seizure free after removal of the temporal lobe and about 50% following extratemporal resection. Ideally, anticonvulsive therapy may be stopped pursuant to surgical intervention. The main focus of this thesis was to compare EEG data, neuroimages, and neuropsychological test results from patients before and after surgery. In addition, histological diagnoses were also undertaken. This resulted in the positive surgical outcome in one young female patient. Although it is not common to treat autoimmune-induced limbic encephalitis by resection of the temporal lobe, as a result of analyzing the patient's data, it became clear that her hippocampus had become atrophied, and this led to persistent epileptic fits. Following the removal of the temporal lobe and hippocampus, the patient recovered and remained seizure free for a year. A further outcome of this work was the publication of an extensive review on neuropathological handling of epilepsy specimens. Importantly, the work helped define clear-cut histological characteristics in brain samples that might positively influence neuroimaging, including: cortical thickness, grey-white matter border, heterotopic neuropil in the deep white matter; and myelination. These findings should help improve presurgical diagnostics, thereby resulting in more a successful outcome of surgical intervention.

Topic: Clinical Neurosciences

P 170 Glucocorticoid receptor function is essential for SOCS2-mediated negative regulation of hepatic GHR signaling

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GHR signaling plays an essential role in somatic growth and development as well as in the maintenance of metabolic homeostasis. Target tissue responsiveness to GH is under regulatory control to circumvent unphysiologic and off-target effects due to prolonged GHR activation. SOCS2, a GH-STAT5-regulated gene, is a key negative regulator of GHR sensitivity. Transcription of distinct GH-STAT5-dependent gene subsets requires coactivator function of the GR. This mechanism involves direct binding of GR to the STAT5 N-terminus. Earlier studies have implied that regulation of Socs2 also requires synergistic action of STAT5 and GR. Therefore, our aim is to investigate the molecular interaction between STAT5 and GR to regulate Socs2 expression. Hepatocyte-specific deletion of the GR causes reduced hepatic Socs2 mRNA expression. Furthermore, STAT5 activity is increased in GR-deficient livers upon exogenous GH administration. We found three STAT5 oligomer binding sites in the murine Socs2 promoter region. Binding of activated STAT5 to the putative binding sites was confirmed by DNA binding assay. Subsequently, we performed transactivation studies using a luciferase reporter construct containing the relevant region of the Socs2 promoter and an additional 3' region to assess the requirement for putative enhancer sequences. Maximum luciferase activity was observed in the presence of activated STAT5b and GR only. This transactivation was severely impaired upon transfection of the aminoterminally truncated STAT5b isoform. Additionally, colocalization of activated STAT5 and GR on the Socs2 promoter was detected in GH/dexamethasone-treated control livers by re-ChIP assays. We show that full Socs2 transactivation requires STAT5-GR protein-protein interaction in hepatocytes. As the liver represents a primary site of GH-mediated STAT5 activation, deregulated GR signaling might lead to imbalanced liver GH sensitivity due to aberrant SOCS2-mediated feedback inhibition.

Topic: Malignant Diseases

P 171 TLR-independent recognition of Streptococcus pyogenes is required for successful inflammatory response

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S. pyogenes is a leading Gram-positive human pathogen which causes a broad range of mostly self-limiting diseases including pharyngitis, scarlet fever or impetigo [1,2]. It may however produce invasive and life-threatening infections such as necrotising fasciitis and toxic shock with over 30% mortality rate. Animal studies demonstrated that the innate immune system, most notably macrophages, dendritic cells and neutrophils, plays an essential role in in a mouse model of invasive S. pyogenes infection [3,4,5]. However, pattern recognition receptors (PRRs) involved in the recognition of S. pyogenes by the cells of the innate immune system are not known. We and others showed that infection of murine bone marrow-derived macrophages and conventional dendritic cells with S. pyogenes caused these cells to produce cytokines, including TNF, IL-6 and IFN-b, and that MyD88 plays a key role therein [6,7,8]. We recently demonstrated that streptococcal nucleic acids are required for IFN-b induction which occurs independently of the known TLRs. In our current study we demonstrate that the production of TNF also proceeds in the individual absence of PRRs usually involved in recognition of bacteria, including TLR2/3/4/5/7/8/9, NOD1 and NOD2. Similar to IFN-b induction, phagocytosis and S. pyogenes-derived nucleic acids are involved in TNF production upon S. pyogenes infection. Our data further reveal that the extent of cytokine induction by different S. pyogenes strains inversely correlates with their virulence. Consistently, an enhancement of the innate response by genetic means [9] results in successful defence against a virulent S. pyogenes strain. Together, our study suggests an approach of targeting the innate immune system for therapeutic exploitation in streptococcal infections.

1.Johansson et al., 2010 2.Wessels, 2011 3.Goldmann et al., 2004 4.Loof et al., 2007 5.Navarini et al., 2009 6.Gratz et al., 2008 7.Loof et al., 2008 8.Gratz et al., 2011 9.Kratochvill et al., 2011

Topic: Molecular Mechanisms of Cell Signaling at the MFPL

P 172 Pharmacokinetic-pharmacodynamic modeling of P-glycoprotein function at the rat blood brain barrier studied with positron emission tomography

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Pharmacokinetic (PK) modeling is the common method of choice to quantitatively analyze data obtained with positron emission tomography (PET) and to verify, that the PET signal indeed represents the underlying physiological, biochemical and pharmacological functions studied. PK modeling software is usually "in-house" developed software and therefore presents a low level of validation. Additionally, based on the individual PK model parameter estimates population averages and variability is generated after each subject is analyzed separately. In this study we used population mixed effect modeling (NONMEM VI – acronym for Nonlinear Mixed Effect Modeling), which is used in standard PK pharmacodynamic (PD) modeling but is not yet routinely used for PET data analysis. NONMEM analyses all subjects simultaneously, and gives a description of the PK in the typical subject as well as the variation in the study population. Data obtained from preclinical (R)-[11C] verapamil (VER) PET used to study in-vivo P-glycoprotein (Pgp) function at the rat blood brain barrier before and after inhibition with the Pgp inhibitor tariquidar (TQD) in a dedicated epilepsy model (naïve and 48 h post status epilepticus) was analyzed. NONMEM allows predicting the rate constants describing the pharmacokinetics of VER and the effects of TQD on VER pharmacokinetics. In the final model TQD was found to decrease the efflux rate constant from the first brain compartment to the plasma compartment Qout (effect described as a categorical covariate) and epilepsy was a significant covariate on the first brain compartment, increasing the volume of distribution VT. NONMEM outcome parameters were reparameterized and compared to PK modeling outcome parameters. NONMEM is a potent and powerful alternative and supplement to PK modeling and should be promoted for PK/PD modeling of PET data.

References 1. Bankstahl, J.P., et al., (2011) J Neurosci, 31(24) 2. Wagner C.C., et al., (2009) JNM, 50(12) 3.Syvänen S., et al., (2011) BMC Medical Imaging, 11(1)

Topic: Preclinical and Clinical Research in Pharmaceutic Development

P 173 DETECTION OF BARTONELLA SPP. IN IXODES RICINUS

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Bartonella species are aerobic, Gram negative, facultative intracellular bacteria, which cause a variety of human and non-human diseases. They are pleomorphic, slightly curved rods belonging to the alpha-2 subgroup of the class Proteobacteria. Until now 24 Bartonella species and 3 subspecies have been described including at least 12 pathogenic for humans. They can cause cat-scratch disease, Carrion\'s disease, trench fever, bacillary angiomatosis and endocarditis. Known Bartonella vectors are cat fleas, fleas of other animals, sand flies and clothes lice. Whether ticks are vectors of Bartonella species remains to be substantiated. In this study 4 strains of Bartonella (B. clarridgeiae, B. grahamii, B. henselae, and B. doshiae) were cultivated and used as positive controls for the design of a Bartonella specific nested and Real-Time PCR assay. For this two target sequences were chosen: the 16S rRNA gen and the 16-23S intergenic spacer region. The primers for the ITS region were used for species differentiation. With these PCR assays ticks were screened from a library consisting of over 10.000 Ixodes ricinus ticks (adult, nymphs and larvae) collected at different locations in Austria. The comparison of the two PCR methods showed a higher sensitivity for nested PCR. Out of 216 examined ticks 23 tested positive for Bartonella, equal to an infection rate of 11%. This result confirms Ixodes ricinus at least as carrier of Bartonella species.

Topic: Other

P 174 Prolyl hydroxylase inhibitors increase the production of vascular endothelial growth factor in human dental pulp cells

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Prolyl hydroxylase (PHD) inhibitors induce a pro-angiogenic response that stimulates bone regeneration. However, if PHD inhibitors enhance pulp regeneration is unknown. Here we evaluated the effects of PHD inhibitors, on the pro-angiogenic capacity of human dental pulp cells (DPCs). To assess the impact of PHD inhibitors on DPCs , cells were exposed to dimethyloxaloylglycine (DMOG), desferrioxamine (DFO), L-mimosine (L-MIM), and cobalt chloride (CoCl2). The effect on viability, proliferation, and protein synthesis was measured by MTT, 3[H] thymidine- and 3[H]leucine incorporation assays. The effect on the pro-angiogenic capacity was evaluated by immunoassays for vascular endothelial growth factor (VEGF). To assess whether PHD inhibitors can induce VEGF production under the influence of pulp capping material, DPCs were treated with the PHD inhibitors in the presence of supernatants from calcium hydroxide. Our results show that PHD inhibitors reduced viability, proliferation and protein synthesis of DPCs at high concentrations. At non-toxic concentrations, PHD inhibitors stimulated the production of VEGF in DPCs. Furthermore, incubation with PHD inhibitors increased the production of VEGF when cells were cultured in the presence of supernatants from calcium hydroxide. In addition we assessed the pro-angiogenic effect of PHD inhibitors in tooth slice organ cultures. Also under these conditions, PHD inhibitors increased VEGF production. Overall, these results suggest that PHD inhibitors increase production of VEGF in cells from the dental pulp thereby provoking a pro-angiogenic environment. Whether this increased pro-angiogenic environment results in enhanced regeneration of the dental pulp requires pre-clinical studies.

Topic: Regeneration of Bones and Joints

P 175 Kinetics of primary and memory IgG1 & IgE antibody responses induced by an allergen derivative in-vivo in a murine model of allergy

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Memory is the hallmark of immunity, yet the cells and molecular mechanisms of immunological memory are still being discussed controversially. In type I allergy, a hypersensitivity disease affecting more than 25% of the population, allergic sensitization involves the class-switch from allergen-specific IgM to IgE producing B cells and plasma cells. The role of allergen-specific T cells in the induction of primary IgE response, a process called allergic sensitization, is well established. Much less is known regarding the regulation of secondary IgE responses. Aim of the study was to establish a murine model which allows dissecting the contribution of allergen-specific T and B cells to secondary IgE response and thereby to analyze the kinetics of primary and secondary antibody responses. Based on hapten-carrier model developed by Benacerraf, we used a 31 amino acid peptide from an important IgE-reactive domain of the major grass pollen allergen Phl p 1 and coupled it to an unrelated carrier molecule KLH. When we sensitized mice with this coupled peptide, allergen-specific IgG1 & IgE antibodies were induced. When rat basophils were loaded with the serum from sensitized mice, allergen-specific degranulation was induced. Next, we investigated the specificity of T cell response in sensitized mice. Using cultured splenocytes, we showed that allergen-specific IgG1 & IgE responses were essentially driven by KLH-specific T cells because no peptide or allergen-specific proliferation was observed. The allergen-specific IgG1 & IgE responses were boosted by immunizing the same KLH-coupled peptide, which thereby mimics a model of secondary IgE response. The described mouse model based on KLH-specific T and B cells producing allergen-specific IgG1 & IgE antibodies can now be used to dissect the contribution of T and B cells to secondary IgE response and eventually to develop therapeutic strategies targeting this process.

Topic: Immunology

P 176 Can serum biomarkers reliably quantify lung contusion in polytraumatized patients?

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PURPOSE: To find a lung specific serum biomarker that correlates highly with the extent of lung contusion in multiple injured patients. INTRODUCTION: Scientific reports show that the sensitivity in diagnosing thoracic trauma in severely injured patients at the time point of admission is far from being satisfactory due to the slow development of morphological alterations in the lung tissue. Therefore a follow-up CT scan should be performed after 24-48h to quantify the loss of functional tissue and to adapt the therapeutic procedures. Particularly lung contusions lead to increased rates of pulmonary complications and impaired outcome. By detecting plasma bound specific biomarkers, secreted immediately after trauma, we see a potential of predicting the progression of a possible respiratory impairment. Thus, an early determination of a specific therapeutic pathway would be possible and may lead to an improved outcome. MATERIAL AND METHODS: 100 patients fulfilling the following criteria will be enrolled: multiple injured patients, age≥18 years, direct admission to our department, ISS≥16, need of stay at the ICU. Directly after admission blood samples are collected. Samples will be centrifuged at 3000g for 15min. Afterwards the plasma will be removed and stored at -80°C until assayed. Five biomarkers of lung specific proteins will be measured in triplets using ELISA (CCP-16, SP-D, KL-6, CYFRA21-1, RAGE). The same procedure will be performed after 24-48h. Lung contusions will be visualized with CT at admission and after 24-48h, when the quantity can be seen to its full extent. Volume analysis will be done manually by defining regions of interest on the axial images and then transformed into a percentage value of injured/healthy lung to avoid variances according to lung volume etc. Statistical analyses will be performed using SPSS 16. A p-value <0.05 will be considered significant.

Topic: Regeneration of Bones and Joints

P 177 Exploratory Analysis of Multiple fMRI Paradigms

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Exploratory analysis of functional brain imaging data is tackling a wealth of open questions. A particularly challenging issue is the understanding of the connectivity structure, its default state and how it is modulated by different cognitive activities. Even-though, typical functional magnetic resonance imaging (fMRI) studies contain multiple paradigms to test for specific activations, development of methods for jointly analyzing multiple paradigms has been largely neglected. Joint analysis techniques have the potential to reveal functional similarity structures and basic principles of the brain's functional organization. We present an approach that allows for exploratory analysis of multiple fMRI paradigms based on Multiple Relational Embedding (MRE). By embedding multiple functional relationships into a single latent space, we can find overlapping functional characteristics, how connectivity structure is shared across multiple paradigms and can measure the combined modularity structure. The latent space enables the application of unsupervised machine learning methods such as clustering or graph theory based network and functional structure analysis. First, the capabilities of this approach were illustrated on a synthetic dataset comprising time-series with 100 time-points using baseline values from N(0,0.5) and activation values from N(1,0.5). The simulation creates two different activation patterns. Furthermore, the technique was used to analyze the functional MRI data of two language related paradigms, acquired during neurosurgical planning to identify language functions in patients with a brain tumor in language specific areas. Multi-paradigm analysis is only one possible application of the proposed exploratory embedding analysis. For neuroimaging data, this technique offers further interesting applications such as finding connections between structural and functional characteristics.

Topic: Clinical Neurosciences

P 178 Role of lipid-derived mediators in obesity-induced adipose tissue inflammation

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Obesity-induced adipose tissue inflammation represents a crucial link between obesity and insulin resistance. A number of potent pro- and anti-inflammatory/resolving lipid mediators such as prostaglandins (PG), lipoxins and resolvins (Rv) exist that arise from n-6 and n-3 polyunsaturated fatty acids (PUFA). Based on the extensive adipose tissue production of free fatty acids we hypothesized that lipid-derived mediators synthesized in adipose tissue play a potential role in obesity-induced adipose tissue inflammation. Possible alterations of lipid mediator levels in obesity were investigated in two mouse models of obesity, namely genetic (db/db) and diet-induced obesity applying solid-phase extraction and HPLC-tandem mass spectrometry. In parallel, gene expression of relevant markers for adipose tissue inflammation was determined using quantitative RT-PCR. Adipose tissue levels of analysed lipid mediators like PGE2, 12-HETE, 15-HETE, 17-HDHA and protectin D1 were significantly reduced while RvE1 precursor 18-HEPE was significantly increased in both mouse models of obesity compared to appropriate lean controls. Dietary n-3 PUFA supplementation increased levels of anti-inflammatory and resolving lipid mediators and their precursors, namely protectin D1, 17-HDHA, 18-HEPE and RvE1 in db/db mice. Moreover, n-3 PUFA supplementation improved insulin sensitivity and decreased adipose tissue inflammation.In conclusion, our data demonstrate that obesity-induced alterations of resolving lipid mediators and their precursors in adipose tissue could contribute to adipose tissue inflammation and consequently insulin resistance in obesity. This research was supported by the European Community's 7th Framework Programme (FP7/2007-2013) under grant agreement n° 201608, by the Austrian National Bank (P12735) and by the Federal Ministry of Economy, Family and Youth and the National Foundation for Research, Technology and Development (all to T.M.S.).

Topic: Endocrinology and Metabolism

P 179 Humanized Model for Respiratory Allergy Using a Human Mugwort-specific T-cell Receptor and HLA-DR1

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Currently, T cell receptor (TCR) transgenic (tg) mice with a murine TCR specific for chicken ovalbumin in the context of a murine restriction element (I-Ad) are frequently used in allergy research. We here aimed to generate double tg mice expressing a human TCR specific for the immuno-dominant epitope of the major mugwort (Artemisia vulgaris) pollen allergen Art v 1 in the context of the human restriction element HLA-DR1 to provide a valid model for studying allergy development and treatment in vivo. To obtain high expression levels the allergen-specific human TCR variable sequences were chimerized with murine TCR constant sequences. Resulting transgenes were cloned into the pTcass vector system and thus put under the transcriptional control of the natural TCR alpha and beta promotor/enhancer elements. Allergen-specific TCR tg founder mice were cross-bread with HLA-DR1+ B10.M-DR1dlAb1-Ea mice. Immunophenotyping of double tg TCR/HLA-DR1 mice revealed clear-cut expression of the Art v 1-specific TRBV18 chain on peripheral blood CD3+ T lymphocytes and HLA-DR1 expression on CD14+ monocytes and B220+ B lymphocytes. In vitro, splenocytes from TCR/HLA-DR1 double tg mice but not of HLA-DR1 single tg mice or wt mice specifically proliferated upon incubation with the human-relevant immuno-dominant Art v 125-36 peptide or whole Art v 1 protein. Allergen-specific cellular proliferation is accompanied by the production of a balanced cytokine milieu including IFN-γ, IL-2, IL-4, IL-6, IL-13 and IL-17. No cytokine secretion was evident upon incubation of splenocytes with a control peptide or medium alone. Importantly, double tg mice are proficient to mount both IgG2a and IgG1, IgE responses when i.p. immunized with antigen plus alum. A fully humanized allergy model will provide important insights into the pathophysiology of allergic diseases. The research was funded by the Austrian Science Fund by SFB F1816-B13, SFB F4609-B19, SFB F1807-B13 and FWF 20011-B13 (Austrian Science Fund), Biomay AG, and the Christian Doppler Research Association.

Topic: Clinical Experimental Oncology

P 180 Detailed hemodynamic characterization of athlete's heart using left ventricular pressure-volume analysis in a rat model

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The development of professional sport and sudden cardiac death cases among athletes aroused emerging interest in sports cardiology. Several research groups investigated exercise training induced left ventricular (LV) hypertrophy in animal models, however only sporadic data exists about detailed hemodynamic measurements. We aimed to establish and validate the rat model of athlete's heart and provide a detailed functional characterization using the modern sophisticated method of pressure-volume (PV) analysis. LV hypertrophy was induced by swimming training (200 min/d for 12 weeks). After completion of the swimming portocol we performed echocardiographic measurements and LV PV analysis using a microtip pressure-conductance catheter to investigate the morphology and function of the LV, respectively. Echocardiographic examinations showed LV concentric hypertrophy according to the wall-thickness values (LV mass index:2.41±0.08 vs. 2.03±0.08g/kg BW, $p<0.05$), which was confirmed by post-mortem measured heart weight and histological morphometry. Invasive hemodynamic measurements showed unchanged heart rate, arterial pressure and LV end-diastolic volume along with decreased end-systolic volume, increased stroke volume (248±14 vs. 201±15µl) and ejection fraction (73±1 vs. 64±2%) in trained rats compared to sedentary controls. The PV loop-derived sensitive, load-independent contractility indexes were found to be significantly increased (preload recruitable stroke work: 77±7 vs. 54±5mmHg). We observed increased LV stroke work (27±1 vs. 20±2mmHg*ml) and maximal power (92±9 vs. 60±6mW) in athlete's heart. Despite the significant hypertrophy, the LV stiffness was not increased, while there was an improvement in active relaxation (Tau: 9.6±0.3 vs. 10.9±0.3ms). According to our results, we established a rat model of physiologic LV hypertrophy. It is the first study, which provides a detailed characterization of functional changes and hemodynamic relations in athlete's heart.

Topic: Cardiovascular and Pulmonary Disease

P 181 Prevention of distant organ failure by postconditioning of small intestine on ischemia reperfusion injury model of rats

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Introduction: Temporary occlusion of the superior mesenteric artery (SMA) results in ischemia reperfusion (IR) injury of the small bowel. Permeability of the bowel is increased during ischemia, causing translocation of huge amount of endotoxin. Subsequent release of reactive oxygen species and inflammatory cytokines after a successful recanalization of the SMA leading to systemic inflammation or multiple organ failure. Objective: Aim of our study was to investigate the remote effects of postconditioning on small intestine IR injury. Methods: Male Wistar rats underwent 60 minutes of warm ischemia of the small bowel followed by 6 hours of reperfusion using atraumatic clamp. Postconditioning was performed immediately at the onset of reperfusion, by repetitive 6 cycles of 10 seconds of reperfusion and reocclusion. The animals were divided into 3 groups: sham operated I-R group and PC group. At the end of reperfusion serum was collected to measure LDH, CK, AST, ALT, creatinine and IL-6 levels. Histological samples were taken from jejunum, ileum, lung, kidney and liver. Antioxidant state was determined from mucosal homogenization of jejunum and ileum. Results: The histological damage of small intestine was moderate in PC group regarding changes of Chiu score (jejunum p=0.011, ileum p=0.024) and serum necroenzyme levels (seLDH p=0.027, seCK p=0.038). The necrosis of liver tissue was milder in PC group (seAST p=0.038 seALT p=0.241) and the renal tubules were intact after PC (seCreatinine p=0.012). Inflammatory response (IL-6 p=0.02) was reduced in PC group. Antioxidant state of the small bowel was improved in the IR group regarding the mucosal tissue reduction capacity (jejunum p=0.003, ileum p=0.008), H-donating ability (jejunum p=0.001, ileum p=0.033) and free SH group (jejunum p=0.001, ileum p=0.012). Conclusions: Postconditioning can reduce the local and remote organ injury via protecting the organs from free radicals and from effect of the systematic inflammation.

Topic: Other

P 182 Pancreatitis-associated protein-ELISA (MucoPAP) as a second-tier test for cystic fibrosis. Results of a four month's period within the Austrian Newborn Screening.

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Background Cystic fibrosis (CF) is one of the most frequent inherited disorders within the Caucasian population. The World Health Organization estimates the prevalence of CF in 1:2,000 to 1:3,000 births in the European Union, and 1:3,500 births in the United States of America. Immunoreactive trypsinogen (IRT) analyzed in dried blood spots is the primary neonatal screening tests for CF. These IRT test show low specificity and additional sweat testing and screening for CFTR-mutations is necessary for confirmation. PAP enzyme-linked immunoassay was recently described as a potential second-tier method to reduce high false-positive rates. Study design We implemented MucoPAP (Dynabio SA, France) as a second-tier test in a 4 month's pilot study in the Austrian Newborn Screening. We screened approximately 24,000 newborns for CF routinely with a first-line IRT assay. All IRT positive samples (IRT >60 ng/dl; n=255), were re-analyzed for PAP using the published protocols by Amsterdam and Heidelberg laboratories, respectively. These screening centers were using following algorithms: IRT > 60 ng/ml and PAP > 1.8 ng/ml or IRT > 100 ng/ml and PAP > 1.0 ng/ml, respectively, or IRT > 60 ng/ml and PAP > 1.0 ng/ml, respectively. Results Using these algorithms, the number of positive screening results would have been decreased from 255 (only IRT assay) to 32 (Amsterdam protocol; decrease of 87.5%) and to 52 (Heidelberg protocol; decrease of 79,6%), respectively. Routinely, 255 IRT positive samples were re-called", and second blood specimens were collected in 236 cases (19 were missing at this time point because it takes up to 4-6 weeks until a second sample is collected), and re-tested for IRT. 37 of 236 samples (15.7%) had still elevated IRT levels. In 27 of 37 newborns (72.9%) a sweat test was performed, and finally seven newborns were diagnosed with CF. These CF patients would have also been detected accurately using the screening algorithms including PAP.

Topic: Clinical Endocrinology, Metabolism and Nutrition

P 183 Expression and regulation of Notch pathway members in human decidualization

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This study aims to describe the expression and function of the Notch pathway in first trimester decidua. The highly conserved Notch pathway is critical in various cellular processes such as cell fate decision, proliferation and invasion, therefore Notch receptors and ligands represent highly intriguing genes regarding feto-maternal interaction. Besides analysis of Notch signalling in the trophoblast-decidual cross-talk, we attempt to elucidate the functional role of the pathway in decidualization of uterine stromal cells. Expression patterns of Notch family members were analysed in human decidual tissue, in a human endometrial stromal cell line (THESC) as well as in human primary stromal cell (HDSCs) isolated from first trimester decidual tissue. Cells were cultivated in the absence or presence of decidualizing stimuli, i.e. cAMP and/or estrogen (E2)/progesterone (P4). Immunofluorescence and Western Blot analyses were performed to investigate localisation and expression of Notch family members and ligands in first trimester human decidua. qRT-PCR and Western Blot were carried out to analyse expression of specific Notch proteins and ligands upon pharmacologically induced differentiation of THESC and HDSC. Notch signalling activity and the effects of its inhibition were assessed in THESC and HDSC using a canonical Notch reporter. Descriptive analyses revealed the expression of Notch receptor 2 and the Notch ligands DLL4 and Jagged2 in human decidual tissue, other members of the Notch pathway were absent. During induced decidualization of THESC and HDSC, cAMP-dependent expression of DLL4 and the E2P4-dependent expression of Jagged 2 could be observed. Notch receptor 2 expression was not affected throughout decidualization.

Topic: Molecular Signal Transduction

P 184 A retrospective data analysis of children and adolescents having immigration background in the acute psychiatry – transcultural risk and resilience factors in relation to suicidal and self-harming behaviour

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One of the most common reasons, why people contact the acute psychiatry is auto aggressive behaviour, such as attempted suicide and self-harming behaviour. In Europe, there exist several comparative studies, which have analyzed suicidal behaviour and self-harming behaviour of immigrants. There are many social and biological causes of suicidal behaviour like family/personal disposition, psychical illnesses and cultural and religious influences. We have done a retrospective analysis, using data of the medical files of the out-patient, who were treated at our clinic. The aim of this study is to collect and evaluate transcultural risk and resilience factors related to suicidal and self-harming behaviour of immigrant children and adolescents who were treated at the out-patient clinic. It is also important to find out the specific differences caused by cultural background (eg. concerning the selected method of committing suicide, triggering factors – which have lead to a suicide attempt, personal and social resources, co morbidity etc.) in connection with suicidal and self-harming behaviour among children/adolescents having an immigration background, and native children/adolescents. This presentation discusses the first results of our study.

Topic: Mental Health and Behavioral Medicine

P 185 Tri a 37, a new wheat food allergen, is a member of plant defence proteins

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Background: Wheat is an important source for IgE-mediated food allergy. Avoidance of wheat products is currently the only therapy for wheat food allergic patients whereas allergen-specific approaches such as immunotherapy would require a detailed knowledge and availability of the disease-causing allergens. Aim of this study was the isolation, identification and characterization of new allergens recognized by wheat food allergic patients for diagnosis and treatment of wheat food allergy. Methods: We screened a wheat cDNA library with serum IgE antibodies from patients suffering from wheat food allergy. The cDNA coding for novel wheat food allergen, alpha-purothionin, could be isolated and identified by sequence analysis. Recombinant alpha-purothionin was expressed, purified and characterized regarding molecular properties. The IgE-reactivity was tested in wheat food allergics, grass pollen allergic patients and non-atopic individuals. Allergen-specific rabbit antibodies were used to screen different cereal and bread extracts. To investigate the allergenic activity, we performed basophil degranulation experiments. Results: In this study we report the isolation of an IgE-reactive cDNA clone coding for a novel wheat food allergen alpha purothionin which belongs to a family of plant defence proteins. Homologue proteins to alpha purothionin could be detected by allergen specific rabbit antibodies in many other cereal and bread extracts. Serum IgE antibodies from wheat food allergic patients reacted specifically with alpha purothionin and allergenic activity was demonstrated in basophil degranulation and skin prick test experiments. Conclusion: Recombinant alpha purothionin may be useful for the diagnosis and possibly immunotherapy of IgE-mediated wheat food allergy. Supported by Thermofisher, Uppsala, Sweden, Christian Doppler Research Association, Austria and the FP7-funded EU project MeDALL

Topic: Immunology

P 186 Development of Therapeutic Attitudes: Teaching and Learning in Psychotherapy

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The aim of the study was to investigate how attitudes of potential trainees (medical students) form to build the basis for further training. We examined how potential trainees differ from trainees in a basic psychotherapeutic training in their therapeutic attitudes (Therapeutic Attitude Scales, TASC-2). We investigated further how qualified psychotherapists enrolling in training differ in their therapeutic attitudes including their direct and indirect attitudes, before and after the training. Results are reported for the TASC-2 scales and for indirect attitudes (associations and connotations). The performance of the potential trainees on the TASC 2- scales implies that a general education in the therapeutic field (medical curriculum) serves to form the base for establishing a therapeutic attitude. Potential trainees tend to a coping perspective and a more humanistic oriented approach. Therapists tend to a clarification perspective. Trainees seem to show a mixed pattern of therapeutic attitudes. The therapeutic attitudes of experienced psychotherapists changed during the course of training. The reported findings demonstrate the interference of therapeutic attitudes via teaching and may indicate a process of systematic change of therapeutic attitudes of potential trainees, trainees, and qualified therapists during the period of learning in the psychotherapeutic field.

Topic: Mental Health and Behavioral Medicine

P 187 IL-10R2 overexpression is restricted to microsatellite-stable colorectal cancer and enhances proliferation upon IL-22

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Background: IL-10R2 is a shared receptor among several members of IL-10 related cytokines. These consist of IL-10, IL-22, IL-26 and IFN-λ proteins. IL-10R2 exerts its function at environmental barriers like the colonic mucosa. IL10RB-/- mice as well as humans with mutations in IL10RB develop enterocolitis, underpinning its importance in mucosal homeostasis. It is ubiquitously expressed at a constant level. In a previous study we demonstrated overexpression of IL-10R2 in CRC tissue. Here we searched for the cause and consequence of IL-10R2 overexpression in CRC. Methods: mRNA and gDNA was extracted from CRCs from 25 patients. DNA was analyzed upon MSI using 8 markers and MSI status was correlated to IL10RA, IL10RB and STAT3 mRNA expression levels. For proliferation assays and RT-PCR HT29 and HCT116 cells were transiently transfected with pcDef3/IL10RB or empty vector. 24h after transfection cells were treated with IL-10, IL-6 and IL-22. Cell count was measured 48h and 72h after transfection. Results: IL-10R2 and STAT3 overexpression in CRC samples inversely correlated with MSI status. In culture HT29, but not HCT116 cells, increased STAT3 phosphorylation, SOCS3 mRNA expression and proliferation upon IL-10R2 overexpression and IL-10/IL-22 stimulation. Immunohistochemistry revealed overexpression of IL-10R2 and IL-22Rα in sporadic CRC samples. Intriguingly, IL-22 a cytokine usually only produced by DCs and T-cells, is expressed by CRC cells in vivo. Of note, colitis-associated cancer samples (n=22) did not overexpress IL-10R2 or IL-22Rα, but do produce IL-22.Conclusion: In sporadic CRC, IL-10R2 overexpression is found in MSS tumors and leads to a proliferative advantage upon IL-10/IL-22. IL-10R2 and IL-22Rα are coexpressed in CRC tumor samples and therefore constitute a functional receptor complex. Preliminary results point to abnormal expression of IL-22 in CRC, permitting autocrine stimulation. This indicates protumorigenic properties of this pathway in CRC.

Topic: Malignant Diseases

P 188 Clustered regularly interspaced short palindromic repeats: Bacteriophage-defence system in Clostridium difficile

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Clustered regularly interspaced short palindromic repeats (CRISPRs) are a common feature in the genomes of many bacteria and almost all archaea. Direct repeat (DR) sequences interspersed by unique spacer sequences derived from extrachromosomal elements like phages are the constituent parts of a CRISPR array building a CRISPR/cas system together with a group of CRISPR associated (cas) genes. Prokaryotic cells possessing a CRISPR/cas system are protected against extrachromosomal elements by this antiviral system if the spacer sequences show 100% sequence similarity to an invading phage or plasmid. During this study, we investigated whole genome sequences (GenBank database) of four different Clostridium difficile isolates of the hypervirulent ribotype 027 (RT027) for the presence of CRISPR arrays and CRISPR associated (cas) genes. The sequences were analyzed by using the online tools "CRISPRfinder" and "Crispi". Detected spacers were blasted (NCBI) to check up if they have an extrachromosomal origin. Investigations of the four isolates of RT027 revealed 100 different spacers located within nine highly conserved CRISPRs whereof only two displayed minimal spacer distinction. Due to this variation 93% of the spacers were detected in each C. difficile strain. Only 7 spacers showed 100% sequence similarity to published bacteriophages or plasmids. The genes cas1 and cas2, known as marker for the presence of a CRISPR array as well as cas3 to cas6 were detected. Spacer elements of CRISPR arrays are suggested to display the history of past invasions by extrachromosomal elements. Interestingly, the four C. difficile strains revealed 93% spacer similarity although three of four strains were isolated in the years 1985, 1988 and 2006. Thereby, we suggest that the CRISPR/ cas system of C. difficile is not a fast evolving antiviral system as proposed for other organisms. Further investigation will be needed to determine the role of the CRISPR system in C. difficile.

Topic: Other

P 189 Lymphangiogenesis in kidney transplants is strikingly different in humans and mice.

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The importance of lymphangiogenesis in transplantation is debated and most consider it a double sided sword. Increased lymphatic vessels could facilitate dendritic cell mobilization to the draining lymph node resulting in increased activation of lymphocytes and more allograft rejection; on the other hand greater numbers of lymphatic vessels has been reported to correlate with better graft outcome. In the course of studies to distinguish between these possibilities we discovered that lymphangiogenesis in human and murine renal allografts were strikingly different. This study characterises those differences. We started investigating this problem using a mouse model of allo-rejection, studying sections of transplanted mouse kidneys and biopsies from human kidney transplants, and discovered that mice do not preform lymphangiogenesis in transplanted kidneys, unlike humans. We show the density of lymphatic vessels in humans increases over time while in mice the density of lymphatic vessels remains constant. The lymphatic vessels present in the mouse do seem functional as the vessels are still able to produce CCL21 attracting immune cells to accumulate in them forcing them to expand, especially 3-7 days after transplant. In humans, lymphangiogenesis allows lymphatic vessels to extend into the tubular-interstitial space but not in mice, the lymphatic vessels remain localised around arterioles. 14-21 days after transplantation, the mice begin forming nodular infiltrates around the lymphatic vessels while podoplanin (a marker for lymphatic vessels) begins to spread throughout the nodule rather than localising solely on the lymphatic vessels. This nodule form what looks like a tertiary lymphoid structure. These structures are sometimes seen in humans but rarely, whereas in mice it seems standard. Lymphatic vessels may be doing two different things in humans and mice yet researchers always assume they are the same; this may be a big mistake.

Topic: Immunology

P 190 Effects of antibodies to lysosomal associated membrane protein 2 on human macrophages

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ANCA (anti-neutrophil cytoplasmic antibodies) associated vasculitis is a severe autoimmune disease and a common cause of piFNGN (pauci-immune focal necrotising glomerulonephritis). Around 90 % of those affected have antibodies to LAMP2 (lysosomal associated membrane protein 2) when the disease is active but their role in pathogenesis is unclear. Injury in piFNGN is caused by neutrophils and macrophages which both express LAMP2. The purpose of this study is to analyse the effect of antibodies to LAMP2 on macrophages. The monoclonal antibody H4B4 binds to LAMP2 on the surface of the monocytic cell line THP1 as well as to human monocyte derived macrophages (MDM) and is rapidly internalised as visualised by confocal microscopy whereas a control monoclonal antibody (CD4) was not: At 15 minutes intracellular IgG was detected in 25 % of H4B4 treated cells compared to 0 % for CD4. Comparable figures after 6 hours for H4B4 and CD4 were 68 % and 26 % respectively demonstrating uptake was antigen specific and not mediated by Fc receptors or pinocytosis. H4B4 was detected exclusively in peripheral compartments at 15 to 30 minutes before trafficking centrally to lysosomes by 1 hour and spreading more diffusely by 3 to 6 hours. Uptake of H4B4 induced apoptosis in THP1 cells as demonstrated by caspase 3 staining. There was evidence of increased autophagy in MDM demonstrated by LC3II in Western Blots. H4B4 did not co-localise with LC3 expressing autophagosomes in these cells but these clustered round H4B4 positive lysosomes. In summary, antibodies to LAMP2 are rapidly and specifically taken up by human macrophages, traffic to lysosomes and induce autophagy and apoptosis. Although the mechanisms await elucidation, the results obtained have implications for the pathogenesis of piFNGN.

Topic: Immunology

P 191 The endosomal transporter CD222 - a novel regulator of T cell activation?

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The endosomal transport is being more and more recognized to play an important role in the complex regulation of T cell activation. CD222, also known as the cation-independent mannose 6-phospate/insulin-like growth factor 2 receptor, is one of the central components of endosomal pathways – CD222 transports its cargo proteins from both the Golgi apparatus and cell surface to lysosomes. Upon T cell activation, CD222 expression is highly upregulated on the cell surface, yet the biological relevance of this membrane accumulation remains elusive. Here we aimed to investigate the impact of CD222 in T cell activation. We found that silencing of CD222 in T cells abrogated T cell effector functions, like cytokine secretion and downregulated calcium flux, whereas the T cell proliferation remained unaffected. Via mass spectrometric analysis we identified several interaction partner candidates for CD222 known to be involved in T cell activation, which we could confirm by co-immunoprecipitation. Using truncated forms of CD222, we determined domains that might be responsible for its immunomodulatory function. Ongoing studies address the molecular mechanisms that account for this function.
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Topic: Immunology

P 192 T cells in Neuromyelitis optica

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Neuromyeltis optica (NMO) is a severely disabling, demyelinating inflammatory disease of the central nervous system (CNS). The diagnostic hallmark of NMO is the presence of antibodies in the serum, which are specifically directed against Aquaporin 4 (AQP4), a water channel enriched on astrocytic endfeet and the glia limitans. These antibodies have been shown to be pathogenic and seem to be directly involved in the pathogenesis of NMO, but how do they get into the brain? Contrary to the opinion that T cells do not play a role in the pathogenesis of NMO because only few can be seen in lesions of NMO-Patients, we could show in our animal models of NMO that, although T cells were used to induce inflammation, acute lesions with AQP4 loss can contain very low numbers of T cells whereas no lesions can be induced with AQP4 antibodies only. It is currently not clear to what extent the antigen-specificity of these T cells contributes to the disease process. So we investigated this matter further and could see that the antigen specificity had impact on the speed of lesion formation, peripheral organ affection and recruitment of other inflammatory cells. Furthermore it seems that the extend of AQP4 loss correlates best with the local availability of sufficient amounts of complement, efficient recruitment of granulocytes and pathogenic AQP4 specific antibodies rather than high T cell numbers or their specificity. Our findings strongly suggest that T cells are part of a complex immunological interplay.

Topic: Neuroscience

P 193 Protein kinases signaling networks involved in learning and memory linked to Alzheimer's disease

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Learning and memory(L&M) are two brain functions absolutely necessary for all species of mammals, to acquire new knowledge, behaviors, synthesize new information for adaptation, habituation and finally survival. Numerous brain mechanisms are required for acquisition, consolidation and retrieval of new memories, including neurotransmitters, proper brain receptors regulation, downstreaming pathways. In the presented study we concentrated at the protein kinases and their pathways involved in learning and memory formation. In addition to check whether their role is really critical we decided to test it in the Alzheimer's disease(AD), as main symptoms of it present difficulties in new memory formation and retention. In current study we have used the behavioral testing of C57B mice in the Multiple T-Maze and sacrificed at different learning stages. Our previous findings are indicating that PKC gamma, NEK7, PKG, MEK3, S6K, PKN, ROCK2, FAK, BMX kinase and others are involved in L&M mechanisms. Levels of these protein kinases were checked using SDS-PAGE Western blotting, analyzed using densitometry and statistics and then checked in AD mouse model hippocampus. The screening methodology have shown that above mentioned protein kinases are involved in different stages of L&M and are significantly different in the AD mouse hippocampus, giving the broad field for further studies of pathways involved in the Alzheimer's disease and other neurodegenerative disorders.

Topic: Neuroscience

P 194 The Oral Health Impact Profile (OHIP) to measure Oral Health-related Quality of Life (OHQoL) in clinical oral implant research

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The Oral Health Impact Profile (OHIP) is the most comprehensive and widely used instrument to measure oral health-related quality of life (OHQoL) currently available. Originally it consists of 49 items organized into 7 subscales (functional limitation, physical discomfort, psychological discomfort, physical disability, psychological disability, social disability and handicap) and responses are based on a Likert scale. In reviewing the current literature, the use of OHIP in the field of clinical oral implants research was evaluated. Of 39 studies identified, 15 used the short version OHIP-14 (38%), 7 used the OHIP-20 (18%), 13 used the full version OHIP-49 (33%) and 4 used the version for edentulous patients OHIP-EDENT (10%), while modified OHIP versions were used in another 2 studies. Further differences were seen regarding outcome definitions used (categorized vs. added evaluation). The majority of investigations (72%) used the OHIP to assess OHQoL in edentulous patients, while only few studies looked at partially edentulous patients (5%) or patients with tooth agenesis (5%). However, investigation often lack within-patient comparison of pre- vs. post-treatment conditions as well as inclusion of negative controls. Although reference values have been suggested, reliable conclusions may only be drawn from comparative effectiveness research. Future studies may use the OHIP more frequently and accurately to gain further knowledge on the impact of oral implant treatment on OHQoL.

Topic: Regeneration of Bones and Joints

P 195 Assessment of Batch to Batch Variation in Polyclonal Antithymocyte Globulin Preparations

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Antithymocyte globulins (ATGs) are widely used to prevent and treat allograft rejection and graft versus host disease. They are purified IgG fractions derived from rabbits immunized with the Jurkat T cell line (ATG-Fresenius S) or human thymocytes (Thymoglobuline). Differences in the amounts of leukocyte reactive antibodies but also in the antigens targeted by ATGs could potentially affect the clinical efficacy of different batches of these polyclonal antibody preparations. In our study, four batches of ATG-Fresenius S and Thymoglobuline were compared regarding their capacity to interact with human leukocytes. Using flow cytometric assays we analysed the reactivity of these ATG preparations with Jurkat cells and with primary leukocytes. In addition, ATGs derived from different batches were probed with a panel of cell lines expressing high levels of ATG antigens. Furthermore, the ability of ATGs to mediate complement-mediated lysis of human monocytes and lymphocytes was also compared. Overall, the high conformity in ATG preparations of both manufacturers found in our study makes variations of different batches of ATGs in respect of their clinical efficacy unlikely. Moreover, the methods described in this study allow for a comprehensive evaluation of ATG preparations in clinical use regarding their antibody composition and their ability to mediate lysis of human leukocytes.

Topic: Immunology

P 196 A novel score of p53 activity increases the accuracy of p53 diagnosis in human breast cancer

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Mutation of the TP53 gene is the most frequent genetic alteration in human cancer and occurs in approximately 20-40% of all human breast tumors. p53 is routinely diagnosed in clinical breast cancer care by immuno histochemistry (IHC). However, the concordance between p53 protein accumulation detected by IHC and TP53 mutation detected by sequencing is less than 75% in breast tumors. Thus, the clinical and prognostic utility of p53 diagnosis by IHC is still debated. In this study, the TP53 gene was sequenced in 114 Austrian breast cancer patients. Moreover, IHC and/or qRT-PCR for p53 and its target genes p21, BAX and MDM2, and qPCR to detect amplifications of negative regulators such as MDM2/4 and overexpression of miRNA125b were performed to further refine the functional status of p53. Additionally, expression of the TP53γ-isoform was determined. Using an alternative scoring method of IHC diagnosis of p53 mutation in the patient collective we could show better correlations with sequencing results as the current standard scoring method. We found inactivating TP53 mutations in 31 out of 114 breast cancer patients (27.2%). Mutant p53-status significantly correlated with estrogen and progesterone receptor negativity, younger age of onset, high tumor grade, a ductal tumor type and poorer disease free survival. Approximately 8% of the tumor samples showed amplifications of MDM2 and/or MDM4. In tumors harboring TP53 mutations and/or MDM2/4 amplifications, the mRNA expression of p53 targets was significantly reduced. High expression of p53 targets was associated with better outcome. TP53γ expression significantly correlates with ER negativity, higher tumor stage and lower p21mRNA and miRNA125b expression. Our planned identification of novel markers of p53 activity, and the development of new IHC-based assays should eventually improve the accuracy of p53 diagnosis in breast cancer.

Topic: Malignant Diseases

P 197 Retargeting T cells to viral glycoproteins for adoptive therapy of human Cytomegalovirus infection

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Cellular immunity is required for controlling human cytomegalovirus (HCMV) infections in vivo. Reactivation of viral disease in immunocompromised patients despite close monitoring followed by preemptive therapy can still cause life-threatening complications. An attractive treatment strategy is the application of virus specific cytotoxic T cells (CTLs) isolated from blood of HCMV positive donors and expanded in vitro for adoptive immunotherapy. However, this therapy is not applicable in the high risk subgroup of stem cell transplant patients characterized by donor CMV seronegativity and patient CMV seropositivity. To develop an adoptive CMV directed immunotherapy we constructed a chimeric antigen receptor (CAR) composed of CD3zeta, CD28 and a single-chain variable fragment (scFv) targeting the glycoprotein B (gB) of HCMV. Activated T cells engrafted with the construct by electroporation of in vitro transcribed RNA showed specific effector functions after coincubation with gB-expressing target cells. These T cells released IFNγ; and TNF after stimulation with HCMV infected human foreskin fibroblasts (HFF), cytokines that inhibited the replication of HCMV in HFF efficiently. In addition redirected T cells showed degranulation and cytotoxic activity after coincubation with target cells. In ongoing experiments we examine differently activated T cells as well as NK cells engaged with our CAR for their potential to lyse infected HFF to identify the most suitable vehicles for CMV directed adoptive immunotherapy.

Topic: Immunology

P 198 Alveolar bone structure of implant sites following either orthodontic tooth movement or tooth extraction

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An edentulous alveolar ridge created by orthodontic separation of teeth appears to be less prone to resorptive changes over time compared to what occurs after conventional extraction of a tooth (Spear et al. 1997). The aim of the study is to analyse quality, structure and remodelling dynamics of orthodontically generated bone by means of histomorphometry of bone biopsies and densitometry of consecutive radiographs. All patients who have underwent appropriate orthodontic treatment with a need for an eventual dental implant will be included in the study. Exclusion criteria are diseases or medication affecting bone metabolism and age of less than 16 years. Biopsies will be performed in the course of the surgical insertion of the implant: Instead of the routine pre-driller a trepan bur with an outer diameter of 3 mm will be used. For histological processing the biopsy will be left in the trepan bur, stored in Schaffer's fixation and marked regarding its bucco-lingual orientation in the bur. Vertical thin sections will be stained with Giemsa solution. After qualitative evaluation and digital image processing a quantitative histomorphometry will be performed. Radiographs (before and after orthodontic tooth movement) will be analysed densitometrically. Findings will be matched to the examination of alveolar bone following tooth extraction in an analogous group of patients without orthodontic treatment (same exclusion criteria).

Topic: Regeneration of Bones and Joints

S 199 A mid-infrared sensor system for detecting changes in melanoma cells treated with anti-225D9.2+-TT antibodies

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We have previously reported on the design and realization of a measurement method with a sensor system for a label-free cell type discrimination. The measurement method is based on the CH2-symmetric and CH2-antisymmetric stretches of tumor cells at wavelengths 3.51µm and 3.42µm, respectively. Experiments on melanoma cell lines exposed to cisplatin showed a significant decrease in CH2-stretch ratio, indicating the great potential for investigating therapy related effects on tumors cells with this method. Here, we present the CH2-stretch ratio measurements we have obtained after the incubation of the melanoma cell line A375 with anti-225D9.2+-TT antibodies. These polyclonal antibodies were obtained after immunization of rabbits with the peptide mimic 225D9.2+ conjugated to tetanus toxoid and showed in vitro and in vivo activity against human melanoma cells. 2x105 A375 melanoma cells were prepared on 6x6 mm2 CaF2 sample slide and incubated overnight with and without anti-225D9.2+-TT antibodies. Four different 1.5mm diameter spots were measured. A significant decrease in the CH2-stretch ratio was seen in the A375 melanoma cells incubated with anti-225D9.2+-TT antibodies compared to untreated melanoma cells (0.70 ± 0.03 and 0.79 ± 0.06, respectively). In conclusion, the result shows that the interaction between antibody and antigen can lead to a biochemical modification in the inner-membrane lipid region and to internal movements of its molecules containing CH2 that can be measured with the mid-infrared sensor system.

Topic: Medical Physics

P 200 Understanding subtype selective allosteric modulation of GABAA receptors

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The gamma-aminobutyric acid type A (GABAA) receptor is the major inhibitory neurotransmitter receptor of the central nervous system. The most abundant subtype contains 2 alpha (a), 2 beta (b) and 1 gamma (g) subunit. Benzodiazepine (Bz)-site ligands bind at the a/g interface and can enhance GABA-induced current and therefore neuronal inhibition. The affinity and efficacy of certain benzodiazepines strongly depend on the type of alpha subunits in the receptors. a2/a3 functionally selective compounds can be anxiolytic without having the side effect of sedation. Recent modelling studies in our lab, resulting in 3D models of a diazepam-bound a1g2 interface of GABAA receptors, provide the basis for investigating the bound states of other subtypes. Modelling and docking studies of a1g2, a2g2 and a3g2 containing receptors and short molecular dynamics (MD) simulations will be performed to understand Bz-ligand interaction with the different alpha subunits. Mutagenesis, radioligand binding assays and electrophysiology in wild type and mutated receptors will be employed to test the predicted structural hypotheses. The electrophysiological experiments are conducted in a parallel project. All experimental data will then be used to validate, and, if necessary, to further optimize the model structures. The residue R228 was identified as unique for a3 among all alpha subunits. According to the model structures, this amino acid has no direct interaction with the ligand, but R228A mutated receptors showed a reduced response to a3 selective compounds. R228 is part of the so-called loop C, a several residue spanning segment forming part of the ligand binding site with highly variable sequence. The long term aim of these studies is the identification of the local molecular mechanism by which the different alpha subtypes are modulated with selective efficiency by certain ligands, thus enabling a rational design of ligands that use this mechanism.

Topic: Neuroscience

P 201 Angiogenesis in chronic thromboembolic pulmonary hypertension (CTEPH)

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Background: Chronic thromboembolic pulmonary hypertension (CTEPH) is a late sequela of venous thromboembolism affecting up to 4 % patients surviving symptomatic pulmonary embolism. CTEPH is characterized by non-resolving thrombi in the pulmonary arteries leading to right heart failure and death. Previous studies in a murine model of stagnant flow venous thrombosis have shown that endothelial cell-specific deletion of vascular endothelial growth factor receptor 2/fetal liver kinase-1 (VEGF-R2/flk-1) leads to misguided thrombus resolution. We hypothesized that thrombus non-resolution in CTEPH results from dysfunctional thrombus angiogenesis. Methods: CTEPH thrombi and unthrombosed pulmonary arteries as reference standards were collected from 11 CTEPH patients undergoing pulmonary endarterectomy at our institution. Patients gave informed consent. Several angiogenesis markers were investigated in CTEPH thrombi using Real Time RT-PCR, gene expression levels were normalized to endogenous 18S-RNA levels. Results: The gene expression levels of angiopoietin-2, VEGF, VEGF-R2/flk-1, podoplanin, platelet endothelial cell adhesion molecule-1 (PECAM-1) and vascular endothelial cadherin were decreased in CTEPH thrombi compared with pulmonary arteries. Furthermore factors involved in proliferative pathways of the vascular cells such as bone morphogenetic protein receptor type 2 (BMPR2) and transforming growth factor beta (TGF-ß1) showed also decreased expression. By contrast, the thrombogenic molecule plasminogen activator inhibitor-1 (PAI-1) showed an increased gene expression level in CTEPH thrombi. Conclusion: Angiogenic molecules are downregulated in CTEPH thrombi compared with parent pulmonary arteries. Downregulation of genes involved in angiogenesis may drive venous thrombus persistence.

Topic: Cardiovascular and Pulmonary Disease

P 202 Pathway analysis: How does Wnt1 perform its anti-lymphangiogenic function in melanoma

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We have previously shown that proto-oncogene protein Wnt-1 down regulates expression of the main lymph angiogenic factor VEGF-C in the melanoma cell lines in vitro and in the melanoma in vivo, leading to decreased lymph-angiogenesis and metastasis (J. Invest. Dermatol. 2012 in press). Preliminary results support a role of calcineurin in the down regulation of VEGF-C. However, the exact signaling mechanism between the Wnt-1 and decreased VEGFC expression remains to be discovered. In an attempt to uncover the signaling route between Wnt-1 and VEGF-C in the melanoma, we treated Wnt-1 over expressing melanoma cells with various pathway inhibitors and analyzed VEGF-C levels by real time PCR. This screening approach led to the surprising result that inhibition of Rho-signaling resulted in an up-regulation of VEGF-C levels. We are currently further addressing this issue by over-expressing dominant negative Rho constructs in order to analyze, if this annihilates Wnt-1 effects on VEGF-C. In parallel, we are currently analyzing expression of full length and truncated VEGF-C promoter constructs, in order to define promoter regions sensitive to Wnt-1. Based on the surprising anti-lymph-angiogenic effect of Wnt-1, we hope that elucidating the exact pathway between Wnt-1 and VEGF-C may open the door to design anti-lymphangiogenic compounds for the treatment of melanoma.

Topic: Vascular Biology

P 203 The effects of religious conviction/spirituality on the coping strategies of cancer patients

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The effects of religious conviction/spirituality on the coping strategies of cancer patients Introduction: The issue of spirituality and religiosity (SpR) in medicine has gained international attention and awareness in recent years. Numerous studies have shown that religious conviction/ spirituality may be a ressource in coping with illness. The aim of this study was to investigate the connection between SpR and coping, quality of life, anxiety and depression in patients diagnosed with cancer. Methods: 60 patients (51,7% female, 48,3% male) diagnosed with lymphoma (n= 43) and multiple myeloma (n= 17) were interviewed using standardized questionnaires to assess anxiety and depression (HADS-D), illness-related coping styles (FKV-LIS), religiosity/spirituality (SpREUK-15) as well as sociodemographic characteristics. Results: 80% of all patients reported being of any denomination, with only 48,3% regarding themselves as religious and/or spiritual. Patients ranked SpR as the third most important coping strategy. Women showed higher prevalence and values for SpR when compared with men (female: 69%, male: 41%). Within the religious/spiritual subgroup, there was a significantly higher value for the item "seeing meaning in an illness". This item correlates strongly with all the items of the SpREUK-15 questionnaire, most of all with the item "positive interpretation of illness" (reflection). Regarding the five illness-related coping styles of the FKV-LIS, no significant differences could be established between the religious/spiritual and the non-religious/-spiritual subgroups. No significant associations could be found between religiosity/spirituality and anxiety/depression, and life satisfaction respectively. Conclusion: The data demonstrate that 1) patients consider SpR as an important coping strategy, 2) women use SpR more frequently then men 3) we could not demonstrate any significant association between SpR, coping, anxiety, depression and quality of life.

Topic: Mental Health and Behavioral Medicine

P 204 Genome-wide genotyping of patients with benign childhood epilepsy with centrotemporal spikes

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Benign childhood epilepsy with centrotemporal spikes (BECTS) is the most common epilepsy syndrome in children. A complex interplay between brain development processes, environmental factors and multiple susceptibility genes are thought to contribute to its development. Although genetic predisposition plays a major role in the etiology of BECTS, it is yet poorly understood. Our aim is to perform a systematic genetic investigation in patients with BECTS within the next years. It is planned to perform genome-wide association analysis, copy number variation (CNV) analysis and exome sequencing. So far, DNA from 248 patients and 200 neurologically healthy controls was genotyped for more than 950000 single nucleotide polymorphisms (SNPs) enriched for exonic markers and common variants at > 5% minor allele frequency (MAF). After SNP and sample quality control a subset of 202 cases and 198 controls was used for a case-control association study (logistic regression analysis). Additionally, copy number variation analysis was performed in all 248 patients and 200 controls. Detailed data will be presented. However, further samples need to be genotyped in order to increase the power. In addition integration of CNV analysis and exome sequencing results need to be conducted for a comprehensive genetic study of BECTS.

Topic: Neuroscience

P 205 Genotyping Lyme borreliosis spirochetes

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Lyme Borreliosis (LB), the most common tick borne disease in the northern hemisphere, is caused by spirochetes of the Borrelia burgdorferi (B. burgdorferi) sensu lato group, a complex that currently comprises 19 genospecies. Whereas in North America a single genospecies, B. burgdorferi sensu stricto, is the cause of LB, in Europe also B. afzelii and B. garinii have been identified and confirmed to cause the disease. However other genospecies such as B. spielmanii, B. bisettii, and B. valaisiana are also suspected to cause LB. Our aim is to identify Borrelia genospecies of clinical isolates as well as from a tick collection representative for Austria. Our study will allow a comprehensive overview of the B. burgdorferi sensu lato genospecies distribution in certain specimens of patients and in ticks collected in Austria. Correlations of the tick results with that obtained from clinical samples may identify certain genospecies as pathogens. Currently we are developing a nested real time PCR approach, based on previously described primer pairs. This approach employs a Borrelia burgdorferi sensu lato specific probe, as well as five genus specific probes (B. burgdorferi, B. lusitaniae, B. afzelii, B. garinii, and B. spielmanii) allowing for detection and identification of Borrelia on the basis of the 5S-23S ribosomal spacer polymorphism. Species not yet identifiable by this method will be determined by sequencing the ribosomal spacer and further typing by multi locus sequence typing (MLST) of housekeeping genes, a method for precise clarification of the taxonomic status of a species bearing the potential for delineation of novel species.

Topic: Other

P 206 The coexistence of Huntigton and Alzheimer disease

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Objectives: Alzheimer disease (AD) and Huntington disease (HD) are both neurodegenerative disorders. For early-onset familial Alzheimer disease most frequently the amyloid precursor protein gene (APP), presenilin 1 (PSEN1) and presenilin 2 (PSEN2) gene mutations are responsible. Huntington disease is a progressive autosomal dominant trinucleotid disease caused by an expanded CAG repeat in exon 1 of the huntingtin (HTT) gene. Methods: A 55 years-old female patient suffering from young onset Alzheimer dementia and her two family members have been investigated. Detailed clinical and laboratory investigation have been performed. All exons of the PSEN1, PSEN2, and 16. and 17. exons of APP genes were sequenced. The CAG repeat number in the HTT gene was detected by PCR and gel electrophoresis. Results: The first symptoms of the proband appeared at the age of 51 years with memory loss, and anxiety. Neurological examination found choreiform hyperkinesis in the limbs, palmomental reflex on both side, non-fluent aphasia, damaged verbal fluency, perseveration, visuospatial disturbance. A novel missense mutation in the PSEN2 gene at the c.1163 T>C (T388M) position have been detected. The affected amino acid is highly conserved in various species and the mutation leads to severe structural change in the protein. In the HTT gene one allele increased 42 CAG repeat number was detected. The PSEN2 gene mutation was found in the proband's 57-years-old paternal aunt and was not present in the proband's sister. The aunt has normal repeat number in the HTT gene, the 50-year-old sister has a reduced penetrance (CAG)n repeat number (37) on one allele. Conclusion: Here we report the coexistence of a novel, putatively pathogenic PSEN2 gene mutation and a pathologically expanded repeat number in the HTT gene. However there are differences in the disease pathways determinated by these two gene mutations, the double hit may have an add-on effect on the clinical phenotype.

Topic: Clinical Neurosciences

P 207 The effect of in vitro-gastro-duodenal digestion of the major shrimp allergen tropomyosin on IgE reactivity and allergenic activity

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Introduction: Resistance to enzymatic digestion in the gut is known as a frequent feature of food allergens. Aim of this study was to investigate IgE reactivity and allergenic activity of the major shrimp allergen, tropomyosin, after gastro-duodenal digestion. Methods: Shrimp extracts were digested in vitro with gastric and pancreatic enzymes and analysed by SDS-PAGE, protein staining and immunoblotting with tropomyosin-specific rabbit antisera. Digestion products were identified by liquid chromatography-ion trap mass spectrometry as well as N-terminal sequencing. The IgE reactivity and the allergenic activity of the digested shrimp extracts were evaluated in IgE immunoblot and humanized RBL (rat basophil leukaemia) cell-release assay experiments. Results: Tropomyosin was the only protein in shrimp extracts which was resistant to gastric digestion. Tropomyosin-specific antibodies and N-terminal sequencing identified 2 fragments of around 18 and 30 kDa as degradation products. Subsequent digestion under duodenal conditions completely degraded tropomyosin within few minutes. Interestingly, gastric digestion did not affect the ability of tropomyosin to bind patients' IgE, indicating that the IgE epitopes of tropomyosin remained intact in the degradation products. In addition, the shrimp extract induced similar basophil degranulation after gastric digestion as the undigested extract. Even after gastric and pancreatic digestion of the extract, the allergenic activity was maintained for some patients. Conclusion: The retained IgE-reactivity and allergenic activity of tropomyosin after gastric digestion explains why it behaves as a major food allergen in sea-food.

Topic: Immunology

P 208 The effects of MDR1 polymorphisms on tacrolimus through levels in long-term kidney transplant recipients

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The multidrug resistance 1 (MDR1) gene codes for P-glycoprotein (P-gp), expressed mostly in liver and engaged in the metabolism of drugs such as the immunosuppressant tacrolimus (TAC). Fifteen single nucleotide polymorphisms (SNPs) in the MDR1 gene, which may significantly alter pharmacokinetics, have been reported. Most influences of SNPs on TAC concentration/dose ratios (C/D ratio; [ng/mL]/[mg/d]) have been observed for the genotypes G2677T/A. Here we report on the allelic distribution and influence on TAC metabolization of the SNPs C1236T, G2677T/A and C3435T in kidney transplant recipients (KTR) who participated in a 24 months open-label, randomized controlled trial at the Division of Nephrology and Dialysis, Department of Medicine III, Medical University Vienna. SNP analysis was performed according to published protocols. Overall, the study population represented a primarily caucasian population with a well-preserved graft function. 148 patients were randomized, 142 consented for genotyping and 141 were included into the intent to treat analysis (55.6 [46.5-65.0] years old; 67% male; 6.2 years [3.2-12.5] since kidney transplantation, estimated glomerular filtration rate of 45.8 [37.9 - 57.6] mL/min/1.73m2). Genotype distributions were C1236T: CC 24.6%, CT 3%, TT 38%, G2677T/A: CC 2.1%, GA 1.4%, GG 39%, GT 13%, TA 0.7%, TT 8.4%, G3435T: CC 14%, CT 4.2%, TT 47%. We did not find any significant associations of MDR1 SNPs and TAC trough levels in KTRs, although power may have been limited to do so.

Topic: POeT - Programme for Organfailure, -replacement and Transplantation

P 209 Automatic analysis of Tumor Budding in Colorectal cancer specimens

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The normal morphology of epithelial structures is lost during cancer progression as tumor cells proliferate in an intense and unorganized manner. Epithelial-mesenchymal transition in cancer cells lead to a loss of cellular adhesion and increases cells' mobility. Single cells or small groups of cells detach from bigger epithelial structures and move into blood- or lymphatic vessels and form new proliferations sites on distant places. Generation of these loose structures of 1-3 tumors cells spread in the stroma is called tumor budding and is observed in both tumor center (intratumoral budding, IB) as well as the invasive front (peritumoral budding). Recent studies suggested the importance of IB for diagnosis and prognosis of colorectal cancer (CRC).Since manual evaluation and counting of tumor budding is a time-consuming and tedious work, our aim was to develop an automatic tool for identification and scoring of budding in tissue sections. For this approach, immunofluorescence staining of keratin-8 was performed on sections from 8 patients, from which 4 already developed liver metastasis. Slide scanning was done with the automated microscopy analysis system TissueFAXS+ (TissueGnostics, GmbH). Existing image processing algorithms were modified and fine-tuned for assessment of different tumor budding features. The tumor center area was drawn on each of the scanned virtual slides and was subject to automatic scoring. With this novel method, similar budding scores were observed in the tumor center and invasive front of CRC specimens in both groups of patients. The new algorithms revealed a robust automatic detection and measurement of budding on all slides. They provide an important tool for CRC analysis in combination with other features like measurement of targets in microenvironment and immune cells. Supported by the FFG Bridge-Project N° 818094

Topic: Other

P 210 Effects of ischemic postconditioning on reperfusion injury after a short and a long ischemic period

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Ischemia-reperfusion (I-R) injury of liver is a clinically significant manifestation of several surgical interventions. The damage degree correlates mainly with the length of the hypoxic period. Ischemic postconditioning (IPO) seems to be an appropriate technique to ameliorate the I-R injury. The aim of this study was to compare the effect of IPO applied after a short and a long-term ischemia in a rat model of liver I-R. 25 Wistar male rats were randomized into 5 groups (A-E). Group A: Sham operated; Group B: ischemia was performed by clamping the hepatic hilum for 45 min followed by 6 hours reperfusion; Group C: after 45 min ischemia IPO was applied (6x10sec) reperfusion lasted 6 hours, Group D: 90 min ischemia followed by 6 hours reperfusion; Group E: IPO was performed after 90 min ischemia accompanied by 6 hours of reperfusion. Hepatic microcirculation was monitored by laser Doppler flowmeter detecting the plateau maximum (PM) and the reperfusion area (RA). Biliary epithelial cell damage was assessed by the measure of bile glucose concentration. Histological alterations of the liver and serum necroenzyme levels were examined. Ischemic postconditioning significantly attenuated both functional and morphological injuries after 45 minutes of ischemia. Group C had significantly higher flow rates during reperfusion (PM: 63%vs.92%;RA: 57%vs.99%) and significantly lower serum ALT levels (870±6U/lvs.484±5U/l). Bile glucose level decreased significantly (p=0,04) with the use of postconditioning in the short term group (C). This protective effect was not observed in the group exposed to a long-term ischemia except for the histological sections where postconditioning reduced the injury level. No significant difference was observed between the results of the group D and E regarding the microcirculation of the liver (PM: 18%vs.27%;RT: 12%vs.16%) or the bile glucose. IPO attenuated the hepatic injury after a short term ischemic period, but not after a long -90 min- ischemia.

Topic: Other

P 211 Ca^{2+} channel impairments in dystrophic cardiomyocytes

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The muscular dystrophies (MDs) are inherited diseases characterized by progressive muscle weakness and degeneration. Besides the relatively well described skeletal muscle degenerative processes, the MDs are also associated with cardiovascular complications including cardiomyopathy and cardiac arrhythmias. The current understanding of the patho-mechanisms is still very limited, but recent research suggests, that dysfunctional ion channels in dystrophic cardiomyocytes considerably contribute to the cardiovascular complications. By using the whole cell patch clamp technique, the functional properties of voltage-gated L-type Ca^{2+}-channels were studied in ventricular cardiomyocytes derived from adult normal and dystrophic mice. Besides the classical dystrophin-deficient mdx mouse model for human Duchenne muscular dystrophy (DMD), we additionally used the dystrophin- and utrophin- deficient mdx-utr mouse (DMD severe), as well as dysferlin-deficient mice as a model for human limb girdle MD 2B. We found that the voltage-dependent inactivation of L-type Ca^{2+}-channels is significantly reduced in dystrophic mdx and mdx-utr cardiomyocytes. Moreover, the current density levels of cardiomyocytes derived from these mouse models are significantly increased. Finally, dysferlin–deficient cardiomyocytes also show significantly increased current densities compared to normal ones. We conclude that L-type Ca^{2+}-channels are significantly impaired in dystrophic cardiomyocytes. These impairments likely contribute to the cardiovascular complications associated with the muscular dystrophies.
The work was supported by the Austrian Science Fund FWF (P23060).

Topic: Molecular Signal Transduction

P 212 Molecular Characterization of the Tumour-Stroma Crosstalk Using a Novel 3D Co-Culture In Vitro Model

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Carcinomas are highly complex structures composed of genetically altered tumour cells and stromal cells. Fibroblasts (NF), cancer-associated fibroblasts (CAFs), endothelial cells, pericytes and inflammatory cells are the major constituents of stroma. The resulting molecular heterogeneity influences the way tumour cells migrate, proliferate and survive during tumour progression. Even resistance to therapeutic intervention has been linked with the molecular crosstalk between tumour cells and stromal cells. In addition, there is preliminary experimental evidence that CAFs might also play a role in stemness and preparation of the metastatic niche. Recently it has been shown that normal dermal fibroblasts can be in fact "educated" by carcinoma cells to express pro-inflammatory genes which orchestrate tumour-promoting inflammation in an NF-κB-dependent manner (Hanahan et al. 2010). Therefore, targeting CAFs is an important novel therapeutic concept. With the aim to identify the underlying molecular mechanisms ("epigenetic make-up of CAFs") in the crosstalk between non-small cell lung cancer (NSCLC) epithelial cells and their corresponding CAFs a novel and complex 3D organotypic (spheroid) in vitro cell culture model system was established. This model is thought to much better recapitulate the in vivo situation as it reflects the complexity and dynamics of human tumours more faithfully than 2D monotypic monolayer cultures. The spheroid-based model allows live imaging, histological examination, biochemical assays as well as functional genomic analyses of the interaction between human tumour and stromal cells embedded in ECM. Transcription profiling of pairs of primary NF and CAFs derived from NSCLC patients identified CAF-specific signature genes that are mainly involved in MAPK-signaling and focal adhesion interactions. These CAFs will be immortalized by h-TERT and used for co-cultivation with NSCLC spheroids to further analyze the molecular crosstalk events.

Topic: Tumorbiology - Oncology

P 213 LRET-based distance measurements in the mammalian glutamate transporter EAAC1

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EAAC1 [Excitatory amino acid carrier (EAAT3)] mediates the regulation of synaptic transmission by reuptake of glutamate in the synaptic cleft. It is distributed in the neuronal membranes and is selectively enriched in the neurons of the hippocampus, cerebellum and the basal ganglia1 They belong to the family of soluble carrier family 1 member 1 (SLC1A1) and are expressed in kidney, a wide variety of epithelial tissues, brain and eyes2. The project is based on the high resolution crystal structure of GltPh,the bacterial orthologue to mammalian glutamate transporters, (Yernool et al., 2004) which provides a structural framework for the determination of the helical movement in EAAC13. The structural rearrangement of the protein is caused by the helical movements which will be assessed by distance measurements using the technique of lanthanide resonance energy transfer (LRET)4. The measured distances will allow us to obtain new insights into the structure function relationship of the glutamate transporters and can be further investigated using different substrates and inhibitors. The results obtained in this project will allow us to understand better the pathological conditions associated with mutations in EAAC1 causing human dicarboxylic aminoaciduria (Peghini et al., 1997)5

Literature 1.Kanai, Y. & Hediger, M. A. The glutamate and neutral amino acid transporter family: physiological and pharmacological implications. Eur. J. Pharmacol. 479, 237–247 (2003). 2.Maragakis, N. & Rothstein, J.D. Glutamate transporters in neurologic Disease: Arch Neurol. 58 , 365-370 (2001). 3.Yernool, D., Boudker, O., Jin, Y., and Gouaux, E. Nature 431, 811–818 (2004). 4.Sandtner W., Bezanilla F., Correa A. M. In Vivo Measurement of Intramolecular Distances Using Genetically Encoded Reporters . Biophys. J. 93, L45–47 (2007). 5.Peghini P, Janzen J, Stoffel W. Glutamate transporter EAAC-1-deficient mice develop dicarboxylic aminoaciduria and behavioral abnormalities but no neurodegeneration. EMBO J 16:3822–3832. (1997).

Topic: Neuroscience

P 214 Arginase I as a key mediator of the innate immune system at the interface to autoimmunity

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The immune system consists of two interplaying arms, the so called innate and the adaptive system. Antigen presenting cells, such as dendritic cells, crosstalk with the adaptive arm by priming naïve T cells. Depending on the specific cytokines they express, various types of T helper cells form and either dampen or enforce the immune response. Recently we identified Arginase I, which is a key enzyme of the urea cycle, to be highly upregulated in mice with PTEN deficient myeloid cells. These mice exhibited a protected phenotype, when we performed collagen induced arthritis as a model for autoimmunity. Arginase I is a marker for alternatively activated macrophages and in murine cells it is mainly induced by Th2 cytokines. Based on this, we are investigating mice with Arginase I deficiency specifically in myeloid cells. Dendritic cells as well as macrophages deficient for Arginase I exhibit higher levels of proinflammatory cytokines IL6 and IL12/23 in a gene dosage dependent manner after LPS stimulation in in vitro experiments. In contrast to that, mice lacking myeloid Arginase I were protected from collagen induced arthritis in vivo. In future experiments we want to induce experimental autoimmune encephalomyelitis (EAE), which is a T-cell mediated disease and collagen induced arthritis (CIA) in mice lacking Arginase or PTEN or Arginase/PTEN (double-deficient). Furthermore we are interested to elucidate the mechanism of Arginase I-mediated protection in arthritis despite showing an enhanced inflammatory response in vitro as well as in vivo.

Topic: Vascular Biology

P 215 Phosphorylation of Kv7.2 determines its regulation via G Proteins

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Kv7 potassium channels, especially Kv7.2, Kv7.3, and Kv7.5, control neuronal excitability. They are tightly regulated by various neurotransmitters acting via G protein-coupled receptors signaling via Ca^{2+}/calmodulin or depletion of membrane phosphatidylinositol-4,5-bisphosphate (PIP2). Moreover, phosphorylation may affect the Kv7 channel function. Yet, information about in vivo phosphorylation sites and their functional implication is lacking. To determine the influence of steady state phosphorylation of the channels, superior cervical ganglion (SCG) neurons were incubated for 30 min in various kinase inhibitors (GW8510 [10µM], SB415286 [1µM], SB203580 [10µM], H7 [10µM]) which block CDK5, GSK3, p38 MAPK, and PKC as well as PKA, respectively. Next, inhibition of the M-currents (through primarily Kv7.2/7.3 heteromers) by oxotremorine M (OxoM) or bradykinin was investigated. Inhibiting CDK5 caused a leftward shift of the concentration response curve for OxoM, but no change in the effect of bradykinin. Likewise, GW8510-treatment shifted the concentration response curve for Kv7.2 channel inhibition via muscarinic M1 receptors heterologously expressed in tsA201 cells to the left. Mass- spectrometric analyses of heterologously expressed and native Kv7.2 channels revealed several phosphorylated amino acid residues in the C-terminus, 5 of them within the putative PIP2 binding site. Serines S427 and S446 were predicted to be targets for cdk5. An alanine mutation of S427, but not that of S446, caused a significant increase in the sensitivity of the channel towards M1 receptor modulation and prevented any further effect of the CDK5 inhibitor. However, these mutations left the channel-voltage dependence unaltered. Thus, the phosphorylation state of serine 427 in the C- terminus of Kv7.2 plays a crucial role in its regulation by M1, but not by B2 bradykinin receptors suggesting that the phosphorylation state of serine 427 determines the affinity of the Kv7.2 C-terminus for PIP2.

Topic: Cell Communication in Health and Disease

P 216 Silk spider Nephila clavipes - Proteome and posttranslational modifications of the spidroins

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The silk of spiders are characterized by diversity of their chemical composition, structure and function. Presents an interesting structure-function relationship of mechanical properties and being very strong and rigid with an exceptional combination of tensile strength and extensibility. Thus, it has attracted interest in human exploration and bioprospecting applications of this material that offers a great potential in biomedical applications and textile industry. The study reported here is a classical bottom-up proteomic approach where proteins from the silk spider Nephila clavipes were extracted and separated by 2-DE; the individual protein spots were proteolytically digested using different enzymes and subsequently identified by using tandem mass spectrometry and database query with the protein search engine MASCOT. Have been identified different spidroins such as spidroin-1, spidroin-2, dragline silk spidroin and flagelliform spidroin. High-sequence coverage of spidroins was revealed and posttranslational modifications such as phosphorylation, hydroxylation, deamidation, methylation and others PTMs have been observed. Thus, performing a chemical prospecting in a systematic way, was possible obtain information from the structural and chemical properties of silk proteins from the N. clavipes spider web, allowing its development in biotechnological applications. Key words: silk protein; spidroins, post translational modifications, phosphorylation

Topic: Molecular Biology in Medicine

P 217 Intraperitoneal injection of saline modulates hippocampal brain receptor complex levels but does not impair performance in the Morris Water Maze

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The involvement of the hippocampus in pain has been demonstrated but key players, i.e. the major brain receptors have not been shown to be modulated by pain. It was therefore the aim of the study to show the concerted action and pattern of brain receptor complex levels in a non-invasive model of moderate pain. C57BL/6J mice were divided into four groups of 14 animals each: trained injected, trained non-injected, yoked injected and yoked non-injected. Animals were tested in the open field and the elevated plus maze for behavioural evaluation and cognitive functions were tested using the Morris Water Maze. Hippocampi were taken 6 h following sacrification. Membrane proteins were prepared by ultracentrifugation and run on blue native gels to keep the native state, blotted to membranes and western blotting was carried out using the primary antibodies against serotonin receptor 5HT1A, muscarinic acetylcholine receptor M1 (mAChR-M1), nicotinic acetylcholine receptor alpha7 (nAChR-alpha7), glutamate (AMPA) receptor (GluR1) and neurokinin receptor 1 (NK-1). There was no difference between performance in behaviour or in the MWM between groups. Brain receptor level changes involved all receptors given above. Pain affected mAChR-M1, GluR1 and NK-1 complex levels when yoked-injected were compared with yoked non-injected animals. Memory mechanisms affected mAChR-M1 complex levels when trained non-injected animals were compared with yoked non-injected controls. Taken together, the neurochemical basis for testing receptor agonists/antagonists on the role of pain and the hippocampus was generated that may be useful for interpretations of the role of this complex area in moderate pain.

Topic: Neuroscience

P 218 Decoding transmembrane immunoglobulin-like glycoprotein CD147 inside the cell

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CD147, also known as basigin or EMMPRIN, is a transmembrane type I immunoglobulin-like glycoprotein, ubiquitously expressed, but highly up-regulated on proliferating tumor cells and T cells upon activation. It is involved in cell survival, differentiation and migration, and thus regulating such diverse processes as implantation, neuronal development, tumor formation and cytokine production. In T cells, CD147 dampens T cell antigen receptor down-stream events (tyrosine phosphorylation, calcium mobilization, interleukin-2 synthesis) and influences the expression of activation-associated surface markers, including CD28. Several of the CD147 interaction partners in the plasma membrane (CD43, CD44, CD326) have been shown to translocate into the nucleus, co-regulating transcription of genes important for proliferation. Analogue to these and other nucleus-targeting molecules, CD147 features a juxtamembrane cluster of positively charged amino acids within its cytoplasmic domain. Bioinformatics analysis of the CD147 primary sequence suggested translocation of a cytoplasmic fragment into the nucleus as well as potential cleavage sites along the molecule. Preliminary Western blotting experiments of cellular fractions from human Jurkat T cells and human embryonic kidney cells depicted a fraction of full-length glycosylated CD147 as well as an ectopically expressed cytoplasmic domain fragment in the nucleus. To study the intracellular trafficking of CD147 further, we analyze wild-type and mutant CD147 in human primary and immortalized cell lines via confocal laser scanning microscopy and subcellular fractionation as well as by co- and chromatin immunoprecipitation experiments and mass spectrometry.
This work is funded by the Cell Communication in Health and Disease (CCHD) PhD program of the Austrian Science Fund.

Topic: Cell Communication in Health and Disease

P 219 Intermediate Monocytes but not TIE2 Expressing Monocytes are Biomarkers for Colorectal Cancer

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Introduction: Based on the differential expression of CD14 and CD16 monocytes can be divided into „classical", „intermediate" and „non-classical" monocytes with distinct biological functions. Expression of the angiopoietin-2 (ANG-2) receptor TIE2 characterizes a pro-angiogenic monocyte subset within the intermediate population. We thus hypothesized that TIE2 expressing monocytes (TEMs) and intermediate monocytes may be elevated in colorectal cancer (CRC) patients and have diagnostic potential. Methods: Monocytes were investigated in healthy volunteers (N=32) and CRC patients with localized (N=24) or metastatic disease (N=37) by flow cytometric detection of CD14, CD16 and TIE2 on peripheral blood leukocytes. Furthermore, proteins (MCP-1, ANG-2, sTIE1, VEGF-A) known to regulate these monocyte populations were measured by enzyme linked immunosorbent assay in platelet poor plasma. In vitro experiments were conducted by exposing PBMCs to cell culture supernatants of primary (SW480, HT-29) or metastatic (SW620) colon cancer cell lines and by subsequent flow cytometric evaluation of monocyte subsets. Results: TEMs were not elevated in the blood of CRC patients. However, intermediate monocytes were significantly increased in cancer patients, with highest values recorded for localized CRC. This finding was further supported by the in vitro analyses demonstrating a more potent induction of intermediate monocytes by primary as opposed to metastatic CRC lines. Furthermore, ANG-2 and VEGF-A were significantly elevated in metastatic CRC, whereas sTIE1 and MCP-1 were not altered in this collective. None of the soluble parameters showed correlations with monocyte subsets. Conclusion: TEMs have no marker potential for early diagnosis or progression of CRC, in contrast to intermediate monocytes which exhibit a prominent marker potential especially in the early stages of CRC. ANG-2 and VEGF-A are associated with metastatic spread, in agreement with their role as angiogenesis regulators.

Topic: Clinical Experimental Oncology

P 220 Evaluation of Fibroblast Growth Factor Receptor 1 (FGFR1) as potential new therapy target in Malignant Pleural Mesothelioma

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MPM is an aggressive asbestos-related malignancy characterized by frequent resistance to chemo- and radiotherapy with poor outcome and limited therapeutic options. FGFs and their receptors have been identified as potential therapy targets in several tumor types but have not been systematically investigated in MPM so far. Aim of this study is to provide a systematic analysis of the expressed FGF and FGFR molecules in MPM and to investigate the impact of blocking FGFR-mediated signals with genetic constructs and FGFR-specific tyrosine kinase inhibitors on MPM cell growth, migration, signaling pathways and sensitivity to radiation and to currently used chemotherapeutic agents. Expression of FGF and FGFR genes was determined by qRT-PCR and expression arrays in MPM cell lines and normal mesothelial cells. Selected FGFs were also verified by immunohistochemistry on tissue samples. FGF2 was used to stimulate, and the specific inhibitor PD166866 and an adenovirus expressing dominant-negative FGFR1 to block FGF signal transduction in MPM cell models and further combined with chemotherapeutics and radiation. MTT, clonogenic, spheroid formation as well as platypus, and transwell assays and videomicroscopy were performed to analyze cell growth, survival and migration. Downstream signaling was investigated by immunoblotting. Expression analysis revealed high levels of FGFR1, FGF2 and FGF18 in all MPM cell lines tested. Stimulation with FGF2 showed remarkably increased migration and significant changes in morphology. Inhibition of FGFR1 lead to significantly decreased proliferation, survival, migration and spheroid formation in all cell lines tested. Combination of FGFR inhibition with chemotherapeutics and radiation increased cytotoxic activity against MPM cell lines.

Topic: Malignant Diseases

P 221 Proteomic analysis of thioredoxin-targeted proteins in Entamoeba histolytica

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Entamoeba histolytica, an intestinal protozoan that is the causative agent of amoebiasis, possesses a functional NADPH-dependent thioredoxin system comprising the dithiol-containing redox proteins thioredoxin (Trx) and thioredoxin reductase (TrxR). Both proteins were found to be covalently modified by the 5-nitroimidazole drug metronidazole which consequently led to the loss of disulfide reducing activity of the TrxR/Trx system and the covalent modification of only a few defined proteins. The aim of the present study was to search systematically for further interaction partners of thioredoxin in order to extend our knowledge about the lethal action of metronidazole in E. histolytica. Based on the Trx-reduction mechanism we constructed an active site mutant of Trx lacking the resolving cysteine residue. The recombinant mutant protein (EhTrxC34S) was immobilized on Ni-NTA resin to capture target proteins from E. histolytica cell extracts after formation of intermolecular disulfide bonds. EhTrxC34S and covalently linked proteins were eluted and visualized by two-dimensional gel electrophoresis and Coomassie Blue staining. Twentyfive out of 80 Trx-captured proteins were analyzed by liquid chromatography-tandem mass spectrometry yielding 18 putative Trx binding partners which are involved in distinct cellular processes. Besides NADPH-dependent alcohol dehydrogenase 1 and 2-Cys peroxiredoxin, which are already known to interact with E. histolytica Trx, we could identify new proteins that had not previously been associated with redox-mediated regulation in E. histolytica such as serine acetyltransferase-1 (EhSAT1) which catalyzes the O-acetylation of serine, the first reaction in the two-step process of cysteine biosynthesis. Since cysteine is the major non-protein thiol and absolutely required for cell viability of E. histolytica, the interaction of Trx with EhSAT1 and its influence on the enzymatic activity will be studied in further detail.

Topic: Immunology

P 222 Feasability of transrectal ultrasound in the assessment of locally advanced cervix cancer in the course of primary adaptive radiochemotherapy

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Purpose: To retrospectively compare the maximum target width and maximum target thickness in patients with locally advanced cervix cancer between magnetic resonance imaging (MRI) and transrectal ultrasonography (TRUS) in the course of primary radiochemotherapy including image-guided adaptive brachytherapy (IGABT). Material and Methods: T2-weighted MRI and TRUS were performed in patients with locally advanced cervix cancer – either (1) at the time of diagnosis before the initiation of external beam radiotherapy, or (2) at the time of brachytherapy before insertion of the applicator or (3) at the time of brachytherapy after insertion of the applicator. Patients treated from 2009 to 2011 were selected for this study based on the availability of MRI and TRUS at the defined time points. The target was defined as the complete macroscopic tumour mass and the remaining cervix and was measured on transversal planes. Descriptive statistics and a linear regression analysis were made between the groups. Results: Images from 17 patients were available for analysis. One patient had to be excluded due to TRUS related artefacts. Mean maximum target width was 4.2cm ± 0.83 (range: 2.8-6.1) and 4.2cm ± 0.79 (range: 3.2-6.0) for MRI and TRUS, respectively. The mean absolute difference in width between TRUS and MRI was 0.0cm ± 0.3 (range: -0.7 – 0.6). Linear regression analysis between TRUS and MRI demonstrated a correlation with a R^2= 0.842. Mean maximum target thickness was 3.3cm ± 1.03 (range: 2.2-5.9) and 3.1cm ± 1.15 (range: 1.9-6.2) for MRI and TRUS, respectively. The mean absolute difference in thickness between TRUS and MRI was -0.2cm ± 0.3 (range: -0.7 – 0.3). Linear regression analysis between TRUS and MRI demonstrated a correlation with a R^2= 0.943. Conclusion: The feasibility of TRUS for the assessment of local target extension could be demonstrated. Comparison of target width and thickness showed a high correlation between TRUS and MRI indicating the potential of TRUS for target definition in IGABT.

Topic: Clinical Experimental Oncology

P 223 Characterization of the major wheat food allergen Tri a 36

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Wheat can be grown in a wide climatic and geographic range and is along with rice and maize, one of the most important cereals worldwide. However, wheat is also known to elicit food allergy, mainly in children but also adults can be affected by wheat food allergy. The aim of our study was to isolate and characterize new wheat food allergens. We screened a wheat seed cDNA library with sera from wheat food allergic patients. The isolated IgE-reactive clones were compared with sequences deposited in GenBank. The deduced aminoacid sequence of one clone showed identity with the C-terminal portion of a low molecular weight glutenin. This protein was expressed in Escherichia coli and purified by Ni-NTA chromatography. The IgE reactivity was checked by dot blot analysis with sera from wheat food allergic patients and the biological activity was tested by basophil activation tests. We analysed the protein stability by examining the IgE reactivity after gastric and duodenal in-vitro digestion assays or after boiling the protein. Extracts from different cereals were prepared to test the IgE cross reactivity of related proteins in these cereals with the recombinant wheat allergen. We expressed and purified a low molecular weight glutenin with the designated allergen nomenclature Tri a 36. We found out, that it is a major allergen recognized by more than 80% of wheat food allergic patients. Tri a 36 shows a high resistance to boiling and digestion. Furthermore, IgE-cross reactivity was detected between Tri a 36 and related proteins in other cereals. In conclusion, we isolated a major wheat food allergen Tri a 36, which is a cross reactive highly heat and digestion stable allergen.
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Topic: Immunology

P 224 The role of Cingulin in endothelial junctions

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Tight junctions regulate the transcellular passage of fluids, molecules and larger solutes or cells across physiologic barriers. They consist of a protein complex of claudins, occludins and JAMs at the cell membrane and a large cytosolic complex of ZO proteins, cingulin and GEFs. The structure and composition of this complex determines barrier function in different parts of the body and also at various levels of the same tissue type. In epithelial cells cingulin serves as scaffold for GEFs, which regulate Rho activation and thereby junction assembly and disassembly. In endothelial cells the role of cingulin has not been investigated yet. We have analyzed cingulin expression in various vascular beds at the protein and mRNA level and found different expression levels in an organ-specific fashion. Cingulin co-localizes with tight junctions only in certain vascular beds. We now aim to correlate expression patterns of cingulin with junction tightness by measuring transendothelial electrical impedance across endothelial mono-layers in situ.

Topic: Vascular Biology

P 225 Prolyl hydroxylase inhibitors decrease formation and activity of osteoclast in murine bone marrow cultures

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Bone regeneration is a multistep-process which involves angiogenesis, activity of osteoblasts and osteoclasts. Pharmacological inhibition of prolyl hydroxylases (PHD) enhanced angiogenesis resulting in increased bone formation. Although data on the effect of PHD inhibitors on angiogenesis and osteoblasts are available, the impact of PHD inhibitors on osteoclast activity and thus bone resorption is not entirely clear. In our study we investigated the effect of PHD inhibitors on osteoclastogenesis and resorption activity of osteoclasts. We induced osteoclastogenesis in murine bone marrow cultures in presence of PHD inhibitors and evaluated the effect on the formation of multinucleated, tartrate-resistant acid phosphatase (TRAP) positive cells and their resorption activity. In addition we measured proliferation of osteoclast progenitors. Our data show that PHD inhibitors reduced formation and activity of multinucleated, TRAP positive cells. Furthermore, PHD inhibitors reduced resorption activity of these osteoclast-like cells. We further found decreased proliferation of osteoclast progenitors. Next we assessed the effect of bone substitute material supplemented with PHD inhibitors. We found that bone substitute material supplemented with PHD inhibitors reduced the number TRAP positive multinucleated cells and decreases proliferation. In conclusion our results show that PHD inhibitors can reduce osteoclastogenesis and resorption activity of osteoclasts. Whether these finding translate into anti-resorptive effects in vivo requires further studies.

Topic: Other

P 226 Iron accumulation in models for inflammation/degeneration of the central nervous system: Does iron impact neurodegeneration?

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Inflammatory processes play a key role in various neurodegenerative diseases such as Multiple Sclerosis, Alzheimer´s Disease and Parkinson´s Disease. Increasing evidence suggests that iron accumulation in the brain might contribute to neurodegeneration. Iron is a potential source of free radicals as it can catalyze the production of hydroxyl radicals under oxidative conditions. This can lead to amplification of tissue injury caused by oxidative damage. We characterized iron storage within the central nervous system (CNS) of animal models of different neurodegenerative diseases. We examined animals with acute inflammation mediated by CD8 or CD4 positive T cells and animals suffering from T cell and antibody mediated chronic inflammation due to active immunization. Further, we studied LPS induced lesions which represent central nervous system disease caused by the innate immune system. In acute and chronic inflammation, iron positive microglia cells and perivascular macrophages were observed. Similarly, we characterized oxidative damage in the different models for inflammation. We could not find evidence for oxidized phospholipids or DNA damage. As iron accumulates progressively with aging, we analyzed iron storage in aged rats. We observed that iron is stored in oligodendrocytes and myelin in certain areas of the brain. To address the question, if iron could influence CNS inflammation, we compared acute CNS inflammation in young and old animals. We found a similar disease course in old compared to young animals whereas in the pathological analysis we detected higher levels of inflammatory infiltrates in young animals. In contrast, we found a tendency for a higher grade of neurodegeneration in the spinal cords of old animals. Iron accumulation is only minimal compared to the human brain in all the tested animal models. These observations necessitate the search for additional animal models mimicking the human situation more closely.

Topic: Cell Communication in Health and Disease

P 227 Kinematic changes in patients with double arthrodesis of the hindfoot for re-alignement of planovalgus deformity.

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Introduction Triple arthrodesis of the hindfoot was considered to be the gold standard of surgical treatment for fixed planovalgus deformity. The double arthrodesis (fusion of subtalar and talonavicular joint) represents a modificaiton that allows for integrity of the calcaneocuboidal joint. In vitro biomechanical studies prooved that the corrective potential of both types of fusion is similar Aim of the present study was to evaluate dynamic plantar pressure distribution in patients who underwent double arthrodesis and (1) to compare it to healthy feet (2) to evaluate the influence of radiographic alignement and (3) to assess functional outcome with validated outcome scores. Methods 16 feet (14 patients) who underwent double fusion were included in this study. Mean age of the patients was 65.8 years (range 44 – 81) and the follow-up period ranged from 18 to 62 months (average 46). Dynamic plantar pressure distribution was assessed using a capacitive pressure platform (emed system (Novel)). Assessed parameters included maximum force (N), peak pressure (kPa), contact area (cm2), contact time (msec) and impulse. The foot was divided into total object, hindfoot, midfoot, the metatarsals and 3 toe regions. Results were compared to an age-and sex matched control group. Clinical and radiographic assessment was performed also. Results Statistically significant differences concerning plantar pressure distribution were found for maximum force of the hindfoot, midfoot and big toe region. Whereas the hindfoot and big toe represented decreased load in the double arthrodesis patients, there was increased load in the midfoot region compared to healthy controls. Clinical and radiographic parameter improved significantly. Conclusions The results of the present study reveal that double arthrodesis represents a sufficient method for correction of planovalgus deformity. However, force transmission of the midfoot is incereased whereas push-off force decreases. Future studies should focus on direct comparison to triple arthrodesis.

Topic: Regeneration of Bones and Joints

P 228 Quantitative reproducibility assessment of RPE atrophy lesions in patients with choroidal neovascularization related to neovascular age-related macular degeneration using polarization-sensitive OCT

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Abstract: Aim: To quantitatively assess the reproducibility of retinal pigment epithelium (RPE) atrophy in patients with choroidal neovascularization (CNV) secondary to neovascular age-related macular degeneration (AMD) using polarization-sensitive spectral-domain OCT (PS-OCT). Methods: Twenty eyes of 20 patients diagnosed with neovascular AMD were included in this prospective cross-sectional study and examined by a PS-OCT prototype, capable of specifically detecting the RPE. Each patient was scanned 5 times at a one day visit. Following RPE segmentation, the respective B-scan located closest to the macular center presenting with RPE atrophy was identified and quantified manually using Autocad 2008. This procedure was followed for the identical B-scan position in all 5 scans per eye and patient. Coefficients of variation analyses, scatter and Bland Altman plots, as well as an inter-observer variability assessment were perfomed. Results: Mean variability of all atrophy lesion dimensions verified from the repeatability measurements was 0.11 mm ((SD (=standard deviation):0.39)). The coefficient of variation (SD/mean) was 0.21 on average (SD: 0.10). Inter-observer variability assessment showed a mean difference of 0.006mm between all patients regarding RPE lesion size evaluation (paired T test: p=0.65). Spearman correlation coefficient was r=0.85 with p<0.001. Results revealed a good overall reproducibility of ~80%. Conclusion: PS-OCT offers good reproducibility in RPE morphology assessment in patients with neovascular AMD and may be suitable for precise RPE evaluation in clinical practice. Additional technical applications such as an eye tracker function or an increase in depth resolution may improve reproducibility.

Topic: Medical Physics

P 229 Predicting the Effects of Stent-Grafting in the Aortic Arch onto Vessel Mobility

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We predict the changes in vessel movement induced by implanting a stent-graft in the aortic arch based on static and dynamic measurements of the aorta acquired before the intervention. We propose a fully automated method to compute a dynamic model of the thoracic aorta from ECG-gated CTA sequences which we use to predict the effects of stent-grafting in the aortic arch onto vessel movement. We base this prediction on shape parameters extracted from the model and information about the movement of the aorta before the intervention. The aorta is segmented in a population of 50 healthy and diseased cases. Image registration between the first and consecutive frames of the CTA sequences is used to compute deformation fields describing its motion during a cardiac cycle. Trajectories of patches of the aorta are extracted from these deformation fields using a surface model. Models computed for each patient are matched in a groupwise manner based on their centerline and landmarks corresponding to the locations of the supra-aortic vessels. From this, we compute a model representing the average shape of the aorta in the dataset, as well as modes of variation describing each specific case. We build a regression model of to the effects of stent-grafting onto vessel movement with distance traveled as prediction- and the compact representation of the shape and motion as regression variables. We have employed the described method to predict the effects of stent-grafting in the aortic arch in 4 cases. To evaluate the accuracy of the model, we measured the mean squared difference between predicted and measured displacement. We obtained a mean prediction error of 32.97%, with a standard deviation of 33.96. We present first results of a fully automated method for modeling and predicting the movement of the thoracic aorta from ECG-gated CTA sequences. The results obtained for predicting the effects of stent-grafing 4 cases are promising. However, a thorough evaluation on a larger set of patients will be necessary to fully assess the capabilities of the proposed approach.

Topic: Medical Informatics, Biostatistics and Complex Systems

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P 230 Angiogenic properties of different Ti surface evaluated by cell co-culture with endothelial cells and osteoblasts

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Aim: Osseointegration following implantation depends on the interaction between angiogenesis, osteogenesis and Ti surface characteristic. The aim of present study is to evaluate the angiogenic properties of Ti surfaces with different characteristics by cell direct contact co-culture with endothelial cell and osteoblast. Material and Methods: Human umbilical vein endothelial cells (HUVECs) and osteoblast-like cells (MG-63) were directly co-cultured for 48h on four different kinds of Ti surfaces: acid-etched (A), hydrophilic A (mA), coarse-gritblasted and acid-etched (SLA) and hydrophilic SLA (mSLA). Proliferation and the expression level of angiogenic genes, von Willebrand Factor (vWF), Thrombomodulin (TM), endothelial cell protein C receptor (EPCR), E-Selectin (E-selectin), VEGF receptor 1 (Flt-1), and VEGF receptor 2 (KDR) in HUVECs were measured by flow cytometry and real time PCR. Results: Cell proliferation on all Ti surfaces was significantly lower than on cell culture plastic. Within surfaces, the proliferation of both cell seemed to be highest on the A surface, followed by SLA, mA and mSLA. The expression of vWF, TM, EPCR, and E-Selectin were significantly higher on A than on all other surfaces. In addition, the expression of EPCR was significantly higher on mA compared to mSLA. KDR was not detected on A, whereas no difference between other surfaces was observed. The expression of Flt-1 was significantly higher on A than on SLA and mSLA. Conclusion: Under conditions of direct co-culture of MG-63 and HUVECs, proliferation and expression of angiogenic genes of HUVECs were promoted by smooth hydrophobic A surface. However, hydrophilicity of Ti surface seems has not effect on angiogenic behaviors of HUVECs.

Topic: Regeneration of Bones and Joints

P 231 Angiogenic potential of fibrin biomatrix delivered VEGF165 in a model of angiogenesis

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The process of angiogenesis has been subject to numerous studies in various animal models due to its pivotal role in tissue regeneration. Focusing on the admission of fibrin bound growth factors, we developed a new model of angiogenesis to standardize „in vivo" testing of angiogenic substances. Utilizing a silicon tube to isolate a vascular bundle, a protected niche is created, providing a standardized surrounding for angiogenesis around the vessels. Newly formed vascular structures within the silicon shielding then can be precisely attributed to the specific local conditions. In the present study, silicon tubes with an inner diameter of 3mm were used to sheath the epigastric bundle of Sprague Dawley rats. The angiogenic growth factor VEGF165 was then administered among fibrin sealant which served as a carrier matrix to provide spatial stability and a prolonged release. Fibrin sealant alone was tested as the growth factor carrier group. An empty group with only the silicon tube put in place served as control group. The angiogenic response was evaluated by immunohistological means (vWF and sma antigen staining). Results showed an increase in newly formed vessels in the VEGF165 group compared to the empty control group. In contrast, a suppression of vascular growth was noted when compared to the fibrin sealant group which seems attributable to high VEGF dosing. The vehicle group yielded promising results, showcasing the potent angiogenic effect of a standalone fibrin matrix. The developed model of angiogenesis seems to be a reliable approach to further evaluate the angiogenic potential of different growth factors in conjunction with various scaffolds.

Topic: Vascular Biology

P 232 Measurements of intramolecular distance changes at atomic level in LeuTAa using Luminescence Resonance Energy Transfer.

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Background: LeuTAa from Aquifex aeolicus is a leucine/alanine transporter. LeuTAa is recognized as a bacterial orthologue of mammalian solute carrier class 6 family proteins (SLC6). SLC6 are integral membrane proteins and of particular pharmacological interest because they are targets of many clinically important drugs. These SLC6 proteins play diverse crucial roles. A leucine transporter LeuTAa from Aquifex aeolicus has been recognized as a bacterial orthologue of mammalian SLC6 family proteins. LeuTAa has been crystallized in different conformations and has been resolved with high resolution. Though with low sequence identity (~20–25%), there are crucial region in transmembrane segments 1, 3, 6 and 8 in LeuTAa where conservation reaches ~50%. For these reasons LeuTAa provides a good structural paradigm to study dynamicity of SLC6-family members in terms of their structure/function relationships to mammalian transporters. Methods and results: In order to address the dynamicity of transport cycle and different proposed models, we planned to measure intramolecular distance changes associated with the dynamic substrate transport cycle. To solve this we employ Luminescence Resonance Energy Transfer (LRET) to measure the changes at atomic level. For LRET based measurements we have introduced LBT (Lanthanide binding Tags) to accommodate Terbium, as the donor element, along with cysteines, where acceptor fluorophores are linked chemically, at selected positions in LeuTAa. After expression and purification of these mutants, we obtained the first distances at atomic resolution. We also confirmed the functional activity of these mutants by using scintillation proximity assay (SPA) in comparison to the wild type LeuTAa before going for LRET measurements. Conclusion and future plan: Taken all together our LRET measurements would help us to validate or propose a dynamic substrate transport model for LeuTAa. Our future plan focusses on the LRET measurements in more native environment i.e. proteoliposomes.

Topic: Molecular Signal Transduction

P 233 A Transcutaneous Energy Transmission System Delivering up to 45 Watt to an Artificial Heart

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As percutaneous drivelines to cardiac prostheses are prone to infections, transcutaneous energy supply systems (TETS) are currently being intensely investigated. Compared to the energy supply for ventricular assist devices (VAD) however, the requirements for pulsatile total artificial hearts (TAH) are even more challenging, both in power demand and pulsatility of the electric load. Aim of the present study is to develop a system that delivers power of up to 45 Watt peak to a pulsatile load. A TET system composed of an input inverter, resonant tanks and an output rectifier forming a DC-DC converter was designed. The inverter and rectifier topologies were varied as well as the turn numbers and turn ratios of the primary and secondary coil of the air core transformer in order to maximize efficiency. For the coil windings different litz wires with different numbers of single strands were used effectively varying the overall wire cross section. An electric model of the TET was developed using MATLAB Simulink and simulations were performed. Based on the simulation results, topologies and coils were chosen and subsequently implemented and tested. The chosen combination of inverter topology, rectifier topology, coil wire diameter and strand numbers led to an efficiency of 85% at a distance of 2cm and a load of 45 Watt using coil diameters of 10cm (transmitter) and 7cm (receiver). However, changing the load from 45 to 5 Watt and the coil separation from 2 to 0 cm caused an increase of the output voltage of 200%. The measurements are in good accordance with the simulation, validating the simulation model. These results show that a TET system delivering 45 Watt is able to operate at 85% efficiency. However, under pulsatile and changing geometric conditions a careful control of output voltage is required.

Topic: Biomedical Engineering

P 234 Influence of gliptins on endostatin, glucose and HbA1c in 35 NIDDM patients

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Background: Gliptins are complete inhibitors of dipeptidyl-peptidase-4 (DPP4) and therefore increase the blood levels and bioavailiability of glucagon-like peptide 1 (GLP-1). Consequently, the insulin production and release rises, glucagon release decreases and blood glucose level recede. Endostatin, a potent angiostatic factor, inhibits endothelial cell proliferation and migration and stimulates endothelial nitric oxide synthase (e-NOS). Methods: The study population consisted of 35 NIDDM-patients (15 female, mean age: 60,13±10,80; 20 male, mean age: 58,10±7,32) who could not reach a HbA1c <7% by a metformin monotherapy. The patients obtained 50 mg Vildagliptin + 1000 mg Metforminhydrochlorid 2x/d (1-0-1) in tablet-form for 6 months. BMI (kg/m2), blood glucose (mg/dl), HbA1c (%), endostatin (ng/ml), intima media thickness (IMT; cm) and physical performance (by ergometry; %) were measured before and after treatment for 6 months. Results: Gliptin treatment was associated with significant decrease in glucose (p<0,01) and HbA1c (p<0,01). HbA1c decreased from 7,70±1,06 to 6,53±0,73% resp. glucose from 140,00±18,78 to 113,47±25,26 mg/dl. Endostatin levels increased significantly from 126,15±35,43 to 145,71±54,67 ng/ml (p<0,04). Conclusion: A 6 months gliptin treatment is associated with a significant increase in venous endostatin levels in patients suffering from NIDDM. If this gliptin-mediated endostatin up-regulation can be interpreted as an additional vasoprotective property it should be elucidated more closely.

Topic: Cardiovascular and Pulmonary Disease

P 235 Influence of sex and etiology on Endostatin serum levels in patients with chronic heart failure (CHF)

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Background: Endostatin, a potent angiostatic factor, inhibits endothelial cell proliferation and migration and stimulates endothelial nitric oxide synthase (e-NOS). Chronic heart failure (CHF) is a vasoconstictive state associated with a significant upregulation of neurohumeral factors such as brain-natriuretic peptide (BNP), predicting morbidity and mortality in CHF patients. Therefore, the aim of the present study was to investigate the impact of sex, etiology and functional heart class in CHF on serum endostatin levels. Methods: Endostatin levels were measured (ng/ml) at rest in 75 individuals, divided into 2 groups: 30 CHF-patients (17dilatative, 13 ischemic; 9 NYHA I, 9 NYHA II, 12 NYHA III) and a control group consisting of 45 "elderly" non smokers (female vs. male). In the CHF group also BNP was measured. Results: In contrast to the control group, which showed no gender specific difference in mean endostatin levels (female: 112,33±23,59; male: 116,55±16,65), male CHF-patients (263,00±115,39) had much higher endostatin levels compared to female CHF-patients (191,36±52,94). Endostatin levels in dCHF were 192,25+/-44,85 compared to iCHF 260,69+/-116,02. Endostatin also showed a positive correlation to BNP-levels in the CHF group (p<0,003). Conclusion: 1) CHF is associated with upregulation of endostatin levels, especially in female patients 2) CHF based on ischemic heart disease is associated with higher Endo serum levels compared to DCHF 3) Furthermore, Endostatin serum levels correlate to BNP levels in CHF patients. Further studies are warranted, to investigate the impact of Endo as prognostic marker in CHF patients

Topic: Cardiovascular and Pulmonary Disease

P 236 Pharmacochaperone-mediated rescue of trafficking deficiency in the ABCB-transporter subfamily

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A folding defect caused by mutation of particular proteins and subsequent impaired trafficking is the pathogenetic principle for a number of monogenic diseases, such as cystic fibrosis and nephrogenic diabetes insipidus. Aberrant folding leads to retrotranslocation to the cytosol and degradation by the ubiquitin-proteasome system. In recent years folding correction by specifically binding small molecules, so called pharmacological chaperones, has been advocated as a therapeutic concept for the treatment of monogenic folding diseases. These small molecules bind to the respective client proteins and correct mutation-induced aberrant folding. Non-synonymous mutations at the transmembrane-domain / nucleotide-binding-domain interface lead to misfolding of at least three ABC-transporters and human disease. Mutations in ABCC7 lead to cystic fibrosis, those in ABCG2 to gout and that in ABCB11 to intrahepatic cholestasis. The analogous trafficking deficient ABCB1 mutant ΔY490 was used as an initial model system, because of its ability to react with a large complement of small solute-like molecules. A number of solute analogues related to the lead compound propafenone have been synthesized and evaluated for their ability to elicit a trafficking rescue. Several compounds are able to restore surface expression to wild type levels. The potency to recover surface expression correlates with the IC50 values for daunomycin efflux inhibition. Therefore IC50 values predict a compound´s ability to rescue trafficking. Therefore IC50 values can be used for the generation of pharmacophore models and database screening. IC50 values can be determined with high accuracy and show a higher dynamic range (6 orders of magnitude) than EC50 values for trafficking correction.

Topic: Molecular Mechanisms of Cell Biology

P 237 Molecular characterization of wheat antigens involved in celiac disease

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The small intestinal mucosa of celiac disease patients is characterised by presence of numerous infiltrating CD4+ T cells. These lamina propria T cells proliferate and secrete IFN-γ when stimulated with gliadin extracts and are implicated for the mucosal damage, villous atrophy and the production of anti-gliadin antibodies. Peptide sequences in the P and Q rich regions of gliadins are known to be T cell stimulatory but due to the heterogeneity of gliadin sequences, several potential stimulatory epitopes and the individual protein antigens yet to be identified and characterized. Thus our aim was to identify and characterize wheat antigens with ability to initiate and sustain celiac disease. The three subtypes of gliadinsα, β, γ, and ω-gliadins are insoluble under physiological conditions and difficult to isolate into pure fractions. Therefore we developed a method wherein the alcohol extracted gliadins was fractionated by ion-exchange chromatography and each generated fraction's reactivity to serum IgA, from clinically well defined CD (active/diet) patients and non-CD patients was analyzed. We identified and generated recombinant gliadins that were disease specific. Epitope mapping studies revealed regions that are immunostimulatory. Recombinant gamma-gliadins will be useful for characterizing the immune response to wheat antigens and develop reliable diagnostic and therapeutic strategies.

Topic: Inflammation and Immunity

P 238 Time-dependent expression of pro- and anti-apoptotic proteins in cortex of adult male Wistar rats after permanent bilateral occlusions

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Objectives: Permanent bilateral occlusion of the common carotid arteries (2VO) in rats is a model for various neurological and cerebrovascular disorders. Neuronal cell death via necrosis and apoptosis that occurs through the alterations in expression of the Bcl-2 family proteins as well caspase 3 might be the main features of pathological conditions associated with ischemia. Activation of Akt pathway has been shown to be essential for the anti-apoptotic effects of hypoxic preconditioning in neuronal cells as well for infarct size reduction. Since chronic cerebral hypoperfusion provokes severe cognitive decline that occurs in aging, vascular dementia and Alzheimer's disease, it is essential to determine whether 2VO insult modulates investigated protein expressions and are these alterations time-depend. Materials and methods: 3 months old male Wistar rats were subjected to permanent bilateral common artery ligation. Alterations in activation of Akt, as well pro- and anti-apoptotic molecules of Bcl-2 family and procaspase 3 protein expressions in cortical crude synaptosomal fraction were monitored via Western blot, 3, 7 and 90 days following the insult. Results and conclusions: 3 days after 2VO no significant difference in the expression of investigated proteins was detected, indicating that this was not sufficient period for modulations of protein expression to occur. A significant increase in expression of Bax and procaspase 3 as well as Bax/Bcl-2 protein ratio was observed after 7 days. Furthermore, 90 days following the 2VO insult, p-Akt and Bcl-2 as well Bcl-2/Bax protein ratio were significantly augmented, implying that neuronal recovery has already begun. Our results implicate that the peak of neurodegenerative processes is at 7th day and that neuroprotective effects of certain substances, which are planed for our further experiments, should be done after this period of time.
Acknowledgment: Supported by the Ministry of Education and Science, Project No 173044.

Topic: Neuroscience

P 239 Assessment of intrafraction prostate and patient motion for IMRT patients with rectal balloon

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Purpose: To investigate intrafraction prostate and whole-patient motion during IMRT (Intensity Modulated Radiotherapy) delivery. Margins accounting for setup uncertainties and intrafraction motion were calculated. Methods: 17 patients with prostate cancer (3 fiducial gold markers within the prostate: 1.2mm diameter, 3mm length) were immobilized in supine position with a knee support. Among them 12 patients received an IMRT treatment and 5 patients a 4-field box treatment. An endorectal balloon was used daily to spare the posterior rectal wall and immobilize the prostate. After first setup based on skin marks, patients were imaged using the ExacTrac stereoscopic imaging system. If the marker displacement exceeded the tolerance of 3mm in relation to the planning CT, patients were shifted accordingly and verification images were taken. Additionally, patients were imaged after treatment and IMRT patients also at halftime of treatment. Prostate and bone drifts were evaluated as a function of treatment time for more than 600 fractions and margins calculated according to van Herk. Results: Patient motion evaluated by bone match was strongly patient dependent, but in general smallest in CC direction. The less patient dependent prostate drifts showed an increase with treatment time in CC and AP direction. In LAT direction the prostate stayed rather stable. Margins resulted in 2.2mm, 3.9mm, 4.3mm for 4-field box, 3.7mm, 2.6mm, 3.6mm for 5-field boost IMRT and 4.2mm, 5.1mm, 6.6mm for 9-field pelvic IMRT in LAT, CC and AP direction, respectively. Mean treatment times were 5.4min, 9.7min and 16.2min for 4-field box, 5-field and 9-field IMRT, respectively. Conclusion: Intrafraction prostate and patient motion causes significant positioning variations. Calculated margins were comparable to results from literature without endorectal balloon. Repositioning of the patients during the treatment or shorter treatment times will be necessary to further reduce the treatment margin.

Topic: Medical Physics

P 240 Calmodulin kinase II modulates amphetamine-induced reverse transport in the dopamine transporter

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The dopamine transporter (DAT) mediates dopamine (DA) reuptake once DA gets released into the synaptic cleft; thereby, DAT regulates the DA content available for synaptic transmission. Certain stimuli, e.g. a change in the ionic composition of the extracellular fluid or psychostimulants like amphetamine can induce the reverse operation and induce outward transport, thereby increasing extracellular dopamine concentrations. Increases of DA in the synaptic cleft are associated with psychosis and believed to underlie the initiation of drug addiction. Influx and efflux of substrate via the DAT are thought to be asymmetrical processes and were shown to possess consensus sites for the regulation by intracellular kinases. It was demonstrated that the loss of N-terminal serines ablates amphetamine-induced reverse transport in the DAT and that Ca^{2+}/Calmodulin dependent protein kinase II a (CaMKIIa) can physically bind the DAT C-terminus and phosphorylate N-terminal serines. Pharmacological inhibition of CaMKIIa with KN93 dramatically reduces amphetamine-induced efflux in both cells stably transfected with the human DAT and in rat striatal slices. Here, we show that CaMKIIa is regulating amphetamine induced DAT mediated efflux in mice with different mutations in the gene of CaMKIIa and in a mouse model of Angelman Syndrome. Either pharmacological inhibition or genetic ablation of CaMKIIa function reduces amphetamine-induced reverse transport in CaMKIIa-deficient mice. We are currently testing in vivo whether these mice respond differently to the psychostimulants amphetamine and cocaine, i.e. whether they have impairments in getting sensitized to the locomotor-stimulating and addictive effects of these psychostimulants.

Topic: Molecular Drug Targets

P 241 EVI1 is a potent modulator of transcriptional and biological responses of human myeloid cells to all-trans retinoic acid

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Ecotropic viral integration site 1 (EVI1) is overexpressed in myeloid leukemias as well as in solid tumours, and is associated with poor treatment response. Previous work has shown that EVI1 was regulated by all trans retinoic acid (ATRA), and in turn enhanced the ATRA induction of the RARβ gene but counteracted the ATRA induction of EVI1 itself. These opposing activities were both mediated through retinoic acid response elements (RAREs) in the regulatory regions of the RARß and EVI1 genes. The goal of the present study was to further characterize the potential of EVI1 to modulate the ATRA response on the transcriptional and functional levels and to explore the molecular mechanisms underlying this modulation. U937 cells with and without ectopic expression of EVI1 were cultured with ATRA or DMSO (solvent) and subjected to microarray analysis, proliferation assays, cell cycle analysis and apoptosis assays. Results were confirmed in HL60 cells. Furthermore, differentiation was analyzed using CD11b staining and morphological analysis. Microarray analyses revealed that the modulation of the transcriptional response to ATRA by EVI1 is not restricted to the RARβ and EVI1 promoters but valid in a more general concept: 44 genes exhibited a modulated ATRA response in the presence of EVI1. Biological effects of ATRA were also modulated by EVI1: EVI1 enhanced ATRA-induced cell cycle arrest and apoptosis in U937 cells. The augmentation of apoptosis was validated in HL60 cells. Furthermore, in this cell line ATRA induced differentiation was enhanced by Evi1. To investigate how EVI1 modulates the ATRA response, proteins interacting with EVI1 in the absence or presence of ATRA will be identified: U937 cells inducibly expressing an HA-tagged version of EVI1 will be used to study the EVI1 interactome through mass spectrometry analysis after enrichment of EVI1-HA from nuclear extracts through affinity purification.

Topic: Malignant Diseases

P 242 Role of GHR in Liver Fibrosis

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Liver fibrosis is identified as the excessive accumulation of extracellular matrix proteins that occur in many chronic liver diseases. Growth hormone receptor (GHR) controls various cellular functions including the transcription of IGF-1 through Stat 5 signalling. Recently, growth hormone resistance has been associated with liver cirrhosis in humans. Furthermore, supplementation of recombinant IGF-1 in cirrhotic animal models was shown to reduce disease severity. To investigate whether modulation of the growth hormone-Igf-1 axis alters the severity of fibrosis we specifically deleted the GHR receptor and challenged these animals by crossing them with a mouse model of inflammatory cholestasis and liver fibrosis (Mdr2 -/- model). Results: GHR-/- Mdr2-/- showed an increase of serum parameters such as Alt, bilirubin and BA which signify liver damage and fibrosis when compared to control mice (Mdr2-/-).Bile duct proliferation and extensive collagen deposition were also observed in GHR-/- Mdr2-/- relative to Mdr2-/-. Additionally, a greater down regulation of the hepatoprotective genes HNF6, EGFR and IGF-1 accompanied by increased apoptosis was seen in GHR-/- Mdr2-/-. Conclusion: Loss of the GHR signalling results in increased liver injury in the Mdr2-/- mouse model of inflammatory cholestasis and liver fibrosis, signifying the possible therapeutic value of this pathway in the development of liver fibrosis treatments.

Topic: Malignant Diseases

P 243 PathogenPCR for improved diagnosis of neonatal sepsis

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Premature infants are particularly susceptible to infections due to immature host defense mechanisms. Approximately 25% of all very low birthweight infants experience at least one systemic infection during their hospital stay. Conventional blood culture is the gold standard for sepsis diagnosis but pathogens are only detected in about 20% of cases and results are usually not available within two days. However, early diagnosis as well as fast and accurate treatment of neonatal sepsis are essential to prevent life-threatening complications. New molecular assays for the detection of blood stream pathogens are promising diagnostic tools for the rapid, sensitive, and specific detection of pathogens. The advantage of fast pathogen detection like PCR systems (5-6 hours, blood culture: about 72 hours) results in benefits, e.g. targeted pathogen defence, less use of broad-spectrum antibiotics and therefore less antibiotic resistence, an important issue in times of multiresistant pathogenes. Therefore, considering patients´ fragility, particularly neonatology needs quick bacteriological results. Nethertheless new tests are not available for premature born children yet due to required blood volume (1,5 ml). The aim of the present study is to adapt and evaluate a commercially available PCR system for use in neonatology. In previous experiments, the blood volume required by the PCR system was minimized from 1,5 ml to 100µl of blood, thus enabling the use in preterm infants. In this study, the diagnostic utility and clinical usefulness of the modified Septifast protocol for the rapid diagnosis of neonatal sepsis will be evaluated.

Topic: Inflammation and Immunity

P 244 The prevalence of root resorption of maxillary incisors caused by impacted maxillary canines

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Objectives: The aim of this study was to evaluate the prevalence of root resorption of maxillary incisors caused by impacted maxillary canines using low-dose dental computed tomography and to gain additional knowledge of the underlying aetiology and the progression of root resorption. Materials and Methods: A total of 440 patients (mean age 24.7 years) with 557 impacted maxillary canines were examined regarding their location and the occurrence of root resorption of maxillary incisors. Results: The frequency of root resorption was 2% of central and 7.7% of lateral maxillary incisors. The location of the 557 impacted canines within the dental arch was palatal in 67.5%, buccal in 15.4% and central in 17.1%. No significant differences could be shown with respect to the width and the shape of the dental follicle of the impacted maxillary canines and the presence of root resorption of incisors. The presence of root resorption of central ($p<0.0001$) and lateral ($p<0.023$) maxillary incisors was significantly correlated with an existing contact relationship of the impacted maxillary canines. Conclusions: Our investigation confirms the theory of prior reports comprising a much larger patient population, hypothesizing that the dental follicle of impacted maxillary canines does not cause resorption of adjacent maxillary incisors per se. Clinical Relevance: Root resorption of maxillary incisors is correlated with effects of contact of the impacted maxillary canines and these findings should be considered in treatment planning. Our findings are consistent with other reports and may develop new treatment approaches for the treatment of this sequela.

Topic: Regeneration of Bones and Joints

P 245 In skin, sensitization to the house dust mite allergen Der p 2 is not solely dependent upon TLR-4 activation

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Background: 10 to 20% of the world population suffers from house dust mite allergen-specific symptoms, which include dermatitis, rhinitis and asthma. Among the allergens from house dust mite species Dermatophagoides pteronyssinus, Der p 2 is a major elicitor of allergic sensitization and symptoms. Der p 2 is a molecular homologue to the TLR4-compound MD-2 and thereby mediates LPS-driven TH2 response. An asthma model in TLR-4-defcient mice has even suggested immune-unresponsiveness to Der p 2 in the absence of TLR-4 expression. Aim: The aim of this project is to investigate whether by similar mechanisms like in the lung Der p 2 may be responsible for mediating allergic dermatitis. Methods: We studied the pathophysiology of sensitization to Der p 2 via the skin. C57BL/6 WT mice were sensitized percutaneously with recombinant Der p 2 and with/without adding LPS. Further, the role of TLR-4 in percutaneous sensitization with Der p 2 was investigated using TLR-4-/- C57BL/6 mice. Allergen-specific antibody responses and skin inflammation of the treated mice was monitored and compared between the groups. Results & Conclusion: In C57BL/6 WT mice, high titers of Der p 2 specific TH2 antibodies (IgE, IgG1) were induced, independently of the presence of low or high LPS content. Furthermore, TLR-4 deficient mice showed equally high levels of IgE and IgG1 as WT-control mice. Moreover, Der p 2 alone has a high allergenic potential on its own and is not dependent on the presence of other allergens in house dust mite extract. Our results suggest that sensitization to Der p 2 via the skin does not solely rely upon the activation of the TLR-4 signalling pathway.

Topic: Immunology

P 246 Signalling network between ERG, cMyc and NF-κB is critical in prostate cancer.

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Activation of transcription factors may play a critical role in the initiation and progression of human cancers, including prostate cancer. The transcription factor ERG (ETS-related gene) is expressed in about 50% of prostate cancers due to a gene fusion placing the coding sequence of ERG under the control of a prostate-specific promoter. Several other transcription factors such as c-Myc, androgen receptor and NFκB are known to be involved in prostate cancer development. These factors might cooperate with each other and can contribute to tumor initiation and progression in several ways including the activation of cell survival mechanisms and influencing proliferation. We generated mice, which overexpress ERG in the prostate to mimic the gene fusion and found that these mice develop low grade prostatic intra-epithelial neoplasias (PIN), which increase with age. ERG influences cell proliferation both in the mouse model and in human cell lines. This may be attributed to a decrease in cyclin D1, c-Myc and E2F1. We find that the overexpression of ERG causes upregulation of E2F, c-Myc and p65-NFκB, implicating ERG as a potential transcriptional regulator of these genes. Furthermore, ERG enhances the nuclear translocation of p65. We show that c-Myc, which is upregulated by ERG, interacts with p65 and AR and that it has a dose dependent effect on NFκB activity. ERG positive vcap cells show resistance to cytotoxic drugs and inhibition of NFκB make them sensitive for chemotherapeutic drug. Our data indicate that the effect of ERG overexpression on prostate cancer development may be due to increased cell survival by activation of NFκB and an increase in cell proliferation due to c-Myc upregulation. Therefore combinatorial and network targeted drug therapy can have better results in clinics

Topic: Molecular Signal Transduction

P 247 Genomics effects of 9-cis-retinoic acid in an adrenocortical cell line

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Background: Adrenocortical cancer is rare, but its prognosis is poor. In our previous meta-analysis of adrenocortical tumor genomics data, retinoic acid signaling was established as a major, novel pathogenic pathway, moreover, adrenocortical cancer was associated with reduced retinoic acid production and signaling. Aim: To examine 9-cis-retinoic acid-induced mRNA expression changes in the NCI-H295R adrenocortical cancer cell line. Materials and methods: Several concentrations of 9-cis-retinoic acid in different treatment periods have been tested on cell viability and for hormone measurements to optimize treatment conditions. Agilent 4x44 K microarray platforms have been used for whole genome gene expression profiling. Microarray results have been analyzed by Genespring software and Ingenuity Pathway Analysis. Microarray results were validated by real-time qRT-PCR. Results: 9-cis-retinoic acid decreased cell viability and cortisol secretion. The 24 hour treatment period and three 9-cis-retinoic acid concentrations ($2.5x10^{-5}$M, $5x10^{-5}$M, $7.5x10^{-5}$M) have been selected. Based on the microarray study, 832, 2953 and 2997 significant gene expression changes have been found between controls and the three concentrations tested, respectively. Pathway analysis revealed four major pathways in association with 9-cis-retinoic acid treatment that correlated with our previous findings: i. cell cycle damage, ii. retinoic acid signaling, iii. changes of hormone secretion and iv. changes of immune response. Conclusion: 9-cis-retinoic acid decreased cell proliferation and hormone secretion in the NCI-H295R cell line. We have identified the most prominent gene expression changes and pathways. These data might raise the possible utility of 9-cis-retinoic acid in the treatment of adrenocortical cancer.

Topic: Tumorbiology - Oncology

P 248 Signalling pathways of the Calcium Sensing receptor in colonocytes

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The calcium sensing receptor (CaSR) which was first cloned by Brown et al. in 1993 from bovine parathyroid glands; is a G-protein coupled receptor (GPCR) that senses changes of extracellular calcium levels. In addition to its main role in regulation of calcium homeostasis, CaSR is also involved in numerous other biological processes. Recent studies focused on elucidating the role of CaSR in gastrointestinal (GI) tract in the maintenance of the normal gut physiology. Not only calcium, but a number of ligands can activate the CaSR and this activated receptor then stimulates a number of downstream cell signalling pathways affecting various cellular functions such as lipid membrane metabolism and cell proliferation. Colon cancer cell lines show differences in signalling pathways activated by CaSR compared with parathyroid cells. Previous work demonstrates that Ca^{2+} inhibits cell proliferation in colon cells; but the pathways or factors which are directly involved in this process are yet to be found. First we will investigate the involvement of the CaSR in the regulation of several kinases such as MAPK, PI3K and NFκB under different ligand treatments using luciferase assay. The activation will be validated with kinase inhibitors and the CaSR involvement will be confirmed by down regulation of the receptor using siRNA. Once the activated pathways are determined we will analyse whether CaSR-regulated membrane lipid metabolisms are ligand dependent. First the membrane lipid composition will be analysed by mass spectroscopy (MS). The possible changes in the membrane structure due to treatment with CaSR agonists will be examined by fluorescence resonance energy transfer (FRET) using fluorescent phospho lipid tags and if the membrane lipid structure changes we will analyse the effect on the membrane lipid metabolism via MS. We also plan to measure the ligand influence G-protein selection by CaSR using FRET and co-immunoprecipitations. Finally, using the resulting data we are planning to construct the first signalling model of the CaSR in colonocytes.
This project is funded by the Marie Curie Initial Training Network grant number FP7-264663.

Topic: Tumorbiology - Oncology

P 249 Visualization of Dendritic mRNA Localization and its Interacting RNA-Binding Proteins in Living Neurons

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Asymmetric localisation of mRNAs is contributing to establish functional subcellular compartments. This is crucial to maintain cellular functions, including synaptic plasticity. During their journey mRNAs are packaged into transport competent ribonucleoprotein particles (transport RNPs) and are prevented from translation until arrival at sites of anticipated need. Other types of cytoplasmic RNA granules include Processing bodies (P-bodies), major sites of mRNA storage and degradation, or stress granules (SGs), which are formed in cells that have undergone cellular stress. I have previously tackled, in collaboration with Dahm and Zeitelhofer, the visualisation of P-bodies in dendrites. A significant number (approx. 30%) of P-bodies is located in close proximity to the base of dendritic spines (the post-synaptic compartment of synapses). These data suggest a role of P-bodies in translation control at synapses indicating a putative impact on synaptogenesis and synaptic plasticity. mRNAs are transported to the basis of dendritic spines, where they might be stored in P-bodies. When appropriate signals arrive, the translational block is relieved and protein is produced. In the absence of such an activating signal the mRNA would be degraded within the P-body. To visualise mRNA localisation in living hippocampal neurons the MS2 imaging system has proven to detect mRNAs on a single molecule resolution. This system is a powerful tool to unravel mRNA kinetics in dendrites, both under physiological conditions and chemically induced neuronal activation. To identify the RNA binding proteins that are necessary and sufficient for dendritic mRNA transport I will apply dominant negative mutants and mouse genetics. This cutting-edge approach, using neurons in culture derived from Staufen2 and Pumilo2 knock-out mice, marks the highlight of my experimental work. Taken together, I am convinced that my work will result in new insight into the mechanism of mRNA transport to the synapse.

Topic: Neuroscience

P 250 Role of Adenosine A2A Receptors in the Modulation of GABA (A) Receptor Function

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GABA (A) receptors (GABA (A)Rs) are ligand gated Cl- channels composed of five subunits, and are the major inhibitory neurotransmitter receptors in the brain. They are extensively targeted in clinical medicine to achieve anxiolysis, anticonvulsion, muscle relaxation, sedation and sleep. Adenosine levels in the brain reflect local network activity, and also rise following compromised cell function, thus serving as a danger signal. Adenosine exerts it´s actions via G-protein coupled adenosine receptors (ARs) that act mainly as modulators of other neurotransmitter systems. Similar to GABA (A)Rs, ARs play a role e.g. in sleep, epilepsy, ischemia and neurodegenerative disorders. Several studies support the conclusion that ARs, particularly Gs-coupled A2A receptors (A2ARs), modulate GABAergic neurotransmission. Besides influencing presynaptic GABA release and reuptake, some electrophysiological findings also suggest a postsynaptic effect on GABA (A)Rs. The aim of our work is to determine the mechanism and functional consequences of a cross-talk between ARs and GABA (A)Rs in neurons. So far, we found extrasynaptic co-localization of A2ARs with GABA (A)R $\Delta$and α, 4 subunits in primary cultures of hippocampal and striatal neurons. Additionally, the surface stability of these subunits seems to be modulated following treatment of neurons with A2AR specific ligands in biotinylation experiments. The A2AR is known to engage in direct interactions with many other receptors via it´s long c-terminal tail. To investigate whether such a heteromer formation also occurs with GABA (A)Rs, we are using co-IP from transfected cell lines and brain tissue, as well as FRET and pull-down experiments. Alternatively, a possible second messenger dependent mechanism will be tested in cell culture. Finally, IHC of mouse brain slices is used to identify co-localization in particular brain regions and cell types. Financial support by the CCHD program of the FWF and the MUV is gratefully acknowledged.

Topic: Cell Communication in Health and Disease

P 251 Histone deacetylases HDAC1 and HDAC2 control Cd8 silencing in CD4 lineage T cells

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Chromatin modifications such as reversible changes in histone acetylation patterns play a key role in the regulation of T cell development and function. Modification of core histones by lysine acetylation is controlled by histone acetyltransferases and histone deacetylases (HDACs), which are considered as transcriptional co-activators and co-repressors, respectively. Eighteen HDACs have been identified in mammals, however dissecting individual roles for each member of the HDAC family in T cells remains a major scientific challenge. Here we show that the combined loss of HDAC1 and HDAC2 in T cells (by deleting conditional alleles with Cd4 – Cre) led to a reduction in peripheral T cell numbers, indicating a role of HDAC1/2 in the regulation of T cell numbers/homeostasis. Moreover, loss of HDAC1/2 led to the appearance of mature CD4+CD8+ double-positive T cells in peripheral lymphoid organs in addition to CD4+ and CD8+ T cells. The aberrant CD4+CD8+ T cell population, which could be detected already in the thymus among the mature CD24-lowTCRbeta-high subset, was MHC class II restricted and up-regulated CD40L after TCR stimulation, thus displaying helper T cell features. Gene expression arrays revealed that HDAC1/2 double-deficient CD4+CD8+ T cells resemble CD4 lineage rather than CD8 lineage T cells, indicating that HDAC1/2 double-deficient CD4+CD8+ T cells represent CD4 lineage cells that express CD8. Treatment of wild-type CD4+ T cells with the class I HDAC inhibitor MS-275 led to the induction of CD8 expression upon activation that was dependent on E8I and Runx complexes, indicating HDAC1/2-dependent maintenance of Cd8 silencing in peripheral CD4+ T cells. Taken together, our results provide genetic evidence for CD8 silencing during CD4 lineage development and show that HDAC1/2 are crucial for the establishment and maintenance of CD8 silencing in CD4+ T cells by counteracting the activity of E8I and Runx complexes.

Topic: Immunology

P 252 Factors determining outcome in patients with heart failure and normal ejection fraction

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Background: Patients with heart failure and normal left ventricular ejection fraction (HFNEF) face an adverse outcome. The aim of the present study was to identify factors that determine prognosis. Methods: Consecutive patients with HFNEF diagnosed according to current ESC guidelines were recruited in our prospective Viennese registry. Death and/or hospitalization for heart failure were defined as primary outcome variables. Outcome groups were compared with respect to potential prognostic predictors using the Student's t-test. Multivariable logistic regression analysis was applied to determine whether parameters of interest were associated with adverse outcome. P<0.05 indicated statistical significance. Results: Between December 2010 and January 2012, 49 HFNEF patients (34 f/ 15 m, mean age 70±8 years) were registered. After a mean follow-up of 5±9 months, 14 (29%) patients were hospitalized or died. Patients in the adverse outcome group were characterized by higher body mass index (BMI, 35±7 versus 29±5, p=0.004), higher systolic pulmonary pressure on echo (sPAP in mmHg, 69±15 versus 55±14, p=0.004), shorter 6-minute walk distance (6-MWD in m, 271±131 versus 364±100, p=0.019), higher transpulmonary gradient (TPG in mmHg, 15±4 versus 12±4, p=0.013) and a higher pulmonary vascular resistance (PVR in dynes.s/cm5, 257±97 versus 198±71, p=0.030). Diabetes mellitus II (DM II, 75% versus 24%, p=0,002) and atrial fibrillation (92% versus 51%, p=0,013) were more prevalent among patients with adverse outcome. In the multivariable regression model, only DM II (OR 25.34[95% CI, 2.06 to 311.45]; p=0.012), BMI (OR 1.25[95% CI, 1.00 to 1.56]; p=0.048), and PVR (OR 1.02[95% CI, 1.00 to 1.05]; p=0.032) remained independent predictors of outcome. Conclusions: Presence of DM II, higher BMI and higher PVR worsen prognosis in HFNEF patients.

Topic: Cardiovascular and Pulmonary Disease

P 253 Moleculare connectivity in patients with unilateral temporal lobe epilepsy investigated with [18F]FDG PET

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The aim of this study was to investigate alterations in metabolic connectivity in patients with unilateral temporal lobe epilepsy (TLE) with FDG PET, disclosing dysfunctional connectivity in brain networks. Furthermore, the strong lateralized effects in TLE provide a proof-of-principle using this new analysis approach, which might be subsequently applied in psychiatric disorders with more subtle alterations in molecular connectivity. 46 unilateral TLE patients (23 female; mean,SD=37.28,±10.65 years) underwent FDG PET for presurgical assessment at the MUW. Differences in metabolism between the epileptic (EH) and healthy hemisphere (HH) were assessed by paired-samples t-test. Proceeding from the region with strongest hypometabolism (= seed region), metabolic connectivity was computed and directly compared between the two hemispheres. More precisely, individual FDG maps were normalized to the global mean uptake. The seed region of the EH showed connectivity with adjacent regions of the temporal lobe,whereas the HH shows autocorrelation only ($p<0.05$ FWE-corrected). Direct comparison revealed a reduced metabolic connectivity of the EH hippocampus to the ventromedial prefrontal cortex (vmPFC), as well as to the prefrontal areas of the contralateral hemisphere. On the other hand, increased correlations were found in adjacent regions of the EH such as hippocampus caput and temporal pole ($t>2.58$, $p<0.01$ uncorrected). Since the hippocampus and vmPFC show strong functional interregional connections and are both part of the default mode network, results may indicate that unilateral TLE impairs parts of the self-referencing network. The increased metabolic connectivity between the hippocampus and the adjacent structures of the TL,may imply the alterating impact of focal epilepsy in this brain regions. This study shows the applicability of this promising analysis method, paving the way to study other diseases in multiple disciplines with different radioligands in the near future.

Topic: Clinical Neurosciences

P 254 Chemoresistance can be induced by bile acid-independent activation of FXR in liver and intestinal cancer cells

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Background and Aims: Up-regulation of genes involved in mechanisms of chemoresistance, including ABC export pumps, limits the clinical usefulness of cytostatic drugs in anticancer treatment. The aim of this study was to investigate the expression of ABC proteins in response to cisplatin treatment and the role of the nuclear receptor FXR in the signaling process. Methods: Gene expression was determined by RT-QPCR and Western-blot in hepatoblastoma (HepG2), hepatocarcinoma (Alexander) and colorectal adenocarcinoma (Caco-2 and LS174T) cells. Results: In intestinal cells the expression of several ABC proteins (mainly MRP2) was stimulated by cisplatin at IC50 (LS174T: 20 μM; Caco-2: 25 μM). A partially cisplatin-resistant subline (LS174T/R) - obtained by long-term exposure to cisplatin and double subcloning - has 500-fold increased expression of MRP2. A 2.9 kb-DNA fragment of the 5'-flanking region of the ABCC2 gene presumably including the promoter (MRP2pr) was cloned and partially deleted constructs linked to a reporter gene were generated. Transfection of LS174T and LS174T/R with complete or partial MRP2pr revealed the ability of cisplatin to activate MRP2pr, which was dependent upon the conserved MRP2pr region. Moreover, basal MRP2pr activity was markedly higher in LS174T/R cells, in which the expression levels of transcription factors p53, c-Myc, AP1, YB1, NRF2, RARα and PXR were similar to that in wild-type cells, whereas these of c/EBPβ, HNF1α, HNF3β and HNF4α were 4-to-6-fold higher. The expression of FXR and its target gene SHP was particularly enhanced (≈200- and ≈50-fold, respectively). GW4064 activates FXR signaling pathway in LS174T/R but not in LS174T cells. When Alexander cells, which do not express FXR, were transfected with human FXR a significant reduction in the sensitivity to cisplatin-induced toxicity was found. This protective effect was further enhanced by activation with GW4064. Transfection with FXR also protected against toxic effect of doxorubicin, mitomycin C or potassium dichromate, but not against paclitaxel or acetaminophen. In Alexander FXR-transfected cells, cisplatin was able to induce the expression of FXR target genes SHP, BSEP, and OSTβ although the effect was weaker than that of GW4064. Moreover, cisplatin induced luciferase expression led by an IR-1 element in HepG2 cells (that express FXR) and in Alexander transfected cells. In primary cultures of human hepatocytes, cisplatin also induced the expression of FXR target genes. Conclusions: Bile acid-independent activation of FXR can be triggered by exposure to some toxic compounds, which may be involved in enhanced chemoresistance in liver and colon cancer cells.

Topic: Other

P 255 Expression of α-SMA in Hepatic Stellate Cells as Their Activation Biomarker with Down-Regulated Immune Response in Human Hydatid Infection.

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Fibrosis is one of the most important pathological manifestations of chronic hydatid infection (CHI). It is believed that inflammation-induced macrophage activation generates stimulatory signals for trans-differentiation of hepatic stellate cells (HSCs). This process has an essential role in tissue fibrosis during chronic liver injuries and can be characterized by expression of cytoplasmic smooth muscle actin-alpha (α-SMA). Yet there has been no In Situ study on human CHI which could show the orchestration of inflammatory infiltrate and contribution of HSCs in parasite-induced liver fibrosis. In brief, a multiple semiquantitative immunohistochemistry assay was performed by applying monoclonal antibodies raised against α-SMA, desmin, CD1a, CD3, CD8, CD20, CD68 and myeloperoxidase by using formalin-fixed paraffin-embedded tissue samples from 25 patients with surgically confirmed liver CHI. Eosinophilia was assessed in hematoxilin-eosine stained tissue sections. Eosinophils and CD1a dendritic cells showed no significant contribution in inflammatory infiltrate surrounding the hydatid lesion. CD3+T cells and B lymphocytes showed relatively higher deposition than other subpopulations such as T CD8+ cells, macrophages and neutrophils around the pericyst. Fibrosis was observed in 100% of patients while α-SMA expressing cells showed a significant accumulation adjacent to the periparasitic fibrous layer in 84.61% of the examined samples. Results achieved by this study could suggest that CHI may induce activation of HSCs which is an underlying mechanism for initiation tissue fibrosis; nonetheless the host-parasite immune interactions evidently result in macrophage inhibitory immune profile.

Topic: Immunology

P 256 LRET-based distance measurements in a bacterial homolog to mammalian glutamate transporter GltPh

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Glutamate transporters are integral membrane proteins that catalyse the concentrative uptake of glutamate from the synapse to intracellular spaces by harnessing pre-existing ion gradients. In the central nervous system glutamate transporters are essential for normal development and function, and are implicated in stroke, epilepsy and neurodegenerative diseases. The crystal structure of a eukaryotic glutamate transporter homologue from Pyrococcus horikoshii is available at various conformations (Yernool et al., 2004) providing a structural framework for the determination of substrate and inhibitor binding to the transporter. Although GltPh has a low overall sequence identity of about 25–30% to mammalian glutamate transporters, the grade of conservation is high in functionally critical regions. The structural rearrangements of the protein during substrate transport largely remains to be characterized. In this study we aim to measure such movements using the technique of lanthanide resonance energy transfer (LRET).

Topic: Neuroscience

P 257 Popliteal artery trauma: Treatment and Outcome

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Introduction: Popliteal vascular trauma is an uncommon but potentially devastating problem that carries a great risk of limb loss. In current literature limb salvage and amputation rates vary widely after traumatic popliteal lesions. Some authors have reported on amputation rates up to 20%. The purpose of this study was to analyse characteristics, epidemiology and clinical outcome of patients with traumatic popliteal arterial injuries over a 20-year period. Materials and Methods:In this study we reviewed the trauma database of Vienna General Hospital and identified all patients with traumatic popliteal artery injuries. Results: From a database of 89 patients with vascular injuries of the lower limbs, an analysis of clinical records revealed that we had 64 patients with popliteal artery injury. Thirty-eight of these patients got injured by a blunt trauma, whereas 26 patients had a penetrating mechanism of injury. Arterial repair was obtained within three and 6 hours in most of the cases. Arterial continuity was restored by using autogenous saphenous vein grafts, vein patches or primery arterial sutures. The overall amputation rate within our study was 14%.Comparing between blunt and penetrating mechanism of trauma we had a significantly higher delayed amputation rate following blunt trauma.Conclusion: The highest amputation rate we have found in patients who have undergone a popliteal artery repair using vein patches, followed by bypasses. The best outcome we have had for popliteal artery injuries treated by primery suture. In conclusion, the critical appraisal of our treatment showed potentially suspect areas of our practices which we try to detect and, with respect to current literature, find the optimal controlled treating strategy of this uncommon injury.

Topic: Regeneration of Bones and Joints

P 258 A proteomic approach to identify novel interactors of the Tec family kinases that play a crucial role in T-cell development and differentiation

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Tec family kinases (TFK) are non-receptor tyrosine kinases expressed primarily in haematopoietic cell lines. Three out of five TFK members – Itk (IL-2 inducible T-cell kinase), Rlk (resting lymphocyte kinase), and Tec, are found in T lymphocytes and play an essential role in efficient downstream T-cell receptor (TCR) signalling. Recent functional studies have indicated both Itk and Rlk as important mediators of pathways that control CD8+ T-cell and natural killer T-cell (NKT) development in the thymus1. Furthermore, impaired T-cell differentiation was found to cause a number of inflammatory diseases, e.g., allergic asthma, dermatitis, HIV proliferation and Epstein Barr virus (EBV) associated lymphoma. The mechanism of TFK-mediated T-cell development, however, is still unknown. Nevertheless, the variety and complexity of TFK-associated diseases highlights the need for a deeper understanding of the TFK T-cell signalling network. Experimental design based on tandem affinity purification, cutting-edge mass spectrometry and proteomics should result in the identification and quantitation of novel protein-protein interactions important for regulating T-cell development and differentiation. Overexpression followed by downregulation of Itk, Rlk, and Tec, in Jurkat Flp-In T cell line, will be utilised prior to mass spectrometric analysis. In addition, global phosphoproteomic and phosphotyrosine enrichment in the Jurkat T-cell and primary cells will be assessed to compare the phosphorylation patterns. Finally, characterisation of common TFK substrates and regulatory proteins should confirm TFK signalling pathway interference upon TCR stimulation and suggest regulatory mechanism essential for adequate T-cell differentiation. 1. Atherly LO, Lucas JA, Felices M, Yin CC, Reiner SL, Berg LJ. The Tec family tyrosine kinases Itk and Rlk regulate the development of conventional CD8+ T cells. Immunity 2006;25(1):79-91.

Topic: Molecular Signal Transduction

P 259 Does periodic coronary venous pressure elevation promote regenerative processes through SAFE pathway initiation?

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Objectives: Pressure-controlled intermittent coronary sinus occlusion (PICSO) has been shown to alter cellular signalling pathways. To assess the possibility of SAFE (survivor activating factor enhancement) - pathway induction, interleukin-6 (IL-6) levels were measured in patients with chronic heart failure with PICSO treatment. Methods: 32 patients undergoing cardiac resynchronization therapy (CRT) by device implantation (diagnosis: predominantly ischemic and dilated cardiomyopathy) were included into a prospective non randomized study, (8 interventional / 24 control group). PICSO was performed for 20 minutes by introducing a balloon catheter into the coronary sinus and after positioning of the left ventricular electrode. Hemodynamic data were obtained through the LIDCO System and PICSO catheter (coronary sinus pressure (CSP)). Coronary venous blood samples were taken and IL-6 and NT-proBNP measured before and after PICSO. Mean patients follow up was 34 months. Results: IL-6 secretion increased significantly after PICSO in comparison to controls (p=0.006). There was no significant linear correlation between the percentage increase of IL-6 and hemodynamic data including the maximal developed coronary venous pressure during PICSO. In long term follow up, we assessed a mortality risk reduction by 80 percent (RR = 0.199, CI (95%) = 0.002-1.642, p = 0.302). Also a trend towards a survival benefit was observed. This benefit particularly included severely diseased patients with NT-proBNP levels above 1500 pg/ml (p=0.080). Conclusion: These results indicate the initiation of mechanotransduction and are in accordance with prior experimental analyses showing enhanced expression of vascular endothelial growth factor and heme oxygenase 1. Hence, this intervention could be the link to molecular corresponding factors that improve survival, reduce the risk for reinfarction and decrease adverse cardiac events after myocardial infarction as observed in previous trials.

Topic: Vascular Biology

P 260 Heme oxygenase 1 in brown adipose tissue

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Obesity and type 2 diabetes mellitus (T2DM) are one of the most important health issues in the western world. Regarding obesity it's very important to understand how the organism regulates energy balance and energy storage including the differentiation of adipocytes. T2DM results in insulin resistance and increased oxidative stress, due to an overproduction of reactive oxygen species (ROS) in mitochondria. As a stress response gene heme oxygenase 1 (HO-1) is induced by oxidative stress and plays a cytoprotective role in response to tissue injuries. HO-1 regulates the oxidative degradation of heme into billiverdin, iron and CO, which all possess beneficial anti-inflammatory and antioxidant properties. HO-1 is not only located in the cytoplasm, but also in mitochondria. Since mature adipocytes harbor a lot of mitochondria it is interesting to link the anti-inflammatory enzyme HO-1 with general mitochondrial functions like OxPhos and ROS production. Additionally, CO binds to oxidases in the mitochondrial respiratory chain, a crucial step in the induction of the anti-inflammatory effect of HO-1. Even though the biggest part of our adipose tissue is white adipose tissue (WAT) which primary function is to store fat in the case of a mismatch in energy supply and utilization, we also have a small amount of brown adipose tissue (BAT). BAT plays an important role in the maintenance of body temperature in animals and human neonates. BAT contains more mitochondria than WAT and it also possesses the tissue specific uncoupling protein 1 (UCP-1). After upregulation of UCP-1 by fatty acids in BAT, the βoxidation in mitochondria is rendered inefficient leading to the production of heat rather than ATP. UCP-1 upregulation correlates with the upregulation of HO-1, so it would be interesting to explore whether HO-1 is necessary to counter the heat-induced stress in BAT. BAT is also present in adults and because of its unique ability it could be a very potent target to treat obesity.

Topic: Cell Communication in Health and Disease

P 261 Quantitative Comparison Of Segmentation Performance In Four Spectral Domain Optical Coherence Tomography Instruments

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Automated detection of the retinal boundaries provides retinal thickness values in spectral-domain optical coherence tomography (SD-OCT) instruments. However, segmentation algorithms are prone to errors. Output of incorrect thickness values may lead to wrong conclusions in clinical practice. Our aim was to compare the segmentation performance of 4 SD-OCT devices. Methods: 29 eyes of 19 patients were imaged each in the same session with Spectralis OCT (Sp, Heidelberg), Cirrus HD-OCT (Ci, Carl Zeiss), 3D-OCT 2000 (3D, Topcon) and RS-3000 (RS, Nidek) instruments. Raw data were evaluated in custom software. We investigated a subfield of 1 mm diameter, centered manually on the fovea, to compare identical regions for each case. Errors in segmentation were corrected manually on each B-scan enclosed in the central mm. Proportions of wrongly segmented A-scans were noted. The clinical relevance of segmentation errors was assessed by comparing center point thickness (CPT) and mean central mm thickness (CMT) between automated segmentation and manual correction.Results: Segmentation errors occurred in 77% and affected on average 29% of A-scans within the central mm, resulting in a mean thickness difference of 24/13µm (CPT/CMT). Regarding inter-device comparisons, the proportions of cases with segmentation errors ranged from 48% (Sp) to 79% (Ci), 86% (3D) and 93% (RS). Mean proportions of A-scans with wrongly detected outer retinal boundary were 30% (Sp), 9% (Ci), 23% (3D) and 10% (RS); proportions for the inner retinal boundary were 11% (Sp), 12% (Ci), 6% (3D) and 21% (RS). Mean deviations in CPT/CMT were 41/28µm (Sp), 17/11µm (Ci), 30/13µm (3D) and 18/8µm (RS).Conclusions: Segmentation errors and consequent clinically relevant CMT deviations are frequent and mandate manual correction. Of note, the correlation between technical accuracy and clinical impact varies strongly between the tested SD-OCT devices.

Topic: Medical Physics

P 262 Simvastatin reduces IL-6 mediated migration of human metastatic melanoma cells

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Since the last decade the number of melanoma patients has increased markedly. High plasma levels of interleukin-6 (IL-6) were associated with bad prognosis and reduction in overall survival of these patients. Statins, HMG-CoA reductase inhibitors, are well-tolerated therapeutics for hypercholesterolemia. We have recently shown that simvastatin triggers apoptosis in human melanoma cells which is paralleled by concentration-dependent alteration in autocrine IL-6 secretion. Here, we investigated in IL-6 signalling and the migration capacity of metastatic human melanoma cells under statin treatment. Experiments were performed with the human metastatic cell lines 518a2 and A375. Cell cycle analysis after IL-6 treatment, demonstrated a significant increase in proliferation of these melanoma cells. Simvastatin exposed cells exhibit morphological changes, leading to a mixed population of sitting and floating cells. FACS analysis of the heteromeric IL-6-receptor (IL-6-R/gp130) showed an increase in the surface expression under simvastatin treatment mainly in the floating cell population. Further, reseeding of the floating population allowed a full recovery in their cell cycle of A375 cells, whereas 518a2 cells committed to apoptosis. In migration assays IL-6 increases proliferation and migration, what was prevented by co-administration of simvastatin. These findings demonstrate that simvastatin is capable to counteract IL-6 mediated migration and proliferation of metastatic melanoma cells.

Topic: Molecular Signal Transduction

P 263 Preclinical studies towards a novel pharmacological therapy for X-linked Adrenoleukodystrophy

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X-linked Adrenoleukodystrophy (X-ALD) is an inherited neurodegenerative disorder caused by mutations in the ABCD1 gene encoding a peroxisomal membrane protein (ALDP), which belongs to the ATP-binding cassette transporter subfamily D (ABCD). The closest homologue of ALDP is the Adrenoleukodystrophy related protein (ALDRP), encoded by the ABCD2 gene. Upon overexpression, ALDRP was able to replace ALDP functionally in vitro in human X-ALD fibroblast and in vivo in an X-ALD mouse model. Currently, the only curative therapy available is bone marrow transplantation, implicating the hematopoietic stem cell lineage in the rescue of brain inflammation. The main objectives of this thesis are: first, to quantify the baseline expression of ABCD1 and ABCD2 in the main populations of blood derived immune cells from healthy subjects and patients; and second, the pharmacological induction of ABCD2 in monocytes and other possible target cells. Specific cell types were isolated by gradient centrifugation and magnetic activated cell sorting (MACS), followed by qRT-PCR to determine mRNA levels. Purity of cell isolations was verified by FACS-analyses. Preliminary results indicate that ABCD1 and ABCD2 are contrarily expressed in all analysed cell types. Whereas monocytes and granulocytes show high levels of ABCD1, the ABCD2 mRNA is barely detectable in these cell types. In contrast, T cells express high levels of ABCD2 and only moderate levels of ABCD1. In compound screens using the THP-1 monocytic cell line, retinoids and HDAC-inhibitors induced ABCD2 consistently. The effect of these compounds on ABCD2 expression will be tested further in primary human monocytes from patients in clinical treatment with those drugs due to unrelated disorders. In conclusion, human primary monocytes appear to be a relevant target for pharmacological compensation through induction of the redundant gene, ABCD2. (Supported by the EU Project Leukotreat No. 241622 and Bundesverein Leukodystrophie)

Topic: Neuroscience

P 264 Prolyl hydroxylase inhibitors decrease plasminogen activation in periodontal fibroblasts

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L-mimosine and dimethyloxaloylglycine (DMOG), both prolyl hydroxylase inhibitors, have recently been recognized as a new approach in the treatment of inflammatory diseases. However, the effect of these prolyl hydroxylase inhibitors on plasminogen activation in periodontal cells is unclear. In this study we assessed the effect of L-mimosine on the plasminogen activation of fibroblasts of the periodontium. Fibroblasts from the gingiva and periodontal ligament were stimulated with L-mimosine and DMOG. The experiments were performed in the presence of interleukin (IL)-1 to simulate pro-inflammatory conditions. Plasminogen activators and plasminogen activator inhibitors were analyzed by zymography and quantitative polymerase chain reaction. Kinetic assay and zymographies showed that prolyl hydroxylase inhibitors reduced urokinase-type plasminogen activator activity in fibroblasts of gingiva and periodontal ligament, also in the presence of IL-1. RT-PCR indicated that the reduced plasminogen activation was mainly a consequence of elevated levels of plasminogen activator inhibitor type 1 and reduced levels of urokinase-type plasminogen activator. Taken together, these findings suggest that periodontal fibroblasts are responsive to prolyl hydroxylase inhibitors that can control the plasminogen activation system.

Topic: Other

P 265 Effects of ischemia and inflammatory mediators on liver cell functions: a comparative in vitro study

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Background and aim: Ischemia/reperfusion injury (IRI) and injury elicited by inflammatory mediators (IM) released during systemic inflammatory response (SIR) are serious problems in trauma and intensive care patients, and often lead to multiple organ failure. It is difficult to dissect the influence of IRI and SIR, because they are always linked to each other due to systemic regulations. PCLS are an in vitro model maintaining cell-cell and cell-extracellular matrix interactions without influences of body\'s systemic processes. The aim of the study was to investigate early pathologic changes caused by either hypoxia-reoxygenation (HR) or IM in PCLS. Methods: HR was induced by incubating PCLS at pO2<1% followed by subsequent reoxygenation. Inflammatory injury was modelled by incubation of PCLS with IM, generated by ex vivo incubation of rat leukocytes with LPS. Mitochondrial function was determined by high resolution respirometry. Total RNA quality was assessed by microcapillary gel electrophoresis; gene expression (IL-6, SOD2, iNOS, HO1) was analyzed by qPCR. The leakage of AST and ALT was measured using standard kits. Results: The upregulation of IL-6 and iNOS confirmed cellular response to IM in PCLS. Such response was absent in HR samples. Hypoxia (but not reoxygenation) caused release of AST and ALT. Reoxygenation drastically impaired mitochondrial function and induced RNA degradation. Similar to HR, IM also caused the release of AST and ALT, but no changes in RNA integrity and mitochondrial respiration. Conclusion: Our data suggest that liver damage caused by either hypoxic or inflammatory insults is based on different mechanisms. These results may help to distinguish between hypoxic and inflammatory origin of damage in complex in vivo systems. This study was supported by FWF grant P21121.

Topic: Molecular Signal Transduction

P 266 Extracellular ATP release as a potential benefit in shockwave treatment enhanced wound healing

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Introduction: Shockwave treatment has been shown to induce release of ATP from Jurkat T cells in vitro. Higher concentrations of extracellular ATP result in complex signaling responses, which finally increase T cell function. Nevertheless, ATP release after shock wave treatment is at least in part affected by cell viability. The open question is whether cell deformation alone causes ATP release, or if shockwaves may also activate certain channels and receptors to release ATP. Thus, the aim of our study was to correlate ATP release after shockwave treatment with cell viability assessment. This should provide a rationale for the mechanisms of ATP release after shock wave treatment. Methods: Human Jurkat T cells were subjected to shock waves using the IVSWT (in vitro shock wave trial) waterbath and the unfocused shock wave device dermagold100 (MTS Germany). Cells were treated with different energies (0.01 mJ/mm2 to 0.19 mJ/mm2) and a different number of pulses (5 to 500). Cell suspensions were centrifuged and the released ATP in the supernatants was measured using a bioluminescent ATP detection kit (Roche). Resuspended cells were used for Live/Dead staining (Invitrogen) and analysed by flow cytometry. Results: ATP release after shock wave treatment increased in a pulse- and energydependent manner. ATP release significantly increased already at a low number of pulses/energies compared to untreated controls. These data do not significantly correlate with the amount of destroyed cells, suggesting ATP is not only released due to cell deformation/ transient membrane permeabilisation during shock wave treatment. Further studies aim to identify mechanosensitive channels possibly involved in ATP release after shock wave treatment. To conclude, ATP release and feedback through certain purinergic receptors could be one of the underlying principles of the beneficial effects of shock wave treatment.

Topic: Molecular Signal Transduction

P 267 Attention in children - the necessity of guidelines for assessment and intervention of attentional disorders

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Attention is a common dysfunction following acquired brain injuries in pediatric patients. However, unclear and undifferentiated terms lead to irritation among patients and specialists and can inhibit effective intervention. The development of guidelines will provide standards for an optimal treatment to meet the child's needs in daily life. Attention will be described in a very detailed manner in order to facilitate learning about predominant problems and considerable indicators for correct description, categorization, diagnostic process, validity of test performance in relationship to daily life and practical implementations for treatment. 900 children and adolescents (including healthy controls and treatment groups) between 6 and 16 years are examined concerning their attentional abilities with respect to differentiated constructs using three levels of evaluation (neuropsychological test, standardized behavioral observation and external estimation through parental ratings).

Topic: Other

P 268 Three-dimensional optical coherence tomography for dermatologic in vivo tumor diagnosis.

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A preliminary clinical trial using optical coherence tomography (OCT) for three dimensional (3D) multi-modal in vivo imaging of pathologic skin lesions has been conducted. A total of 49 lesions from 27 patients were acquired, including basal cell carcinoma (26), squamous cell carcinoma (6), actinic keratosis (5), dysplastic nevi (4), seborrhoic keratosis (3), mycosis fungoides (2), lentigo maligna melanoma (1), merkel cell carcinoma (1) and Bowen`s disease (1). State-of-the-art OCT operating at a centre wavelength of 1300 nm was capable of acquiring 3D images depicting the layered architecture of skin with axial and transverse resolutions ~ 8 µm and ~ 20 µm, respectively, from a volume of 7 x 3.5 x 1.5 mm3 at a frame rate of 46 Hz. The clinical diagnostic potential of OCT in dermatological studies for pre-screening over relatively large areas of skin using 3D high resolution OCT to identify suspicious regions at microscopic level of the respective regions could be demonstrated by this study.

Topic: Dermatology

P 269 Fluid replacement with colloids and its impact on platelets, hemostasis and renal function

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Background: Colloids are frequently used solutions to perform fluid resuscitation. The discussion on the optimal management of IV fluid resuscitation perpetuates. Part of the concern is the effect of colloids on hemostasis, platelet function and renal function. But also allergic reactions are discussed. Little alterations are out of danger for healthy volunteers but can have a great impact on critically ill patients. The most frequently used colloidal plasma substitutes in Europe and US are gelatine, albumin and hydroxyethyl starch (HES) solutions. Objectives: The aim is to analyse the effects of HES 130 in a balanced solution to the two other commonly used colloids gelatine and albumin. The hypothesis is, that rapidly degradable HES solutions do not have more interaction on hemostasis than human albumin and gelatine. Another goal is to investigate, if HES 130 does not harm the renal function more than albumin or gelatine. Methods: HES 130 in a balanced solution, gelatine in saline and albumin in saline will be analysed regarding hemostasis, platelet function and renal function. 12 healthy volunteers are going to obtain in 3 times colloid infusion in different osmotic pressure adapted volumes after venesection of 6ml/kg. The different colloids will be given according to their different osmotic pressures. Blood will be taken from a cubital vein before, during and after the infusion. Impedance aggregometry and thrombelastometry will be performed to assess platelet function. Hematocrit, fibrinogen and pH will be analysed, too. Renal parameters NGAL, IL18, serum cystatin C and serum creatinine will be analysed. Originality and relevance: Comparisons between the three colloids in one trial are rare in literature. Most studies are performed in situations of critically ill patients, which makes it difficult to have a homogeneous group for comparison. Also compensatory mechanisms can be missing in critically ill patients.

Topic: Clinical Neurosciences

P 270 Is iron a key player in neurodegeneration?

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Multiple sclerosis (MS) is the most common demyelinating disease, affecting approximately 1 in 1000 people. MS is associated with inflammatory and degenerative processes. Mitochondrial damage and iron-mediated oxidative stress are thought to enhance the tissue injury. However, the exact mechanisms and key players are still not defined. The animal model for multiple sclerosis is called experimental autoimmune encephalomyelitis (EAE). To elicit EAE, myelin basic protein-specific T-cells are injected into rats, leading to inflammatory lesions in the central nervous system (CNS). In contrast to MS, EAE lesions show hardly any signs of neurodegeneration. We hypothesize that this is due to differences in iron-mediated tissue injury as the iron load in the rat CNS is much lower than in the human CNS. To investigate our assumption, we compared the expression profiles of iron homeostasis genes of MS lesions with those of EAE lesions. Iron homeostasis comprises several pathways, including import, storage, and export of iron. The expression profiles of iron importers differ between MS and EAE, suggesting the use of distinct import mechanisms. Additionally, ferric reductases and ferroxidases are essential for iron shuffling across membranes. Ceruloplasmin, for example, is highly upregulated in EAE. In MS lesions however, hephaestin and the amyloid precursor protein (APP) seem to be the predominantly expressed ferroxidases. Iron storage is mediated by light- and heavy-chain ferritin molecules, the former one being upregulated in EAE and the latter one being upregulated in MS. Ferroportin is the only known iron exporter and shows a downregulated expression in EAE, which cannot be seen in our MS data. Cumulatively, our data show that the expression profile of iron homeostasis genes differ between MS and EAE. They further support the hypothesis that iron can act as a key player in neurodegenerative processes.

Topic: Cell Communication in Health and Disease

P 271 Characterization of long-term survivors on subcutaneous treprostinil

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Background: Randomized controlled trials have resulted in improved outcomes in pulmonary arterial hypertension (PAH), however they are biased by stringent inclusion criteria, pre-specified patient subsets and study durations. We have prospectively collected patients in a database who were started on first-line s.c. treprostinil if they met the following inclusion criteria at diagnosis: NYHA functional class III or more, a right atrial pressure of ≥10mmHg and/or cardiac index ≤2.2L/min.m2. To understand the effect of treatment, we characterize patients with severe pulmonary hypertension at least 5 years on subcutaneous treprostinil. Patients and Methods: Treprostinil dose adjustments were driven by patients' symptoms and side effects. Results: Between June 1,1999 and January 31, 2012, 143 patients were included in the registry. Of those, 36 patients (25.2%) were at least 5 years on subcutaneous prostacyclin with a mean dose of 49ng/kg/min (19-125ng/kg/min) (Table 1). 13 patients (36.1%) were classified as idiopathic PAH, 13 patients (36.1%) as chronic thromboembolic pulmonary hypertension, 7 patients (19.4%) as PAH associated with congenital heart disease, 2 patients (5.6%) as PAH associated with connective tissue disease and 1 patient (2.8%) as porto-pulmonary hypertension. Mean age was 52 years (30-82). The majority of patients (29; 81%) were female. Three patients (8.3%) were hemodynamic responders at baseline. Treatment effects on exercise capacity, functional class and hemodynamics were still significant at five years over baseline. From five years on, Kaplan-Meier overall survival rates were 88%, 71%, 63% and 55%, at 7, 8, 9 and 10 years, respectively. Conclusion: Long-term survivors on first-line subcutaneous treprostinil are predominantly female, with severe baseline hemodynamic parameters. First-line treatment of severe pre-capillary pulmonary hypertension with subcutaneous treprostinil remains safe and efficacious over many years.

Topic: Cardiovascular and Pulmonary Disease

P 272 Biological activity of cytokines applied as ‚natural adjuvants' bound to Virus-like nanoparticles is critically influenced by their membrane-anchor characteristics

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Decoration of Virus-like nanoparticles (VNP) with functionally active, immunomodulatory molecules enhances particle immunogenicity and promotes selective immune recognition of the antigens they bear. In this study we sought to investigate the influence of membrane characteristics of artificially membrane-bound cytokines to improve the immunogenicity of VNP co-expressing viral antigens. One (1Ig), two (2Ig) or four (4Ig) immunoglobulin(Ig)-like domains of CD16b were inserted between the model cytokine IL-2 and the minimal GPI-anchor acceptor sequence of CD16b (GPI). We identified a membrane anchor – "2IgGPI" – conferring an up to tenfold increase of targeting of IL-2 into VNP, when compared to the minimal GPI-anchor acceptor sequence. This effect was particularly prominent in cases where additional molecules were targeted onto VNP and space seemed to be restricted. When co-expressed on VNP with H2-Db presenting the lymphocytic choriomeningitis virus glycoprotein(LCMV-GP)33-41 peptide, IL-2::2IgGPI was superior to IL-2::GPI to induce proliferation of primary LCMV-GP-specific P14 TCR transgenic T-cells in vitro, particularly when antigen was limited. Correspondingly, the proportion of IFNγ; producing, cytotoxic CD8+ T cells was significantly increased. Upon in vivo challenge of mice with antigen-specific VNP co-expressing IL-2::2IgGPI larger proportions of LCMV-specific T-cells proliferated when compared to IL-2::GPI. Corresponding loss-of-function variants of IL-2 (C92A) induced consistently less pronounced T-cell proliferation. The requirements for the optimal biological activity of artificially membrane-anchored cytokines decorating VNP used as convenient immunization platform cannot be predicted a priori but has to be evaluated in vitro and in vivo with scrutiny.
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Topic: Immunology

P 273 Persistence of allergen-specific IgE responses in HIV infected patients with low CD4+ cell counts

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Background: HIV-Infection leads to progressive reduction of CD4-positive T-cells, causing a less efficient immune response to pathogens and finally resulting in a failure of T-cell –dependent immunity. Objective: To investigate whether allergic HIV- positive patients with very low CD4-counts (≤200) continue to produce allergen-specific IgE antibodies. Methods Allergic and HIV-infected Zimbabwean patients with CD4+ T cell counts of >200 (n=67) and with ≤200 (n=27) were analysed. IgE-mediated allergy was diagnosed based on case history and positive skin prick test to allergens. Serological analysis was performed using the CLA® assay (containing a panel of 36 allergen extracts), nitrocellulose dotted with purified allergens and on the allergen chip (Thermo Fisher Scientific) containing a panel of 176 naturally purified and recombinant allergens. Allergen – specific IgE levels for seasonal allergens (Art v 1, Art v 3, Bet v 1, Cup a 1, Cyn d 1, Ole e 1, Phl p 1) were quantified by ImmunoCAP analysis in sera from patients were follow up sera were available obtained at different time points of the year. CD4 / CD8 counts were assessed by flow cytometry. Results: Predominant symptoms of allergy were IgE-mediated allergic rhinoconjunctivitis and urticaria, whereas T-cell mediated symptoms such as atopic dermatitis were not seen in allergic HIV-infected patients. Most patients showed IgE reactivity to allergen from the following sources in the chip: House dust mites, Bermuda grass pollen, olive tree pollen and timothy grass pollen. ImmunoCAP monitoring of seasonal allergens showed that IgE responses increased after allergen exposure. Conclusion Results indicate that an intact T-cell repertoire is not required for persistent allergen-specific IgE production.

Topic: Inflammation and Immunity

P 274 Interaction of the transmembrane serine protease testisin with the serpin Protein C Inhibitor (PCI)

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Protein C inhibitor (PCI) is a multi-specific protease inhibitor and a member of the serpin (serine protease inhibitor) family. Human PCI is expressed in various tissues and present in many body fluids. It inhibits a variety of proteases and seems to be involved in the regulation of important biological processes in- and outside vascular biology. Recently we could show that PCI also interacts with the transmembrane serine protease enteropeptidase. In rodents PCI is exclusively synthesized in the reproductive tract at high concentrations. Male PCI -/- mice are infertile. Those mice produce malformed sperm and their blood-testis barrier is disrupted. The observed changes might be explained by unopposed proteolytic activity in the absence of PCI, as well as altered retinoid signaling, since PCI is also a retinoic acid (RA)-binding protein. As PCI interacts with transmembrane proteases and is present at high concentrations in the reproductive organs, we are interested in the potential interaction of PCI with testisin. Testisin is a transmembrane serine protease expressed predominantly in testis, where it exhibits tryptase activity. Deficiency of testisin impairs sperm maturation in epididymis and fertilizing ability. Here we show that PCI is degraded by testisin and no complex formation is observed. Therefore PCI acts as substrate for testisin, leading to the generation of a variety of cleavage products, which might exhibit regulatory functions. The recognition sequence of testisin is not known. Interestingly the reactive center loop of PCI, which is normally recognized as potential substrate of target proteases, is not cleaved by testisin, but PCI is cleaved close to the N terminal end. The N-terminal A-helix of PCI is considered to contribute to RA binding. The exact role of PCI in RA-transport or RA-signaling is still unclear. Therefore our future plan is to investigate the potential effect of PCI cleavage by testisin in RA metabolism.

Topic: Vascular Biology

P 275 The influence of local cooling on capsaicin induced pain and epidermal nerve fibre density reduction

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Introduction: Topical high dose capsaicin is approved for the treatment of peripheral neuropathic pain. Pain reduction is associated with a reversible reduction of epidermal nerve fibres (ENF), which reappear within 12 weeks. Like heat (>43°C), acidosis, alcohol and exogenous and endogenous agonists capsaicin activates TRPV1-receptors and initiates depolarization of C-fibres and Aδ -fibres mediated by influx of sodium and calcium ions. This provokes a distinct burning sensation during the application of topical high dose capsaicin, which appears to correlate with a decrease in heat threshold. It should be tested if cooling down the skin by 5-10°C during application of capsaicin results in a reliable prevention of burning pain sensation without changing the reduction of ENF density. Methods: 4 quarters of a topical capsaicin 8% patch were applied for 60 minutes to both thighs of 12 healthy volunteers. After randomization one of both thighs was locally cooled during application. At distinct time points during exposure pain was ascertained for each exposure area on visual analogue scale. One week later skin biopsies were taken to compare ENF density to reference biopsies. Biopsies were immunostained with antibodies against Protein Gene Product (PGP) 9.5 and analysed according to the current diagnostic guidelines. Results: The evaluation of application pain of the 12 subjects showed significant lower values of more than 30% indicated for the cooled exposure areas compared to the non-cooled areas. The study could be finished without collecting further participants as previously designed. ENF density reduction after capsaicin exposure did not differ between the cooled and non-cooled areas. Conclusion: Local cooling markedly prevents application site pain caused by topical high dose capsaicin, probably by reducing skin temperature below the capsaicin induced decreased heat pain threshold. However cooling does not reduce the effect of capsaicin on reduction of ENF density.

Topic: Clinical Neurosciences

P 276 Hypertension in children is underdiagnosed. Can education help?

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Prevalence of hypertension in children is rising. Without adequate therapy hypertension leads to end organ damage and is related to a higher cardiovascular risk. Thus, diagnosing hypertension is crucially. Since there are often very few symptoms, blood pressure measurement must be part of the routine pediatric medical examination. But not only the right measurement, but especially the evaluation of measured blood pressure values is crucial. Pediatricians are forced to used blood pressure percentiles. According to literature, up to 74% of hypertensive blood pressure levels stay unnoticed and hypertension stays undiagnosed. Project: Using a questionnaire we want to evaluate whether pediatricians measure blood pressure in children and on which occasions. Furthermore, we want to learn whether the definition of hypertension in children is present amongst doctors, if percentiles for blood pressure values are used to identify hypertensive blood pressure and how these are managed. In a next step we want to assess whether educating pediatricians and offering web-based learning tools would result in less undiagnosed hypertensive children.

Topic: POeT - Programme for Organfailure, -replacement and Transplantation

P 277 Overproduction of Th-1 and Th-17 cytokines in the peripheral blood of psoriatic patients is not confined to the skin-homing T cell subset

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Current understanding of the pathogenesis of psoriasis implies that skin-infiltrating T helper 1 (Th1) and T helper 17 (Th17) cells are the pathogenic cell populations in psoriasis. However, essential questions concerning their modes of activation and how these cells are contributing to the major pathologic features of psoriasis have still to be elucidated. To address this issue, we stimulated different T cell subsets of psoriatic patients and analyzed their cytokine secretion profiles as well as their expression of skin-homing molecules. By comparing peripheral blood leukocytes from healthy and psoriatic individuals we observed only a slight increase in numbers of IFN-γ+ cells but significant elevations of IL-17+ and IL-22+ T cells in psoriatics with active and, less so, in those with low-grade disease. In addition, increased numbers of CLA+ (cutaneous lymphocyte antigen) cells in the Th1 and Th17 populations of psoriatics with active disease indicate a skin-tropism of these subpopulations. Surprisingly we found that the percentages of peripheral IL-17+ and IL-22+ cells were increased also in the CLA- memory T cell subset. Using migration assays we could show that CLA+ and CLA- memory T cell subsets isolated from psoriatic patients migrate better towards the chemokine CCL20 when compared with healthy controls. Our findings emphasize the relevance of Th17 cells in the pathogenesis of psoriasis and allude to the existence of a systemic inflammatory response in patients with severe disease.

Topic: Dermatology

P 278 Human allergen-specific T cell clones show high heterogeneity and plasticity within the Th2 lineage

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1. Background: The important roles of allergen-specific Th1, Th2 and Treg cells in IgE-mediated allergy have been well defined in the past. However, the involvement of Th17 cells in Type I allergy is not clear yet. 2. Aim: To investigate the existence of allergen-specific Th17 cells. 3. Methods: Supernatants of 40 allergen-reactive CD4+ clones expanded from peripheral blood of allergic patients were assessed for cytokine production after specific stimulation using the Luminex System. T cell receptors of the clones were sequenced. The expression of lineage-specific transcription factors of the clones was analyzed by qPCR and compared to in-vitro differentiated Th1, Th2 or Th17 cells. 4. Results: Nine of 40 allergen-specific clones produced IL-17 upon specific stimulation. Interestingly, these clones concomitantly synthesized substantial amounts of IL-4. In addition to IL-4, the clones also produced TNF-alpha, IL-6, IL-10 or IFN-gamma. Of note, two allergen-specific clones established from the same blood sample of an allergic individual under exactly the same culture conditions expressed identical T cell receptors implying that they derived from the same precursor cell. One of these clones produced high amounts of both, IL-4 and IL-17 whereas the other clone produced only low amounts of IL-4 and did not produce IL-17. Analysis of the expression of lineage-specific transcription factors confirmed that both clones belonged to the Th2-subset. 5. Conclusion: We found no evidence for allergen-specific Th17 cells causing IgE-mediated allergy. However, we identified a subset of allergen-specific IL-4+IL-17+ T cells and observed high heterogeneity and plasticity of allergen-specific Th2 clones.

Topic: Inflammation and Immunity

P 279 Lipid-protein interactions in cell membranes

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Detergent resistant membrane domains, often referred to as 'lipid rafts' are distinct regions within a cell membrane. These are described to be rich in cholesterol and sphingolipids and play a crucial role in particular in cell signaling. By establishing specialized platforms within a membrane that act as an isolation shell, the cell allows lateral association of some proteins while excluding others. Thereby ensuring directional and selective signaling. There exist so called lipid raft markers, proteins which show almost exclusive lipid raft association. Cytosolic proteins that preferably partition into lipid rafts contain lipid modifications, such as acylation, in order to insert into the inner leaflet of the membrane, e.g. the Src-family kinase Lck by palmitoylation. Surface molecules on the other hand such as CD48, CD55 or CD59 insert into the outer leaflet of the membrane via glycosylphosphatidylinositol (GPI) – anchors. GPI anchored proteins generally are said to be 'raftophilic', however some of them are located within rafts only to a minor extent. Hence it still remains unclear what exactly determines lipid raft association. We therefore aim to decipher whether the molecular determinants are enclosed within the protein domain or whether the GPI anchor itself or its anchoring site, etc. determines raft association. Since the T cell receptor complex is a well-studied raft-dependent signaling complex, our investigations focus on T cells. First we want to generate GPI-anchor swap mutants containing fluorescent proteins, which we further want to analyze by biochemical approaches, microscopy and single cell visualization (micropatterning). This work is supported by the Austrian Science Fund grant I00300 that is part of the LIPIDPROD project of the EuroMEMBRANE program of the European Science Foundation

Topic: Immunology

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