

**Luxembourg,
January 27th to 30th, 2010**

**New Conference Center Kirchberg
NCCK - Luxembourg**

***Inflammation 2010
Inflammatory cell signaling mechanisms as
therapeutic targets***

**Proceedings
and
Program**

**Editor
Marc Diederich**

**Organized by
Recherches Scientifiques Luxembourg**



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NCK - Luxembourg

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Inflammatory cell signaling mechanisms

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Editor

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Preface

In 1998, we organized the first specialized meeting in the field of signal transduction and gene expression in Luxembourg. This type of meeting was originally thought to teach doctoral students of the **molecular and cellular biology master training program** of the University of Nancy I (France).

Since then, more than **5000 fundamental, clinical and industrial researchers** were gathering in Luxembourg for eight different meetings in order to discuss therapeutic applications in the field of signal transduction, transcription and translation related to novel therapeutic applications. These meetings allow new insights into this rapidly moving field. Novel antibodies against receptors, protein kinase inhibitors, antisense oligonucleotides and siRNA targeting both signal transduction and gene expression will certainly enhance therapeutic approaches for the next century.

For the **2010** edition of meeting, with **more than 400 participants**, I am convinced that this meeting will be a great success.

Welcome to Luxembourg!

Marc Diederich

Acknowledgments

This meeting has been realized under the aegis of:

Recherches Scientifiques asbl

The Fondation de "Recherche Cancer et Sang"

Association An Haerz fir kriibskrank Kanner

Corena network

Redcat consortium

We have the pleasure to acknowledge support from:

Kuwait Petroleum SA - Luxembourg

The City of Luxembourg

The Fondation de Recherche "Cancer et Sang"

ENZO LIFE SCIENCES AG

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Canon Luxembourg SA

Your onsite organization team:

Liliane Hermes-Gotting, Marie-Anne Olinger, Nicolas Fangille, Jenny Ghelfi, Christina Grigorakaki, Cindy Grandjenette, Barbora Orlikova, François Gaascht, Franck Morceau, Estelle Henry, Marie-Hélène Teiten, Serge Eifes, Tom Juncker, Michael Schnekenburger, Marc Schumacher, Cyril Sobolewski, Claudia Cerella, Sébastien Chateauvieux, Noémie Legrand, Mareike Kelkel, Tommy Karius and Marc Diederich.

General Information

Meeting Venue

All meeting sessions are held at the **New Congress Center Kirchberg**

4, Place de l'Europe

L-1499 Luxembourg-Kirchberg

Onsite Phone: +352 4302 57567

Onsite Fax: + 352 4302 57568

This new building is hidden located behind the Philharmonie Concert Hall (left side) and is in fact integrated into the basis of the white Tower building (see map with photos for details).

Registration will take place at the registration desk open daily (9h00-19h00).

Coffee breaks will be served in the exhibit area daily (See general program for details).

Lunch

For the participants that pre-paid lunch, lunch is served from 13h00 - 15h00. Additional tickets are NOT available at the registration desk.

Exhibits are open daily from January 27th to 30th, 2010.

The Exhibit opens on Wednesday January 27th (Coffee break 10h30) and ends on Friday January 29st, 2010 after the afternoon coffee break.

Posters

All posters will be up from Wednesday January 27th until Friday January 29th:

Wednesday January 27th 14h00 – 16h00: All posters

Thursday January 28th 14h00 – 16h00: Posters with even numbers

Friday January 29th 14h00 – 16h00: Posters with odd numbers

On Friday January 29th 16h00, all poster presenters are requested to recover their posters (the boards will be taken away).

Welcome reception offered by the City of Luxembourg (expo area)

On Wednesday January 27, 2010 from 19h30-20h30 at the expo surface.

Gala Dinner

The Gala Dinner will take place at the Hôtel Le Royal 12, boulevard Royal L-2449 Luxembourg Tel: + 352 24 16 16-1, Fax: + 352 22 59 48 on Thursday, January 28th starting at 20h30. Additional tickets are NOT available at the registration desk.

Transportation

Our bus shuttles will bring the participants from the hotels to the meeting center in the morning and back to the hotels or city center in the evening. **Please note** that the busses leave from your hotel depending on the distance to the meeting center (Check timetable for details). The busses are red, orange and yellow and are from the bus company "Demy Cars".

Taxis can be called from the registration desk.

How to reach the congress center?

- By taxi:

from the airport (Luxembourg - Findel) in about 15 minutes.

from the railroad station (about 15 minutes)

- By bus: take bus line number 16 (every 20 minutes from the city center)

- By car:

From France: Highway A4 from Metz, take Highway A1 (E44) direction Trier Plateau de Kirchberg Aéroport, choose exit number "8", orient towards "Quartier European Sud Luxembourg-Centre"

From Belgium: Highway A411 from Brussels, take Highway A6 (E44) direction Trier Plateau de Kirchberg Aéroport, choose exit number "8", orient towards "Quartier European Sud Luxembourg Centre"

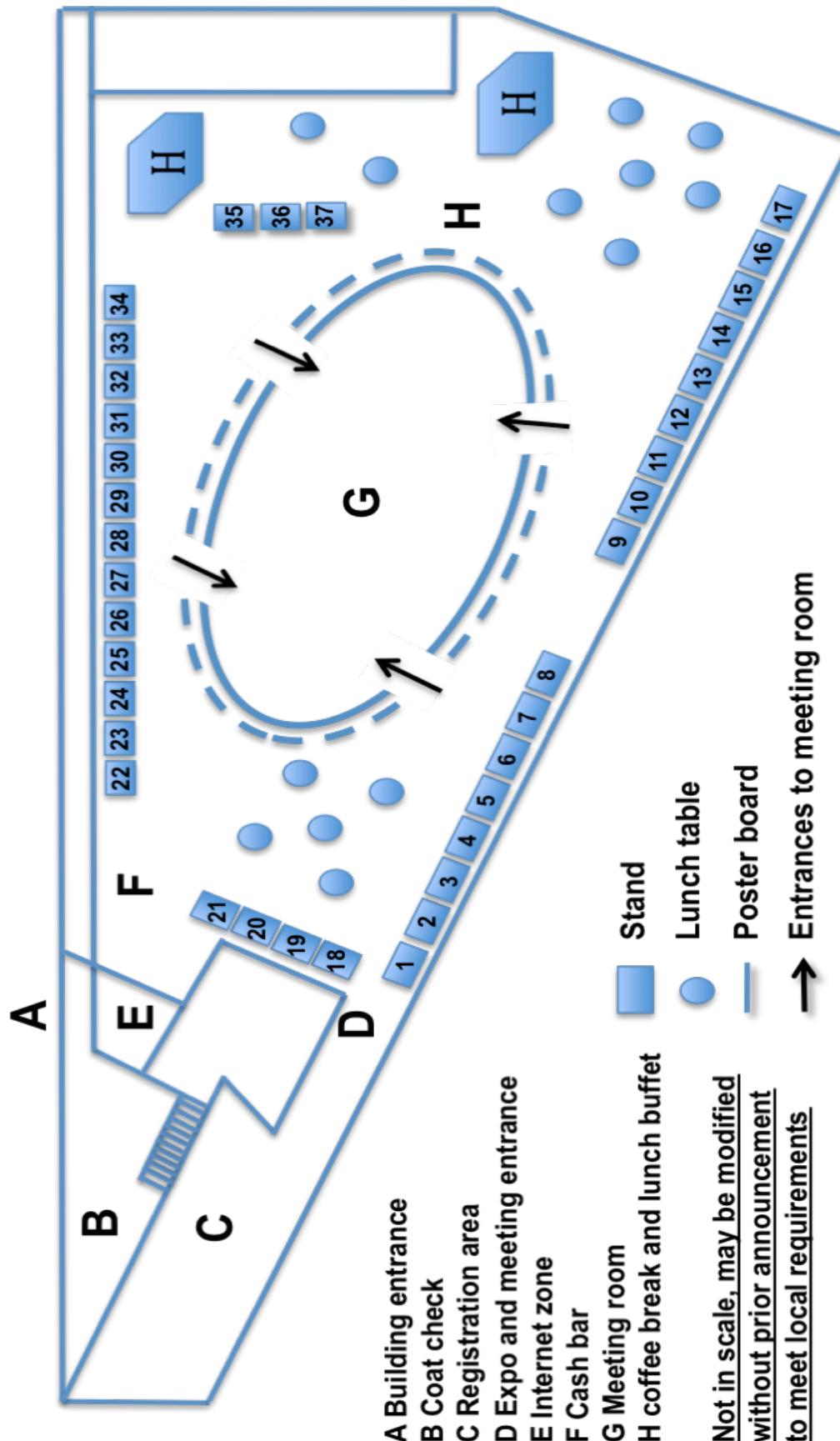
From Germany: Highway A6 (E44) from Trier, direction Luxembourg, choose exit number "8", orient towards "Quartier European Sud Luxembourg Centre".

Exhibition

- | | |
|----------------------|----------------------------------|
| 1. & 2. | BD Biosciences |
| 3. | Prophac |
| 4. | Merck Chemicals Ltd. |
| 5 & 6 | AXXORA Europe |
| 7. & 8. | Enzo Life Sciences AG |
| 9. | Promega Benelux |
| 10. | Portland Press Limited |
| 11. & 12. | Leica Microsystems |
| 13. | Polyplus-transfection |
| 14. | AMS Biotechnology |
| 15. | Sigma |
| 16. | InvivoGen |
| 17. | BIOKE / CST |
| 18. | Jackson ImmunoResearch |
| 19. | Active Motif |
| 20. | Olympus Belgium |
| 21. | GENTAUR |
| 22. | R&D Systems |
| 23. | Eurogentec |
| 24. | Bio-Connect |
| 25. | Bio-Rad |
| 26. | Promocell GmbH |
| 27. & 28. | GREINER BIO-ONE |
| 29. & 30. | PerkinElmer Bio-discovery |
| 31. & 32. | tebu-bio |
| 33. | Berthold |
| 34. | Millipore |
| 35. & 36. | VWR International |
| 37. | Westburg |

Many thanks to all exhibitors

Please visit our Expo !



Scientific Program

Inflammation 2010

Wednesday January 27th, 2010

8h00 – 19h00: Registration

Keynote session:

Chair: Marc Diederich (LBMCC, Luxembourg)

9h30 – 10h30: Jurg Tschopp (University of Lausanne, Lausanne, Switzerland): The inflammasomes: Guardians of our body

10h30 – 11h00: Coffee break

Session 1: Regulation of cell signaling pathways I

Chair: Young-Joon Surh (Seoul National University, Korea)

11h00 – 11h30: Young-Joon Surh (Seoul National University, Korea): Redox regulation of pro-inflammatory and anti-inflammatory signaling

11h30 – 12h00: Massimo Locati (Fondazione Humanitas per la Ricerca, Rozzano, Italy): Signaling and adaptive responses via atypical chemokine receptor D6

12h00 – 12h30: Matthias Gaestel (Hannover Medical School, Germany): The role of MAPKAP kinases MK2 and MK3 in inflammation and beyond

12h30 – 13h00: Thad Stappenbeck (Washington University, St Louis, USA): Role of epithelial growth factors in the maintenance of colonic homeostasis

13h00 – 16h00: Lunch, workshops and posters

Workshops:

14h00 – 15h00: Becton Dickinson

15h00 – 16h00: Promega

Posters:

14h00 – 16h00: Poster session
(free viewing)

Session 2: Regulation of cell signaling pathways II

Chair: Hye-Kyung Na (Sungshin Women's University, Seoul, Korea)

16h00 – 16h30: Véronique Baud (Institut Cochin-INSERM U567, Paris, France): Novel roles of the alternative NF-kappaB pathway

16h30 – 17h00: Rudi Beyaert (Ghent University - Department for Biomedical Molecular Biology, Belgium): The NF-kB inhibitor A20 as a peacekeeper in inflammation and immunity

17h00 – 17h30: Hye-Kyung Na (Sungshin Women's University, Seoul, Korea): Role of IkappaB kinase in 4-Hydroxyestradiol-induced migration and transformation of human mammary epithelial cells

17h30 – 18h00: Wouter de Jonge (Academic Medical Center, Amsterdam, The Netherlands): Acetylcholine modulates innate immune cell activation via Jak/Stat and NF-kB signaling pathways

18h00 – 18h20: Roberto Gambari (Ferrara University, Italy): Decoy PNA-DNA-PNA chimeras targeting NF-kB transcription factors inhibit IL-8 gene expression in cystic fibrosis IB3-1 infected with Pseudomonas aeruginosa

18h20 – 18h40: Emmanuel Dejardin (GIGA-Research, University of Liège, Belgium): TNFL-Induced p100 processing (TIPP) relies on the internalization of the cognate TNFR

18h40 - 19h00: **Hyeyoung Kim** (Yonsei University, Seoul, Korea): Effect of lipid-associated membrane proteins from *Mycoplasma pneumoniae* on IL-8 expression in glutamine-deficient human alveolar epithelial A549 cells

19h00 – 19h30: Official talks and inauguration

19h30 – 20h30: Reception offered by the City of Luxembourg

20h30 – 21h00: Shuttles leave to the hotels from the meeting center

Thursday January 28th, 2010

Shuttle bus from the hotels to the meeting center (see map for details)

8h00 – 19h00: Registration

Session 3: Inflammatory mediators

Chair: **Bharat B. Aggarwal** (M. D. Anderson Cancer Center, USA)

8h30 – 9h00: **Bharat B. Aggarwal** (M. D. Anderson Cancer Center, USA): Targeting Inflammatory Pathways for Prevention and Treatment of Cancer

9h00 – 9h30: **Sankar Ghosh** (Columbia University, New York, USA): Regulation of inflammatory signaling by TNF receptor

9h30 – 10h00: **Edward A. Dennis** (University of California, USA): TLR4 Initiated Phospholipase A2 Signaling via Pro- and Anti- Inflammatory Eicosanoids

10h00 – 10h30: **Zigang Dong** (Homel Institute, University of Minnesota, USA): Prevention of skin cancer: from basic research to clinical trials

10h30 – 11h00: Coffee break

Session 4: Epigenetic and transcriptional control in inflammatory diseases

Chair: **Jonathan Turner** (Institute of Immunology, Luxembourg)

11h00 – 11h30: **Ajay Goel** (Baylor University Medical Center, Dallas, USA): Epigenetic Changes by Dietary Agents

11h30 – 12h00: **Guy Haegeman** (University of Ghent, Belgium): Impact of chromatin on inflammatory gene expression

12h00 – 12h20: **Jonathan Turner** (Institute of Immunology, Luxembourg): Methylation of the (anti-inflammatory) glucocorticoid receptor promoter

12h20 – 12h40: **Oliver H. Krämer** (Friedrich-Schiller-University Jena, Germany): Crosstalk between stimulated NF-κB and the tumor suppressor p53

12h40 – 13h00: **Alexander Remels** (Maastricht University, the Netherlands): TNF-α impairs regulation of skeletal muscle oxidative capacity through activation of NF-κB

13h00 – 15h00: Lunch, Workshops and Posters

Workshops:

13h00 – 14h00: IBA

14h00 – 15h00: Polyplus-Transfection

15h00 – 16h00: AMS Biotechnology

Posters:

14h00 – 16h00: Poster session

(All posters with even numbers: 2, 4, 6)

Session 5: Virus infections and innate immunity
Chair: Jacques Piette (Université de Liège, Belgium)

16h00 – 16h30: **Jacques Piette** (Université de Liège, Belgium): Varicella-zoster virus interferes with innate immune signaling pathways

16h30 – 17h00: **Andrew Bowie** (Trinity College Dublin, Ireland): Novel regulators of pattern recognition receptor signaling

17h00 – 17h30: **Christian Münz** (University of Zürich, Switzerland): Priming of protective and tumor specific T cell responses in mice with human immune system component

17h30 – 18h00: **Johannes Bode** (University of Düsseldorf, Germany): Role of the interplay of MK2 and MK3 for IRF3 dependent effects of LPS

18h00 – 18h30: **Yong Sang Song** (Seoul National University, Korea): Human papillomavirus 16 E5 oncoprotein as an inducer of inflammatory cell signaling

18h30 – 19h00: **Stephen J. Galli** (Stanford University, USA): Mast cells as negative regulators of innate and adaptive immune responses

19h00: Shuttles leave to the hotels from the meeting center

Friday January 29th, 2010

Shuttle bus from the hotels to the meeting center (see map for details)

8h00 – 19h00: Registration

Session 6: Chronic Inflammatory pathologies – Part I

Chair: Kapil Mehta (M.D. Anderson Cancer Center, Houston, USA)

8h30 – 9h00: **Seth Masters** (National Institutes of Arthritis and Musculoskeletal and Skin Diseases, USA): New additions and mechanistic insight into the spectrum of auto-inflammatory disease

9h00 – 9h30: **Decio L. Eizirik** (Université Libre de Bruxelles, Belgium): Signal transduction of inflammation-mediated beta-cell apoptosis in type 1 diabetes

9h30 – 10h00: **Sushovan Guha** (M.D. Anderson Cancer Center, Houston, USA): G protein-coupled receptor mediated signaling pathways in pancreatic cancer

10h00 – 10h30: **Kapil Mehta** (M.D. Anderson Cancer Center, Houston, USA): Tissue transglutaminase (TG2): a proinflammatory protein promotes cell survival and invasive signaling in cancer cells

10h30 – 11h00: Coffee break

Session 7: Chronic Inflammatory pathologies – Part II

Chair: Seth Masters (National Institutes of Arthritis and Musculoskeletal and Skin Diseases, USA)

11h00 – 11h30: **Sunil Krishnan** (M.D. Anderson Cancer Center, Houston, USA): Targeting Inflammatory Pathways for Radiosensitization of Cancer

- 11h30 – 11h50:** **Franck Morceau** (LBMCC, Luxembourg): TNF alpha and anemia
- 11h50 – 12h10:** **Małgorzata Rogalińska** (Medical University of Vienna, Austria): Usefulness of differential scanning calorimetry for evaluation of treatment efficacy and development of personalized therapy of chronic lymphocytic leukemia
- 12h10 – 12h30:** **Jenny E. Gumperz** (University of Wisconsin School of Medicine and Public Health, USA): Recognition of Lyso-phosphatidylcholine by Human Natural Killer T Lymphocytes
- 12h30 – 12h50:** **George Hajishengallis** (University of Louisville, USA): Complement pathways involved in periodontal inflammation
- 12h50 – 13h10:** **Salahaddin Mahmudi-Azer** (University of Calgary, Alberta, Canada): Redefining Eosinophil Crystalloid Granules as a Potential New Functional Unit in Extracellular Inflammatory Events
- 13h10 – 13h30:** **Alfonso Pompella** (Dept. of Experimental Pathology, University of Pisa, Italy): A safe procedure® for measurement of S-nitrosoglutathione, the central metabolite in S-nitrosothiols formation and bioactivity

13h30 – 16h00: Lunch, workshops and posters

Workshops:	Posters:
14h00 – 15h00: Bio-Rad	14h00 – 16h00: Poster session
15h00 – 16h00: GE Healthcare	(All posters with odd numbers: 1, 3, 5)

Session 8: Regulation of cell signaling pathways III

Chair: Ivana Scovassi (IGM-CNR, Pavia, Italy)

- 16h00 – 16h30:** **Varsha Gandhi** (M.D. Anderson Cancer Center, Houston, TX, USA): Targeting Bcl-2 Family Survival Proteins
- 16h30 – 17h00:** **Peter Friedl** (Radboud University Nijmegen Medical Centre, The Netherlands): Killing of cancer cells by CTL
- 17h00 – 17h30:** **Ivana Scovassi** (IGM-CNR, Pavia, Italy): PARP inhibitors: new tools to protect from inflammation
- 17h30 – 17h50:** **Claudia Cerella** (LBMCC, Luxembourg): Anti-apoptotic effect of COX2 inhibitors
- 17h50 – 18h10:** **Alicia Torriglia** (INSERM U 872, Paris, France): Glucocorticoids induce cell death in the retina
- 18h10 – 18h30:** **Béatrice Charreau** (Université de Nantes, France): Inflammation Dysregulates Notch Signaling in Endothelial Cells: Implication of Notch2 and Notch4 to endothelial dysfunction
- 18h30 – 18h50:** **Thomas Luft** (German Cancer Research Center, Heidelberg, Germany): The second signal in antigen-presenting cells: Complementary JAK1 directs pro- and anti-inflammatory cytokine production induced by CD40L
- 18h50 – 19h10:** **Iris Behrmann** (Life Sciences Research Unit, University of Luxembourg): Hypoxia-inducible factor 1a is upregulated by Oncostatin M and participates in Oncostatin M signalling

19h15: Shuttles leave to the hotels from the meeting center

Saturday January 30th, 2010

Shuttle bus from the hotels to the meeting center (see map for details)

8h00 – 13h00: Registration

Session 9: Natural compounds and inflammation cell signalling I

Chair: Norbert Latruffe (University of Burgundy, France)

8h30 – 9h00: **Lina Ghibelli** (University of Rome Tor Vergata, Italy): Novel signaling pathways induced by melatonin in leukocytes: inflammatory implications and pharmacological perspectives.

9h00 – 9h30: **De-Xing Hou** (Kagoshima University, Japan): Flavonoids as anti-inflammatory compounds: targeting signaling and molecules.

9h30 - 9h50: **Norbert Latruffe** (INSERM U 866; University of Burgundy, France): Responses of cell targets to resveratrol; involvement of common or separated mechanisms?

9h50 – 10h10: **Francesco Peri** (University of Milano Bicocca, Italy): A chemical genetic approach to the study of the TLR4 pathway: new chemical entities targeting selectively the CD14 and MD-2 receptors

10h10 – 10h30: **Flavia Radogna** (University of Rome Tor Vergata, Italy): Interference between nitric oxide synthase and lipoxygenase pathways leads to reciprocal abrogation of the effects of melatonin and magnetic fields in leukocytes

10h30 – 11h00: Coffee break

Session 10: Natural compounds and inflammation cell signalling II

Chair: Józefa Węsierska-Gądek

11h00 – 11h20: **Assam El-Osta** (Baker IDI Heart and Diabetes Institute, Melbourne, Australia): The legacy of hyperglycemia is associated with persistent gene-activating epigenetic marks on the NFkB-p65 gene

11h20 – 11h40: **Marc Schumacher** (LBMCC, Luxembourg): Marine compounds as anti-inflammatory and anti-cancer agents

11h40 – 12h00: **Veera R. Konda** (KinDex Therapeutics, USA): Substituted 1,3-cyclopentadienes analogues differentially inhibit inflammation in vitro and collagen-induced arthritis in mice

12h00 – 12h20: **Józefa Węsierska-Gądek** (Medical University of Vienna, Austria): Impact of selective CDK inhibitors on functional status of cellular factors promoting survival of cancer cells

12h20 – 12h40: **Dietmar Fuchs** (University Clinics, Innsbruck, Austria): In vitro testing for anti-inflammatory properties of compounds employing peripheral blood mononuclear cells freshly isolated from healthy donors

12h40 – 13h00: **Stephan Immenschuh** (Hannover Medical School, Germany): Inhibition and genetic deficiency of p38 MAPK induces the anti-inflammatory heme oxygenase-1 gene via the redox-regulated transcription factor Nrf2 in macrophages

13h00 – 14h00: Lunch and end of the meeting

14h00: Shuttles leave to the hotels from the meeting center

**Oral presentations
(in chronological order)**

The inflammasomes: Guardians of our body?

Jurg Tschopp

Department of Biochemistry, University of Lausanne, Switzerland

The NOD-like receptors (NLR) are a family of intracellular sensors of microbial motifs and 'danger signals' that have emerged as being crucial components of the innate immune responses and inflammation. Several NLRs (NALPs and IPAF) form a caspase-1-activating multiprotein complex, termed inflammasome that processes proinflammatory cytokines including IL-1 β . Amongst the various inflammasomes, the NALP3 inflammasome is particularly qualified to sense a plethora of diverse molecules, ranging from bacterial muramyldipeptide to monosodium urate crystals. The important role of the NALP3 inflammasome is emphasized by the identification of mutations in the NALP3 gene that are associated with a susceptibility to inflammatory disorders. These and other issues related to the inflammasome will be presented.

Redox Regulation of Pro-inflammatory and Anti-inflammatory Signaling

Young-Joon Surh

WCU Department of Biopharmaceutical Sciences & Molecular Medicine and National Research Laboratory of Molecular Carcinogenesis & Chemoprevention, College of Pharmacy, Seoul National University, Seoul 151-742, Korea (surh@plaza.snu.ac.kr)

The implication of inflammatory cell/tissue damage in pathophysiology of human metabolic disorders is under intense investigation both at the research level and in clinical practice. Numerous studies have been reported with the global biochemical profiling technologies, such as DNA microarray, proteomics, metabolomics, lipidomics, etc., to identify and characterize a series of critical molecules/changes in the inflammatory signaling. It is by gaining this type of mechanistic understanding of a disease that researchers will unlock the keys to discovering new diagnostics and therapeutic strategies for the management of inflammation-associated metabolic disorders. One of the key molecules involved in mediating inflammation signaling is NF-κB. Accumulating data support the cross-talk between NF-κB and other redox-sensitive transcription factors. Nuclear factor E2-related factor-2 (Nrf2) plays a crucial role in regulating phase-2 detoxifying/antioxidant gene induction. This transcription factor is sequestered in the cytoplasm as an inactive complex with the inhibitory protein Keap1. Upon activation, Nrf2 binds to antioxidant responsive element (ARE) sites, leading to the coordinated up-regulation of down-stream target genes that boost cellular antioxidant potential. Recent studies have revealed that Nrf2 also has an anti-inflammatory function and hence represents an important therapeutic target for the inflammatory disorders. Many dietary phytonutrients can induce ARE-driven antioxidant/phase-2 detoxifying gene expression, thereby fortifying cellular defence against oxidative insult. Cysteine thiols present in various transcription factors and their regulators function as redox sensors in fine-tuning of transcriptional regulation of many genes essential for maintaining cellular homeostasis. Thus, oxidation or covalent modification of thiol groups present in the above redox-sensitive transcription factors and their regulating molecules can provide a unique strategy for molecular target-based chemoprevention and cytoprotection.

References

1. Kim, J., Cha, Y.-N. and Surh, Y.-J. A protective role of nuclear factor erythroid 2-related factor-2 (Nrf2) in inflammatory disorders. *Mutat. Res.*, in press.
2. Kundu, J.K. and Surh, Y.-J. (2008) Inflammation: Gearing the journey to cancer. *Mutat. Res.-Review*, 659: 15-30
3. Surh, Y.-J. and Na, H.-K. (2008) NF-kappaB and Nrf2 as prime molecular targets for chemoprevention and cytoprotection with anti-inflammatory and antioxidant phytochemicals. *Genes and Nutrition*, 2: 313-317.
4. Na, H.-K. and Surh, Y.-J. (2006) Transcriptional regulation via cysteine thiol modification: a novel molecular strategy for chemoprevention and cytoprotection. *Mol. Carcinog.*, 45: 368-380.

Signalling and adaptive responses via atypical chemokine receptor D6

Massimo Locati^{1,2}, Elena M Borroni^{1,2}, Benedetta Savino^{1,2}, Stefania Vetrano², Paul Proost³, Sofie Struyf³, Silvio Danese², Raffaella Bonecchi^{1,2}, Alberto Mantovani^{1,2}.

¹ **Dept. Translational Medicine, University of Milan, Italy;** ² **IRCCS Istituto Clinico Humanitas, Rozzano, Italy;** ³ **Lab. Molecular Immunology, Rega Institute, Leuven, Belgium.**

Chemokines play a major role in the induction of an appropriate immune response by coordinating leukocyte recruitment. The control of the chemokine system relies on several mechanisms, including chemokine processing by proteases and their degradation by chemokine decoy receptors. D6 is a chemokine decoy receptor which recognizes and degrades most inflammatory chemokines agonist at CCR1 to CCR5. CCL14 is a homeostatic chemokine present at high concentrations in the serum with a weak agonist activity on CCR1, converted by plasmin and UPA-mediated processing in the potent CCR1/CCR3/CCR5 agonist CCL14^(9–74) under inflammatory conditions. CCL14^(9–74) is further processed and inactivated by dipeptidyl peptidase IV/CD26 that generates CCL14^(11–74). D6 efficiently binds CCL14 and all its truncated isoforms, but only CCL14^(9–74) induces adaptive up-regulation of D6 expression on the cell membrane and is rapidly and efficiently degraded, while degradation of the biologically inactive isoforms CCL14^(1–74) and CCL14^(11–74) is very inefficient. Analysis of a panel of truncated CC chemokine isoforms revealed that D6-mediated chemokine degradation does not correlate with binding affinity, and is positively correlated with D6 adaptive up-regulation and that a proline residue in position 2 of D6 ligands is dispensable for binding but crucial for D6 adaptive up-regulation and efficient degradation. In the SDS model of experimental colitis, D6^{-/-} mice show significantly higher levels of several pro-inflammatory chemokines and are impaired in the ability to resolve colitis. By bone marrow chimeric mice showed that the contribution of D6 relies on the stromal/lymphatic compartment, with no involvement of D6 on hemopoietic cells. Finally, after administration of the carcinogen azoxymethane, D6^{-/-} mice show increased susceptibility to develop colitis-associated cancer in the distal segment of the colon. Thus, D6 requires an agonist-dependent adaptive up-regulation to efficiently degrade its ligands, cooperates with proteases in the control of inflammatory chemokines, and being expressed on lymphatic vessels plays a key role in the control of intestinal inflammation and inflammation-associated colon cancer.

The role of MAPKAP kinases MK2 and MK3 in inflammation and beyond

Matthias Gaestel

Institute of Biochemistry, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany, e-mail: gaestel.matthias@mh-hannover.de

Downstream of mitogen-activated protein kinases (MAPKs), three structurally related MAPK-activated protein kinases (MAPKAPKs or MKs) — MK2, MK3 and MK5 — signal to diverse cellular targets. Although there is no known common function for all three MKs, these kinases are involved in regulation of gene expression at the transcriptional and post-transcriptional level, control cytoskeletal architecture and cell-cycle progression, and are implicated in inflammation and cancer.

MK2 and MK3 are phosphorylated and activated by p38MAPK-alpha/beta and, in turn phosphorylate various substrates involved in diverse cellular processes. In addition to forwarding of the p38-signal by MK2/3, protein complex formation between MK2/3 and p38 mutually stabilizes these enzymes and affects p38 signaling in general. Among the substrates of MK2/3, there are mRNA-AU-rich-element (ARE)-binding proteins, such as tristetraprolin (TTP), which regulate mRNA-stability and translation in a phosphorylation-dependent manner. Phosphorylation by MK2 stabilizes TTP and ARE-mRNAs by their exclusion from a default degradation pathway. MK2/3 also contribute to the *de novo* synthesis of TTP and of further immediate early genes by stimulating SRF-dependent transcription. Apart from this, MK2/3 bind to polycomb repressive complex and are involved chromatin remodeling necessary for stem cell renewal.

Both p38 MAPK-alpha and MK2 are elements of TLR- and cytokine-signaling and are therefore preferential target molecules to treat chronic inflammation involved in asthma, rheumatoid arthritis, inflammatory bowel disease, atherosclerosis, Alzheimer's disease, ischemic heart and brain diseases as well as cancer by orally available small molecules. Inhibitors against p38 MAPK have been tested in animal models and in the clinics, block acute and chronic inflammation efficiently, but show side effects such as liver toxicity and skin rash which might result from "on target"-effects. Thus, targets downstream to p38 MAPK-alpha, such as MK2, become more interesting for anti-inflammatory therapy.

Growth factor-dependent control of *Ptgs2* expression in colonic mesenchymal stem cells

Monica R. Walker, Terrence Riehl, William Stenson, Thaddeus S. Stappenbeck

Washington University in St. Louis, St. Louis, MO, USA.

For colonic homeostasis, the mucosal immune system must be regulated to control improper response to a large and diverse population of indigenous microbes. This process requires multiple inhibitory pathways. We hypothesized that prostaglandin-endoperoxide synthase 2 (*Ptgs2*, also known as Cox-2) and its downstream products play a role in this process because of the well-recognized role of this pathway in the response to injury that occurs in a variety of systems. We previously found that a partially defined population of colonic stromal cells constitutively expressed high levels of *Ptgs2* and that the location of these cells was mediated by Myd88 signaling. We have now found that these colonic stromal cells were mesenchymal stem cells (MSCs). These colonic MSCs expressed high *Ptgs2* levels not through interaction with bacterial products, but instead as a consequence of mRNA stabilization downstream of fibroblast growth factor 9 (Fgf9), a growth factor that is constitutively expressed by the intestinal epithelium. This stabilization was mediated partially through a mechanism involving endogenous CUG binding protein 2 (CUGbp2). These studies suggest that Fgf9 is an important factor in the regulation of *Ptgs2* in colonic MSCs and may be a factor involved in its constitutive expression *in vivo*.

Novel roles of the alternative NF-kappaB pathway

Veronique Baud

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The NF-κB inhibitor A20 as a peacekeeper in inflammation and immunity

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NF-κB dependent gene expression plays a key role in immunity, and inappropriate NF-κB activity has been linked with many autoimmune and inflammatory diseases. Multiple mechanisms normally ensure the proper termination of NF-κB activation. In this context, the intracellular ubiquitin-editing protein A20 (also known as TNFAIP3) is a key player in the negative feedback regulation of NF-κB signaling in response to multiple stimuli. A20 also regulates tumor necrosis factor (TNF)-induced apoptosis. Recent genome wide association studies indicate human *A20/TNFAIP3* as a susceptibility locus for common inflammatory diseases such as Crohn's disease and rheumatoid arthritis, suggesting that A20 deficiency might contribute to the development and progression of human autoimmune and inflammatory diseases. Understanding the mechanism of action, regulation, and biological function of A20 in different tissues and cell types is therefore of great importance. A20 is believed to exert its NF-κB inhibitory function by acting as a de-ubiquitinating enzyme. We and others have shown that specific A20-binding proteins can target A20 to ubiquitinated substrates in the NF-κB signalling pathway. A20 can be further regulated by phosphorylation and MALT1 paracaspase mediated cleavage. To understand the physiological function of A20 in specific cell types we have also generated multiple cell and tissue specific A20 knockout mice. Recent in vitro and in vivo experiments illustrating the mechanism of action of A20 and its role in different immunopathologies will be presented during the meeting.

Role of IkappaB Kinase in 4-Hydroxyestradiol-induced Migration and Transformation of Human Mammary Epithelial Cells

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There are multiple lines of evidence suggesting estrogen as a prime risk factor for the development of human breast cancer. Estrogen is converted by cytochrome P450 1B1 to 4-hydroxyestradiol (4-OHE₂), a putative carcinogenic metabolite of estrogen. This catechol estrogen metabolite is oxidized further to produce a reactive quinone via semiquinone. Redox-cycling between 4-OHE₂ and its quinoid generates reactive oxygen species (ROS). ROS not only cause oxidative DNA damage but also promote neoplastic transformation of initiated cells. Therefore, we hypothesized that 4-OHE₂ can induce neoplastic transformation in human mammary epithelial (MCF10A) cells via ROS generation which may activate the NF- κ B signaling pathway. 4-OHE₂ induced anchorage-independent colony formation in MCF-10A cells. MCF-10A cells treated with 4-OHE₂ exhibited increased accumulation of intracellular ROS. The antioxidant *N*-acetyl-L-cysteine (NAC) inhibited the neoplastic transformation induced by 4-OHE₂. ROS overproduced by 4-OHE₂ increased the nuclear translocation of NF- κ B and its DNA binding through induction of I κ B kinase- α (IKK α) and IKK β activities. The inhibition of the IKK activities with Bay 11-7082 significantly reduced the anchorage-independent growth induced by 4-OHE₂. In addition to blocking the colony formation induced by 4-OHE₂, Bay 11-7082 suppressed migration of MCF-10A cells in a dose dependent manner. The 4-OHE₂-induced activation of ERK and Akt resulted in enhanced IKK activities and phosphorylation of I κ B α , thereby inducing NF- κ B activation as well as the anchorage-independent growth of MCF-10A cells. In conclusion, ROS concomitantly overproduced during redox cycling of 4-OHE₂ activates IKK signaling which may contribute to neoplastic transformation of MCF-10A cells.

Acetylcholine modulates innate immune cell activation via Jak/Stat and NF- κ B signaling pathways

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Inflammatory cell activation and resolution of inflammation is tightly regulated by comprehensive anti-inflammatory mechanisms that can be harnessed for the treatment of infectious and inflammatory disorders. Previous studies indicate that the vagal nerve, involved in control of heart rate, hormone secretion, and gastrointestinal motility, is also an immunomodulator. In experimental models of inflammatory diseases, vagal nerve stimulation attenuates the production of proinflammatory cytokines and inhibits the inflammatory process in several models of inflammatory disease, such as sepsis, ileus, pancreatitis, and colitis. Acetylcholine, the principal neurotransmitter of the vagal nerve, controls immune cell functions via activation of nicotinic acetylcholine receptors that are expressed on several myeloid immune cell types such as tissue macrophages and dendritic cells. Acetylcholine released by efferent vagal signaling has been shown to modulate Jak-Stat3 and 4 signaling and down regulate macrophage NF- κ B activation and inflammatory cytokine production. In addition, acetylcholine inhibits dendritic cell APC function. From a pharmacological perspective, nicotinic agonists are more efficient than acetylcholine at inhibiting inflammatory signaling and the production of proinflammatory cytokines. This “nicotinic anti-inflammatory pathway” may have clinical implications as treatment with nicotinic agonists can modulate the production of proinflammatory cytokines from immune cells. I will review the recent advances in cholinergic modulation of cellular immune cell activation and function and the signaling pathways involved. These data support the design of more specific receptor-selective nicotinic agonists that can incline anti-inflammatory effects while eluding neurotoxicity.

Decoy PNA-DNA-PNA chimeras targeting NF-kB transcription factors inhibit IL-8 gene expression in cystic fibrosis IB3-1 infected with *Pseudomonas aeruginosa*

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There is a growing interest in developing therapies against CF in order to reduce the excessive inflammatory response in the airways of CF patients. This inflammatory process is characterized by production and release of cytokines and chemokines, among which interleukin 8 (IL-8) represent one of the most important. Since transcription factor NF-kB plays a critical role in IL-8 expression, the transcription factor decoy (TFD) strategy might be of interest. TFD is based on biomolecules mimicking the target sites of transcription factors (TFs) and able to interfere with TF activity when delivered to target cells. In this respect PNA-DNA-PNA chimeras, based on peptide-nucleic acids (PNAs), are molecules of great interest for several points of view: (a) unlike PNAs, they can be complexed with liposomes and microspheres; (b) unlike ODNs, they are resistant to DNases, serum and cytoplasmic extracts; (c) unlike PNA/PNA and PNA/DNA hybrids, they are potent decoy molecules. By using electrophoretic mobility shift assay and RT-PCR analysis we have demonstrated that the PDP/PDP chimera is a strong inhibitor of IL-8 gene expression in *P.aeruginosa* infected IB3-1 cells even in the absence of protection with lipofectamine. Moreover, unlike ODN-based decoys, PDP/PDP chimeras are fully resistant to serum and cytoplasmic extracts. This information is of great impact for the development of stable decoy molecules to be used in non-viral gene therapy as inhibitors of IL-8 pro-inflammatory activity.

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TNFL-Induced p100 processing (TIPP) relies on the internalization of the cognate TNFR

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Several members of the TNFR family can activate distinct NF-kappaB complexes through the classical and the alternative pathway. However, how a single receptor engages both pathways is still poorly understood. In this study, we further characterized the mechanism by which TNFR induce the activation of the alternative NF-kappaB pathway. We undertook to identify the cytosolic region of LTbetaR that mediates the activation of p100 processing. We identified a region encompassing amino acid 345 to 359, which does not display any known structural features. However, cell imaging and biochemical studies revealed that this region played a dual role by controlling LTbetaR trafficking and TRAF recruitment. Using si RNA and pharmacological approaches we demonstrated that LTbetaR-induced p100 processing relied on an internalization route that is dynamin and microtubules dependent but clathrin independent. We also identified a new atypical TRAF binding site involved in the recruitment of TRAF-2 and -3. This region allows to alleviating the inhibitory function of the complex TRAF2/3-c-IAP1/2 associated to NIK. In vivo, we found that the maturation of mesenteric stromal VCAM-1^{low} ICAM-1^{low} MadCAM-1- cells into VCAM-1^{high} ICAM-1^{high} MadCAM-1+ organizer cells correlated to an activation of RelB and a down-regulation of cell surface LTbetaR. The dynamin-dependent internalization of TNFR is a conserved mechanism required for the induction of the alternative pathway since dynasore prevented Lymphotoxin-, CD40L-, Tweak- and BAFF-induced p100 processing while the classical NF-kappaB was unaffected. Thus, our data shed light on a new biological function of intracellular trafficking for building an adapted NF-kappaB-mediated immune response.

Effect of lipid-associated membrane proteins from *Mycoplasma pneumoniae* on IL-8 expression in glutamine-deficient human alveolar epithelial A549 cells

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Mycoplasma pneumoniae is one of the most frequent causes of respiratory disease. Lipid-associated membrane proteins (LAMP) from *M. pneumoniae* activated inflammatory signalings through toll-like receptor 2 in human monocytic THP-1 cells. Glutamine is an essential amino acid during inflammatory conditions and its deficiency occurs during the periods of critical illness. Interleukin-8 (IL-8) is a neutrophil chemotactic and activating peptide and contributes to the pulmonary inflammation. IL-8 expression is mediated with Janus kinase (JAK)/Signal Transducers of Activated Transcription (STAT) pathway in various cells. The present study aims to investigate whether LAMP induces IL-8 expression with the activation of JAK/STAT, and whether glutamine deficiency aggravates LAMP-induced IL-8 induction in the human alveolar epithelial A549 cells. As a result, LAMP increased IL-8 expression in A549 cells in a time-dependent manner, which was augmented in the absence of glutamine. The activation of JAK2/STAT3 was observed in LAMP-stimulated cells. The effect of LAMP on JAK/STAT activation was potentiated in the cells cultured in the absence of glutamine. Glutamine supplementation inhibited JAK/STAT activation in the cells treated with LAMP and cultured in the absence of glutamine. The JAK2 inhibitor, AG490 inhibited LAMP-induced activation of JAK/STAT3 and IL-8 expression in A549 cells. In conclusion, LAMP of *M. pneumoniae* induced IL-8 expression mediated by JAK2/STAT3 activation in A549 cells. Glutamine deficiency augments LAMP-induced signaling for IL-8 expression in A549 cells. Glutamine supplementation may be beneficial for preventing and/or development of pulmonary inflammation.

Targeting Inflammatory Pathways for Prevention and Treatment of Cancer

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Chronic infections, obesity, alcohol, tobacco, radiation, environmental pollutants, and high-calorie diet have been recognized as major risk factors for the most common types of cancers. All these risk factors are linked to cancer through inflammation. While acute inflammation that persists for short-term mediates host defense against infections, chronic inflammation that lasts for long-term can predispose the host to various chronic illnesses, including cancer. Linkage between cancer and inflammation is indicated by numerous lines of evidence; first, transcription factors NF- κ B and STAT3, two major pathways for inflammation, are activated by most cancer risk factors; second, an inflammatory condition precedes most cancers; third, NF- κ B and STAT3 are constitutively active in most cancers; fourth, hypoxia and acidic conditions found in solid tumors activate NF- κ B; fifth, chemotherapeutic agents and gamma irradiation activate NF- κ B and lead to chemoresistance and radioresistance; sixth, most gene products linked to inflammation, survival, proliferation, invasion, angiogenesis, and metastasis are regulated by NF- κ B and STAT3; seventh, suppression of NF- κ B and STAT3 inhibits the proliferation and invasion of tumors; and eighth, most chemopreventive agents mediate their effects through inhibition of NF- κ B and STAT3 activation pathways. Thus suppression of these proinflammatory pathways may provide opportunities for both prevention and treatment of cancer. We will discuss the potential of nutraceuticals derived from spices and from traditional Indian and Chinese medicine in suppression of inflammatory pathways and their role in prevention and therapy of cancer.

Regulation of inflammatory signaling by TNF receptor

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Tumor necrosis factor receptor 1 (TNFR1) has the capacity to activate both cellular survival and death pathways. Forced overexpression of TNFR1 induces cell death and it has been unclear how signaling capability is maintained while preventing spontaneous apoptosis. We have found that TNFR1 is subject to rapid ongoing turnover thereby tightly regulating receptor protein levels. Constitutive trafficking of TNFR1 leads to lysosomal degradation through a mechanism that is distinct from ligand-induced receptor downregulation. Ligand-engagement however leads to shuttling of the receptor to a signaling endosome that provides a platform for recruitment and assembly of signaling complexes. Therefore TNF receptor 1 can switch between two fates, ligand-independent constitutive degradation, or ligand-dependent receptor downregulation that accompanies signal-transduction.

TLR4 Initiated Phospholipase A₂ Signaling and Eicosanoid Lipidomics

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As part of the LIPID MAPS Consortium (www.lipidmaps.com) [Schmelzer *et al.* (2007) *Lipidomics Methods in Enzymology*, **432**, 169-181], we have developed a robust and comprehensive approach to lipidomic analysis [Raetz *et al.* (2006) *J. Lipid Research*, **47**, 1097-111] of hundreds of fatty acids and eicosanoids, including their numerous metabolites arising from an array of cyclooxygenases, lipoxygenases, cytochrome P450s and non-enzymatic oxidation producing isoprostanes, as well as combinations thereof [Buczynski *et al.* (2009) *J. Lipid Res.* **50**, 1015-1038]. The LC/MS approach to eicosanoid analysis [Deems *et al.* (2007) *Lipidomics Methods in Enzymology*, **432**, 59-82] and GC/MS approach to fatty acid analysis [Quehenberger *et al.* (2008) *Prostaglandins Leukotrienes and Essential Fatty Acids*, **79**, 123-129] have been used to discover novel lipids such as the family of dihomoprostaglandins [Harkewicz *et al.* (2007) *J. Biol. Chem.*, **282**, 2899-2910]. We can now apply these techniques generally in the context of an overall omics analysis of macrophages [Dennis,E.A. (2009) *Proc.Natl.Acad.Sci.U.S.A.*, **106**, 2089-2090] integrating transcriptomics, proteomics, and metabolomics of lipid metabolites. We will discuss the application of lipidomic analysis to characterize cellular lipid signaling in agonist stimulated RAW264.7 murine macrophages and thioglycolate elicited and bone marrow derived primary macrophages which activates phospholipase A₂ [Burke,J. and Dennis E.A., (2009) *J Lipid Res.* **50 Suppl**, S237-S242]. Numerous eicosanoids produced through COX and 5-LO were detected either intracellularly or in the media following stimulation with various agonists including Toll-like receptors (TLR), G protein-coupled receptors, purinergic receptors and combinations thereof. Synergy between Ca²⁺ release and TLR pathways was detected and discovered to be independent of NF-kB induced protein synthesis using lipidomics analysis and help to explain macrophage priming and synergy [Buczynski *et al.* (2007) *J. Biol. Chem.*, **282**, 22834-22847]. New data describing novel synergy between TLR4 and purinergic receptor signaling in primary macrophages will be described. We will also illustrate the application of lipidomics analysis to the analysis of lipid signaling in tissues with an example of joint tissues from mice suffering Lyme Disease caused by *Borrelia burgdorferi* [Blaho *et al.* (2009) *J. Biol. Chem.*, **284**, 21599-21612] in which a comprehensive lipidomics analysis of the metabolites produced demonstrates the profile of both pro-inflammatory and anti-inflammatory (resolving) signaling lipid molecules. New data on KO mice and pharmacological intervention demonstrating crosstalk between the COX and LOX cascade will be presented. Supported by LIPID MAPS Glue Grant U54 GM069338, R01 GM020501, and R01 GM064611 from NIH.

Prevention of skin cancer: from basic research to clinical trials

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Skin cancer is the most common cancer in the world. Incidence rates of skin cancer, especially non-melanoma skin cancer (NMSC) are increasing rapidly. The major etiological factor of human skin cancer is exposure to sunlight. We have carefully studied the solar ultraviolet A and B (UVA and UVB)-induced signal transduction pathways and their role in UV-induced skin carcinogenesis. We have found that CB1/2, protein kinase C, and EGFR are directly activated by UV irradiation. The absence of the CB1/2, protein kinase C, or EGFR result in decreased signals downstream of these receptors. More importantly, genetic removal of the CB1/2 receptors in mice results in a dramatic resistance to UVB-induced inflammation and a significant decrease in UVB-induced skin cancer. The transient receptor potential channel vanilloid subfamily 1 (TRPV1) is expressed highly in neuronal tissues, but is also expressed in the epidermis, dermal blood vessels, human keratinocytes and other tissues. The absence of TRPV1 in mice results in a striking increase in skin carcinogenesis. UV light strongly activates many protein kinases, including the MAP kinases JNK1/2, p38 and ERK1/2. JNK1 deficient mice develop more UV-induced tumors than JNK wildtype mice or JNK2 deficient mice. UVA or UVB induces activation of AP-1, p70S6 kinases and COX-2. Blocking these signals with small molecule inhibitors also decreased UV-induced skin carcinogenesis. By using these approaches, we have discovered many chemopreventive agents such as EGCG, myricetin, quercetin, [6]-gingerol, and resveratrol for preventing UV-induced skin carcinogenesis. In collaboration with Drs. David Alberts and Tim Bowden, we have continually put these chemoprevention agents into clinical trials. The agents act on specific molecular targets and pathways associated with skin carcinogenesis. Because most of these agents are natural compounds isolated from fruits and vegetables, and target- or pathway-specific, we believe that these agents can more effectively prevent human skin cancer with fewer side effects.

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Epigenetic changes by dietary agents

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Epigenetics is the study of the regulation of gene activity that is not dependent on nucleotide sequence; this may include heritable changes in gene activity and expression but also long-term alterations in the transcriptional potential of a cell that are not heritable. These features are potentially reversible and may affect genomic stability and expression of genes. Three types of epigenetic changes that we currently best understand include, alterations in the levels of DNA methylation, histone modifications and micro RNAs (miRNAs). In recent years, epigenetics researchers have made great strides in understanding the many molecular sequences and patterns that determine which genes can be turned on and off. This work has made it increasingly clear that in addition to genetic changes, the epigenome is just as critical as the DNA to healthy human development. Unlike irreversible genetic changes, epigenetic changes are thought to possibly be reversible by the environment, diet or pharmacological intervention and alter susceptibility to multiple diseases, including human cancers. However, very limited experimental evidence currently exists that addresses the molecular mechanisms involved in diet-induced epigenetic alterations in in-vitro or pre-clinical studies. Epigenetic events have also emerged as key mechanisms in cancer development, and a better understanding of these mechanisms is fundamental to our ability to successfully diagnose, treat, and prevent cancer. The growing interest in cancer epigenetics stems from several factors. First, epigenetic changes are implicated virtually at every step of tumor development and progression. Second, epigenetic changes including DNA hypermethylation are an early event in tumor development, and may precede development of the *genetic hallmarks of cancer*. Third, a key distinguishing feature of epigenetic changes in contrast to genetic changes is that these are reversible; therefore, aberrant DNA methylation, histone acetylation and methylation provide an exciting opportunity for the development of novel strategies for cancer prevention. Fourth, recent studies have recognized that a cross-talk exists between various epigenetic processes. It is now known that DNA methylation and histone modification processes may cooperate to regulate gene expression. Similarly, expression of miRNAs may be regulated by DNA methylation. Collectively, this means that dietary intervention of even a single epigenetic process is likely to influence other epigenetic processes in a positive manner. In light of this, there is a substantial interest in the development of safe and effective inhibitors of DNA methyl transferases (DNMT), an enzyme that catalyzes the transfer of methyl groups to cytosines at CpG sequences during DNA methylation-induced silencing of genes. Similar efforts are underway to develop histone deacetylase (HDAC) inhibitors that can prevent de-acetylation of lysine residues in histone tails that represent the ‘active marks’ for chromatin and unrestricted gene expression. Given the increasing evidence that epigenetic changes induced by environment, lifestyle and diet may play a major role in oncogenic transformation and cancer development, and it is suggested that over two-thirds of the cancer incidence can be accounted for by the environmental and dietary factors alone. Among all these factors, diet is probably the most important environmental factor which may influence carcinogenesis because diet is readily modifiable and have the potential to modulate multiple epigenetic processes. Polyphenols in food plants are a versatile group of phytochemicals with many potentially beneficial activities in terms of disease prevention. Dietary polyphenols (bioflavonoids) have antioxidant and anti-inflammatory properties that might explain their beneficial effects. However, the actual therapeutic potential of these compounds may not have been completely realized due to lack of understanding on the effects of these agents on epigenetic modifications. Recent, but limited evidence indicates that some of the polyphenols, including curcumin (from turmeric), genestein (from soy), EGCG (from green tea), diallyl disulfide (from garlic), sulforaphane (from broccoli) and resveratrol (from grapes) may induce epigenetic changes in various cancer cell lines. In this presentation, some of these studies on the role of the dietary polyphenols on epigenetic alterations will be described, which will provide a strong scientific foundation for preclinical and human clinical intervention studies in future.

Impact of chromatin on inflammatory gene expression

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Interleukin-6 (IL6) is a pluri-potent cytokine, the expression of which needs to be tightly regulated in order to keep the organism in a balanced and homeostatic condition. Indeed, dysregulation of IL6 leads to a variety of affections, like chronic inflammation, autoimmune and cardiovascular diseases, cancer progression, osteoporosis, etc. The IL6 gene promoter contains a plethora of transcription factor-binding sites, among which NF- κ B is the most important factor for induction of the gene by inflammatory stimuli, like e.g. TNF. NF- κ B is in majority a cytoplasmic protein complex (composed of a p50 and a p65 subunit), which upon inflammatory stimulation migrates to the nucleus and occupies its position on a variety of gene promoters. In previous work, we have shown that, in addition to this cytoplasmic activation step, the transcriptional potency of NF- κ B is codetermined by the activated MAP kinase pathway, more particularly by the nuclear kinase MSK1, that phosphorylates the p65 subunit at Ser 276 (to generate a fully transcription-competent enhanceosome), as well as the Histon-3 tails at Ser 10 (which is the onset of chromatin relaxation). Not only the local (i.e. the gene-specific) induced chromatin relaxation, but also the entire nucleosomal arrangement along the chromatin fiber is determinative for the (cell-specific) levels of gene expression within a given cell type. Indeed, upon comparison of the IL6 gene promoter status in two different breast cancer cell lines, i.e. the low IL6-expressing cell type MCF-7 and the aggressive tumor cell line MDA-MB-231 that shows abundant IL6 expression, we found that, upon DNaseI digestion and restriction enzyme accessibility assays, high IL6 expression correlates with an increased number of hypersensitive sites, and a higher accessibility of transcription factors, nuclear cofactors and polymerase II to the IL6 gene promoter. Finally, we could ‘revert’ this open chromatin configuration and the complementary abundant IL6 expression by means of a natural compound, i.e. Withaferin A.

Methylation of the (anti-inflammatory) glucocorticoid receptor promoter

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Glucocorticoids (GC) are key endogenous anti-inflammatory mediators, acting through their associated receptor, the glucocorticoid receptor (GR, NR3C1). Tissue specific GC actions are dependent on local GR levels. GR levels are transcriptionally controlled through the highly variable 5' gene region. This region codes for 11 alternative untranslated first exons. Seven of these exons are located within a CpG island immediately upstream of exon 2. We have shown that the 7 regions we have proposed as promoters are active in a dual luciferase reporter gene system, and that patch promoter methylation completely silences them. Investigation of the 128 CpG dinucleotides contained within the 5' promoters known to be active in PBMCs in 26 donors revealed stochastic and individual methylation patterns, however, the majority of CpG dinucleotides were methylated at levels >25% in at least one donor. The majority of evolutionarily conserved, and proven active transcription factor binding sites within these promoters contain methylatable CpG sites, suggesting that methylation orchestrates alternative first exon usage, silencing, and controlling tissue specific expression. The heterogeneity of methylation observed may reflect epigenetic mechanisms of *GR* fine tuning, programmed by early life environment and events. Glucocorticoid levels are controlled by an autocrine loop, and hippocampal GR levels play a key role in controlling this loop, and it has previously been proposed that epigenetic regulation of hippocampal GR levels is important. In a series of 6 post mortem human brains, we were able to show that there is no detectable hippocampal methylation. This would suggest that epigenetic control of the GR is limited to peripheral glucocorticoid target tissues, rather than central tissues controlling GC levels.

Crosstalk between stimulated NF-κB and the tumor suppressor p53

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NF-κB and p53 critically determine cancer development and progression. Defining the crosstalk between these transcription factors can expand our knowledge on molecular mechanisms of tumorigenesis. We show that induction of replicational stress activates NF-κB p65 and triggers its interaction with p53 in the nucleus. Experiments with knock-out cells demonstrate that p65 and p53 are both required for enhanced NF-κB activity during S-phase checkpoint activation involving ATM and CHK1. Accordingly, the pro-inflammatory cytokine TNF-α also triggers formation of a transcriptionally active complex containing nuclear p65 and p53 on κB response elements. Albeit p53 appears not required for NF-κB activation in the cytosol, TNF-induced binding of p65 to chromatin and NF-κB-dependent gene expression remarkably require p53. Hence, p53 expression unexpectedly controls nuclear functions of NF-κB p65 activated by atypical and classical stimuli. Remarkably, data from gain- and loss-of function approaches argue that anti-apoptotic NF-κB p65 activity is constitutively evoked by a p53 hot-spot mutant frequently found in tumors. Our observations suggest explanations for the outstanding question why p53 mutations rather than p53 deletions arise in tumors of various origins.

TNF- α impairs regulation of skeletal muscle oxidative capacity through activation of NF- κ B

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Skeletal muscle structure and function are known to deteriorate in individuals with chronic diseases such as COPD. A plethora of abnormalities have been described including a fiber type shift from type I (oxidative) to type II (glycolytic) fibers and decreased activity of enzymes involved in substrate oxidation, illustrative of the loss of muscle oxidative capacity in this disorder. Other common denominators of this chronic disease are enhanced circulatory levels of inflammatory mediators (e.g. TNF- α) and loss of muscle mass. Inflammation, in particular the cytokine TNF- α , has been linked to muscle atrophy. The impact of local inflammation on muscle oxidative metabolism however is unknown. Therefore, we aimed to identify the impact of TNF- α -induced inflammatory signaling on muscle oxidative capacity and elucidate underlying mechanisms. An *in vitro* model of adult skeletal muscle revealed that cellular respiration rates are diminished upon chronic TNF- α stimulation in a NF- κ B dependent manner. Oxidative phosphorylation (OXPHOS) protein content, myosin heavy chain (MyHC)-I, but not -II, protein levels as well as mRNA transcript levels were also decreased under chronic inflammatory conditions. Moreover, chronic TNF- α stimulation resulted in decreased mRNA expression levels of major regulators of muscle oxidative capacity including peroxisome proliferator-activated receptor alpha (PPAR- α) and its co-activator molecule PGC-1 α as well as the mitochondrial transcription factor A (Tfam). Additionally, PPAR transcriptional activity levels as well as promoter activation of master regulators of mitochondrial biogenesis (Tfam and NRF-1) decreased dramatically in response to chronic TNF- α stimulation in a NF- κ B dependent manner. In conclusion, we have for the first time linked inflammatory signaling and muscle oxidative capacity and elucidated a cell signaling mechanism to explain this link.

Varicella-Zoster Virus interference with the NF-κB and IRF signalling pathways.

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The varicella zoster virus (VZV) is a human alpha-Herpesvirus responsible for two diseases. It causes varicella (chicken pox), establishes latency in sensory ganglia and may reactivate to cause herpes zoster (shingles) in the host. Although Herpesvirus immune evasion has been known for many years, the precise mechanisms by which VZV could interfere with two main signaling pathways, i.e. NF-κB and IRF have not been studied yet. The down-regulation of the ICAM-1 expression in the center of a varicella lesion despite the presence of pro-inflammatory cytokines such as TNF α was the first observation suggesting a possible interference with the NF-κB pathway. Indeed, ICAM-1 gene transcription is regulated via NF-κB and we have shown in vitro that in VZV- infected cell cultures ICAM-1 gene transcription induced by TNF α is inhibited. Analysis of NF-κB activation showed that despite the virus-induced nuclear translocation of p65, p52 and c-Rel, p50 did not translocate in response to TNF α . Unexpectedly, these nuclear subunits were unable to bind the proximal NF-κB site of the *icam-1* promoter, despite an increased acetylation of the promoter in response to TNF α . Interestingly, VZV induced nuclear accumulation of the NF-κB inhibitor p100 that could partly explain the inhibition of ICAM-1 gene transcription. VZV was also shown capable of interfering with IRF3 activation. In VZV-infected cells, the hyper-phosphorylated forms of IRF3 detected in the nucleus do not form active dimers, are not transcriptionally active and thereby do not lead to the release of interferon (IFN)- β in the supernatant. The hyper-phosphorylation of IRF3 is not occurring in its C-terminal part and is not involving the classical TKB-1/IKK ϵ complex. By using viral kinase-deficient mutants of VZV, an active dimeric form of IRF3 can be recovered in the nucleus of infected cells demonstrating that VZV-encoded viral kinases could participate to the viral interference with the IRF pathways. These results showed that by targeting NF-κB and IRF3 signalling pathways, VZV could obviously interfere with the innate immune response and likely gain several advantages for its replication.

Novel regulators of pattern recognition receptor signalling

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The innate immune system recognises viral infection by making use of pattern recognition receptors (PRRs), which initiate signalling cascades that control transcription factor activation. For example, Toll-Like Receptors (TLRs) and RIG-I-like receptors (RLRs) recognise various viral components and stimulate the activation of transcription factors such as NF κ B. Since PRR signalling pathways have a key role in controlling not only innate immunity and pathogen-induced inflammation but probably also sterile inflammation induced by endogenous danger signals, understanding how these signalling pathways operate is an important goal. Many viruses, having co-evolved with their host, encode proteins that block or subvert these inflammatory signalling cascades. Large DNA viruses, such as vaccinia virus (VACV), encode a particularly ample repertoire of immunomodulatory proteins. We have been investigating and exploiting the strategies used by VACV to inhibit and manipulate host PRR responses. This is important for a number of reasons. First, this is contributing to our understanding of viral pathogenesis. Second, understanding how viruses manipulate and inhibit PRR detection pathways has led to further insights into how these host pathways function. Third, some of the identified host targets of viral proteins might turn out to be new drug targets for treating a range of diseases. Finally, the viral proteins themselves, or derivatives of them, may have therapeutic use in suppressing inappropriate PRR signalling, since viral proteins have been optimised during evolution to optimally inhibit their targets, which is analogous to a naturally occurring drug development programme. We have identified both novel VACV inhibitors of PRR signalling, and also novel components of host innate immunity, which will be discussed.

Priming of protective T cell responses against virus-induced tumors in mice with human immune system components

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Many pathogens that cause human disease infect only humans. In order to identify the mechanisms of immune protection against these pathogens and also to evaluate promising vaccine candidates, a small animal model would be desirable. We demonstrate here that primary T cell responses in mice with reconstituted human immune system components control infection with the oncogenic and persistent Epstein Barr virus (EBV). These cytotoxic and IFN-gamma producing T cell responses were HLA restricted and specific for EBV derived peptides. In HLA-A2 transgenic animals and similar to human EBV carriers, T cell responses against lytic EBV antigens dominated over recognition of latent EBV antigens. T cell depletion resulted in elevated viral loads and emergence of EBV associated lymphoproliferative disease. Both loss of CD4⁺ and CD8⁺ T cells abolished immune control. Therefore this mouse model recapitulates features of symptomatic primary EBV infection, and generates T cell mediated immune control that resists oncogenic transformation.

Role of the interplay of MK2 and MK3 for IRF3 dependent effects of LPS

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Apart from being essential constituents of the innate immune response against virus type I interferons are also released from macrophages in response to lipopolysaccharide (LPS). This LPS-induced production of type I IFN has been proposed to mediate LPS-induced IL-10 release and subsequent IL-10-induced activation of signal-transducer and activator of transcription (STAT) 3 in macrophages. As a key molecule of IL-10 signaling STAT3 activation is considered to be crucial for negative regulation of pro-inflammatory mediator release in macrophages and neutrophils. In the present study evidence is provided that activation of the mitogen-activated protein kinase activated protein kinase (MK) 2 plays a key role for regulation of LPS-induced synthesis of IFN-β and IL-27 and is also essential for delayed activation of STAT3. Thereby, the data suggest that in macrophages LPS-induced STAT3 activation requires expression of type I IFN and, in the absence of MK2, does not implicitly depend on IL-10 release. Studies in macrophages deficient for MK2, MK3 or for both MK2 and MK3 further reveal that MK2 not directly mediates IFN-β gene expression but rather deters MK3 from impeding IRF3 expression as well as LPS-induced activation of IRF3 and nuclear translocation of the NF-κappaB subunit p65, which both are well known to be important for regulation of IFN-β and IL-27 gene expression. This is in contrast to the regulation of LPS-induced expression of IL-10 or IL-6 as demonstrated in this study or the recently reported regulation of TNFα biosynthesis and stabilization of p38MAPK protein levels, which indeed are shown to be MK2-dependent but were MK3 is demonstrated to cooperate with MK2. Using IFN-α receptor I deficient macrophages the present study further corroborates that type I IFNs are required for LPS-induced activation of STAT3 and further points to a key role of the p38MAPK/MK2 pathway and the interrelationship of MK2 and MK3 in orchestrating the inflammatory macrophage response and its termination. This is particularly since activation of p38MAPK also mediates up-regulation of suppressor of cytokine signalling (SOCS)3, an endogenous inhibitor of Jak/STAT-signalling that prevents STAT3 from being untimely activated by LPS-induced factors, that also mediate STAT3 activation, such as IL-6, which in contrast to IL-10-mediated STAT3 activation is known to be sensitive towards SOCS3. This p38MAPK-dependent regulation of LPS-induced SOCS3 expression also involves activation of MK2 as demonstrated in macrophages deficient for MK2 or MK3 and MK3, where LPS-induced SOCS3 expression is almost completely abrogated.

Human Papillomavirus 16 E5 Oncoprotein as an Inducer of Inflammatory Cell Signaling

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Over the past decade, a series of studies have suggested that the cyclooxygenase-2 (COX-2) plays an important role in carcinogenesis and cancer progression in various malignancies, including cervical cancer. Human papillomavirus (HPV) E6 and E7 oncoproteins, which play key roles in cervical carcinogenesis by modulating tumor suppressors, were recently demonstrated to stimulate COX-2 transcription. However, the effect of HPV E5 oncoprotein on COX-2 expression has not been clearly understood. First, we investigated the effect of HPV 16 E5 on COX-2 expression. Our results revealed that transfection of cells (HaCaT, C33A, HEK 293 cells) with HPV 16 E5 induced significantly higher expression of COX-2 mRNA/protein and PGE₂. This effect was mediated by the activation of EGFR-signaling pathway, which, in turn, increased COX-2 promoter activity with NF-kB and AP-1 as critical transcription factors. In addition, we further evaluated the effect of HPV 16 E5 on the expression of prostaglandin E₂ (PGE₂) receptors. E5-expressing C33A cervical cells showed significantly increased levels of EP4 mRNA and protein. Also, EP4 protein expression was increased in human cervical cancer tissues. We found that EGFR, COX-2, PGE₂, EP2 and EP4, protein kinase A (PKA), CREB and CRE are involved in the induction of EP4 receptor protein by HPV 16 E5. Activation of EP4 by E5 increased anchorage-independent colony formation and VEGF expression, both of which are required for tumor growth, angiogenesis and metastasis. These results suggest that HPV 16 E5-associated inflammatory signaling pathways, including NF-kB and EP4 receptor, may be alternative chemopreventive and therapeutic targets for cervical cancer in the light of recent side effects associated with the use of COX-2 inhibitors.

Mast cells as negative regulators of innate and adaptive immune responses

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Mast cells are most commonly thought of as cells which can markedly amplify the local or systemic manifestations of IgE-associated immune responses, such as in the context of anaphylaxis, atopic asthma or other allergic disorders. Mast cells also can enhance the features of many innate immune responses, such as those which eliminate certain bacterial pathogens. In settings such as these, mast cells can be regarded as positive regulators of innate or adaptive immune responses. Recently, it has become apparent that mast cells also can perform roles well beyond that of being an “amplifier” of inflammation in innate or adaptive immunity. One of these newly recognized functions is to diminish the toxicity and mortality associated with either high concentrations of endogenous peptides (e.g., endothelin-1 or neuropeptides) or exposure to the venom of certain poisonous snakes or the honey bee. In these settings, mast cells limit morbidity and death at least in part by providing proteases that degrade the endogenous peptides or components of the venom. Such innate functions of the mast cell may represent an ancient and still important function of this lineage. In addition, mast cells can limit the magnitude and/or promote the resolution of certain innate or acquired (or “adaptive”) immune responses by representing a source of interleukin-10 and other products which can mediate a potentially wide variety of anti-inflammatory or immunosuppressive effects. Such negative immunoregulatory functions can prevent these innate or adaptive immune responses from severely damaging the affected skin. Accordingly, a new picture of the function of mast cells is emerging — these cells have the potential to turn innate or adaptive immune responses off, as well as to turn them on. In this presentation, I will briefly describe mouse models used to analyse mast-cell function *in vivo* and to identify immunomodulatory roles for mast cells during specific immune responses. Based on this evidence, I will discuss three hypotheses: that the potential to perform negative, as well as positive, immunomodulatory functions is a basic property of the mast-cell lineage; that mast cells can enhance and/or later help to limit certain innate and acquired immune responses; and that the extent to which mast cells actually perform such positive or negative immunomodulatory functions during specific immune responses *in vivo* is highly dependent on the individual biological setting.

New additions and mechanistic insight into the spectrum of autoinflammatory disease

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Although the innate immune system is absolutely required to fight infection and maintain homeostasis, it can cause autoinflammatory disease when inappropriately activated by genetic mutation, or exogenous and endogenous components. Recently we discovered a new autoinflammatory disease caused by mutations affecting the IL-1 receptor antagonist (*IL-1RA*). Normally this cytokine blocks the action of IL-1, a potent pro-inflammatory signal. Patients without functional IL-1Ra suffer from potentially lethal systemic inflammation with significant skin and bone pathology. Fortunately recombinant IL-1Ra (anakinra) can be administered and resolves all disease manifestations. Anakinra has also been observed to ameliorate type 2 diabetes (T2D), suggesting that IL-1 is an important inflammatory mediator of this disease. While it has been long known that pancreatic beta cells rapidly die upon encountering even low levels of IL-1, the mechanism by which IL-1 is generated in T2D is not clear. We have now identified several endogenous factors that are associated with T2D and can regulate the Nlrp3 (Nalp3) inflammasome to activate and secrete IL-1b. A major role for the innate immune system during infection is to promote a successful adaptive immune response. Once this is established however, inhibitory signals need to be sent back to the innate immune cells so as to limit potentially damaging inflammation. Although T-cells can inhibit the inflammasome by direct cell contact with innate immune cells, the role of a dominant T-cell cytokine, IFNg, is highly controversial. We can now reconcile the conflicting data by showing that IFNg does inhibit IL-1b production, but that this is only transient. This leads us to establish a new mechanism by which IFNg provides an anti-inflammatory signal in several models of inflammatory disease that depend on IL-1 and Th17 cells. Therefore our results highlight the importance of IL-1 in chronic inflammatory pathologies, and new mechanisms by which its release can be inhibited by the adaptive immune system, or triggered in autoinflammatory disease.

Signal transduction of inflammation-mediated beta-cell apoptosis in type 1 diabetes

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Pancreatic beta cells die by apoptosis in early type 1 diabetes mellitus (T1DM). Recent observations by our group suggest that beta cell apoptosis depends on the parallel and/or sequential up- and down-regulation of hundreds of genes. By performing microarray analysis and detailed promoter studies in primary rat beta cells and in human islets exposed for different time points to the cytokines interleukin-1b (IL-1b), tumor necrosis factor-a (TNF-a) and interferon-g (IFN-g) we observed that beta cells respond to damage by triggering several genes involved in defense/repair and endoplasmic reticulum stress, by decreasing their most differentiated functions and their capacity for growth and regeneration, and by inducing expression of diverse cytokines and chemokines. Several of these effects of cytokines are regulated by the transcription factors NF-kB and STAT-1, and by blocking NF-kB or STAT-1 we prevented cytokine-induced beta cell death. Subsequent experiments, combining NF-kB or STAT-1 blocking and microarray analysis, suggested that both transcription factors function as “master switches” of respectively IL-1b and IFN-g effects on beta cells, controlling networks of transcription factors and effector genes that trigger apoptosis and inflammation. Other transcription factors, such as JunB, function as “protective regulators”, decreasing expression of pro-apoptotic genes and modulating endoplasmic reticulum stress and mitochondrial pathways of cell death. Downstream activation of beta cell death depends on mitochondrial pathways regulated, by a large extent, by DP5/Hrk and another Bcl-2 family member presently under study. By combining functional studies with microarray and proteomic analysis, performed with or without targeted perturbations of the system, it will be eventually possible to fully map the interacting networks of genes and proteins leading to beta-cell death and amplification of the immune assault.

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G protein-coupled receptor mediated signaling pathways in pancreatic cancer

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Gastrointestinal peptides including mammalian bombesin-like peptides, cholecystokinin, gastrin, and neuropeptides (NT) stimulate DNA synthesis and cell proliferation in cultured cells and are implicated as growth factors in a number of fundamental processes including development, inflammation, tissue regeneration, and neoplastic transformation. These agonists bind to G protein-coupled receptors (GPCRs) that promote G_q-mediated activation of β isoforms of phospholipase C to produce two second messengers: Inositol (1,4,5) trisphosphate {Ins (1, 4, 5) P₃} that mobilizes Ca²⁺ from internal stores, and diacylglycerol that activates the classic and new isoforms of the protein kinase C (PKC) family. PKCs play a critical part in transducing GPCR-induced signals into activation of protein kinase cascades. Protein kinase D (PKD), a serine/threonine protein kinase with distinct structural and enzymological properties, is activated by phosphorylation in living cells through a new PKC-dependent signal transduction pathway. GPCR agonists including NT induce a rapid and striking activation of PKD by PKC. These results indicate that PKD functions downstream from PKCs and identify a new phosphorylation cascade that is activated by gastrointestinal peptide agonists. The growth-promoting effects of neuropeptides and the elucidation of the signaling pathways that mediate their effects assume an added importance because these agonists and their receptors are increasingly implicated in sustaining the proliferation of clinically aggressive solid tumors including those from lung, pancreas, and colon. Neuropeptides and growth factors promote activation of PKD in multiple neoplasias including pancreatic cancer (PaCa). We showed that GPCRs stimulated PKD-dependent mitogenic signaling pathways in PaCa. PKD significantly induced resistance to CD95-dependent apoptosis in PaCa and promoted PaCa cell proliferation. PKD also plays a potential role in cancer cell invasion and motility. PKD is activated by VEGF in endothelial cells and is necessary for tumor-associated angiogenesis. PKD activation also initiates NF-κB signaling pathway, triggering cell survival responses. We will demonstrate for the first time *in vivo* efficacy of an orally administered small-molecule inhibitor of PKD with no observed toxicity in animal models of PaCa. This presentation will summarize PKD-dependent signaling pathways in PaCa both *in vitro* and *in vivo* with implications for novel therapeutic indications.

Tissue transglutaminase (TG2): a proinflammatory protein promotes cell survival and invasive signaling in cancer cells

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Drug resistance and metastasis pose major impediment in successful treatment of cancer. Therefore, identification of cancer-coded genes whose expression contributes to these phenotypes may yield immediate clinical benefits. *TGM2* is one such gene whose expression is upregulated in several cancer cell types.

TGM2 codes a functionally complex protein (tissue transglutaminase or TG2), which has both intracellular and extracellular functions. A positive correlation between chemoresistance and metastatic potential of certain cancers with TG2 expression has been demonstrated. TG2 expression has been shown to exert anti-apoptotic effects on cells while siRNA-mediated downregulation of TG2 or treatment with TG2 inhibitors sensitized these cells to drug-induced apoptosis. These results imply that aberrant expression of TG2 in cancer cells promotes drug resistance and metastatic phenotype.

Working on the mechanisms by which TG2 promotes drug resistance and metastasis, we found that TG2 expression is associated with constitutive activation of cell survival signaling in FAK/Akt axis. In addition, TG2 expression resulted in NF- κ B activation -the transcription factor known to regulate genes involved in cell survival and invasion.

Based on these results, we reasoned that interruption of TG2-mediated signaling could be therapeutically beneficial for treating chemoresistant and metastatic tumors. Indeed, downregulation of endogenous TG2 by liposomal-siRNA was effective in blocking the growth of orthotopically growing tumors. Notably, downregulation of TG2 by liposomal-siRNA significantly enhanced the therapeutic efficacy of drugs and inhibited metastatic spread of tumors to distant sites. Evidence using cancer cell lines, orthotopically growing tumors and genetically engineered mouse models will be discussed to support the biological and therapeutic significance of TG2 in drug resistant and metastatic tumors.

Key words – chemoresistance, metastasis, siRNA

Targeting Inflammatory Pathways for Radiosensitization of Cancer

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Although radiation therapy is an integral component of treatment of patients with many different types of cancer, inherent and/or acquired resistance to the cytotoxic effects of radiation therapy is increasingly recognized as a significant impediment to effective cancer treatment. Inherent resistance is mediated by constitutive expression of oncogenic, proliferative and anti-apoptotic proteins/pathways whereas inducible resistance refers to transient induction of proteins/pathways following exposure to radiation. We and others have reported that the transcription factor NF- κ B, a central molecular mediator of inflammatory signaling, is potently activated transiently and in a dose-dependent manner in cancer cells following radiation therapy. This inducible NF- κ B protects cells from subsequent fractions of radiation by transcriptionally regulating a wide spectrum of pro-survival genes regulating inflammation, anti-apoptosis, invasion and angiogenesis pathways. In colorectal cancer cells, we have targeted this radiation-inducible resistance mediated by NF- κ B using curcumin, a natural product derived from the dietary spice turmeric. Treatment of colorectal cancer cells *in vitro* and *in vivo* with radiation in the presence of curcumin inhibits inducible NF- κ B signaling and increases the efficacy of treatment by modulating proliferation, invasion, and metastasis. Not only is curcumin pharmacologically safe, it may also mitigate some of the side-effects of radiation. For instance, emerging evidence suggests that fatigue experienced during radiation therapy is mediated by activation of a proinflammatory cytokine network that includes IL-1 β , TNF- α and IL-6. Temporal patterns of increase in soluble TNF-R1 serum levels correlate with the development of treatment-related symptoms in rectal cancer patients. Also, treatment-related fatigue levels independently predict response to treatment suggesting a correlation between tumor burden and symptom burden. Although additional data is needed to conclusively link radiation-induced inflammation and proinflammatory cytokines to central nervous system signals that generate fatigue and symptom burden, much of the available evidence points to a prominent role played by proinflammatory cytokines in acute side-effects of radiation. Together with inhibition of radiation resistance in tumor cells, targeting inflammatory pathways may widen the therapeutic window of treatment by reducing side-effects. Lastly, patient survival and quality-of-life are influenced by radiation-induced chronic injury to normal tissues and vasculature. Uncontrolled and chronic radiation-induced inflammatory signaling and oxidative stress have been implicated in tissue atrophy, fibrosis, and necrosis. Anti-inflammatory therapies have the potential to prevent or mitigate radiation-induced chronic normal tissue injury.

Tumor Necrosis Factor-alpha triggers anemia by affecting GATA-1 activity

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Anemia of cancer and chronic inflammatory diseases is a frequent complication affecting quality of life. For cancer patients it represents a particularly bad prognostic. Low level of erythropoietin is considered as one of the causes of anemia in these pathologies. The deficiency in erythropoietin production results from pro-inflammatory cytokines effect. However, few data is available concerning molecular mechanisms involved in cytokine-mediated anemia. The pro-inflammatory cytokine tumor necrosis factor α (TNF α) was particularly linked to inflammation and cancer related anemia. Our studies revealed molecular mechanisms linked to the inhibition of erythroid differentiation by TNF α . We showed that the inhibition of Erythropoietin (Epo)-mediated differentiation by TNF α lead to a downregulation of hemoglobin synthesis and was correlated to a modulation of key erythroid transcription factors. Thus, a reverse of the transcription factor GATA-1/GATA-2 balance normally present during erythropoiesis, as well as a downregulation of the cofactor of GATA-1, friend of GATA-1 (FOG-1) was observed after TNF α treatment of K562, HEL and TF-1 cell lines. Moreover, we showed a reduction of GATA-1/FOG-1 interaction due to a reduced transcription of GATA-1 and a proteasome dependent FOG-1 degradation after TNF α treatment. These changes lead to an inhibition of erythroid gene expression including Epo receptor (EpoR), α - and γ -globin, Erythroid-associated factor (ERAF), hydroxymethylbilane synthetase (HMBS), and glycophorin A (GPA). Furthermore, the inhibitory effect of TNF α has been studied on CD34+ hematopoietic stem cells collected from cord blood. Cells were cultured and differentiated in the presence of hrEpo and experimental results confirmed that TNF α affects GATA-1 activity in these cells. Overall, these data contributed to a better understanding of pro-inflammatory cytokine-dependent anemia, by giving first hints about key erythroid transcription factor modulations after TNF α treatment.

Usefulness of differential scanning calorimetry for evaluation of treatment efficacy and development of personalized therapy of chronic lymphocytic leukemia

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Chronic lymphocytic leukemia (CLL) is the most frequent type of leukemia in Europe and North America among elderly people. The etiology of this disease is still not known. This heterogeneous disease characterized by a strong accumulation of mainly resting B lymphocytes in peripheral blood is caused by disruption of mechanisms controlling homeostasis between production and destruction of B-cells. The strong between patient's diversity in the disease progression, response to anticancer therapy, and outcome is a main difficulty in elaboration of efficacious treatment. Therefore, it is important to develop personalized therapeutic protocols based on the individual patient's specific traits and sensitivity to medication. For this purpose, we describe in this study a combination of a few techniques (flow cytometry, cell viability test, differential scanning calorimetry (DSC), and immunoblotting) useful for a rapid monitoring of the efficacy of treatment of leukemic cells. CLL samples were *ex vivo* exposed to purine analogs combined with mafosfamide i.e. CM (cladribine + mafosfamide), FM (fludarabine + mafosfamide), and additionally to R-roscoxitine, an inhibitor of cyclin-dependent kinases, with the objective of triggering apoptosis. The changes in chromatin complex induced upon treatments were detected by DSC, a simple thermal technique. A significant decrease or even loss of transition at $95\pm3^{\circ}\text{C}$ in thermal scans of nuclear fraction preparations occurred in drug-treated cells. The extent of thermal changes differed between three medications. Furthermore, weak or absence of changes in thermal profiles was observed in nuclei originating from CLL cells resistant to the treatment. Remarkably, the changes in thermal profiles coincided with a marked decrease of the number of viable cells, DNA fragmentation, and changes in cellular levels as well as functional status of apoptosis-related proteins (caspase-9, caspase-3, PARP-1, and Bcl-2). Our results evidence the advantage of DSC in the evaluation of the treatment efficacy and indicate that its application could facilitate the choice of the most effective therapy for individual patients under *ex vivo* conditions. Considering the fact that this method is non-invasive, it could be also applied for direct and rapid evaluation of the clinical response.

Recognition of Lyso-phosphatidylcholine by Human Natural Killer T Lymphocytes

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NKT cells are innate T lymphocytes that have been shown to contribute to anti-tumor responses, and to have potent immuno-regulatory properties. NKT cells can be specifically activated by CD1d-mediated presentation of certain foreign microbial lipids, but it is also clear that recognition of self antigens presented by CD1d molecules on myeloid-lineage cells is an important physiological route of NKT cell activation. However, the molecular identity of specific auto-antigens that stimulate human NKT cells has remained unclear. Here, we have analyzed the ability of human NKT cells to recognize lipid species that were identified as ligands bound to secreted human CD1d molecules. The most clearly antigenic species was lyso-phosphatidylcholine (LPC). LPC recognition was dose-dependent and required presentation by CD1d. Di-acylated phosphatidylcholine and lyso-phosphoglycerols differing in the chemistry of the head group stimulated only weak responses from human NKT cells, although lyso-sphingomyelin, which shares the choline head group of LPC, was also recognized. Thus, NKT cell responses appeared specific for cholinated lyso-phospholipids. Antigen presenting cells pulsed with LPC were capable of stimulating increased cytokine responses by NKT cell clones and by freshly isolated peripheral blood lymphocytes. Inhibition of phospholipase A₂ (PLA₂) enzymes associated with human peripheral blood monocytes resulted in markedly reduced activation of NKT cells, suggesting that PLA₂ activity provides a source of the LPC that is presented by CD1d molecules. These results suggests that NKT cells are attuned to highly conserved lipid signaling pathways that are fundamental to normal physiological processes and are markedly up-regulated during inflammation.

Complement pathways involved in periodontal inflammation

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Periodontal disease arises from excessive host inflammatory responses to subgingival pathogenic bacteria, such as *Porphyromonas gingivalis*. In addition to leading to inflammatory destruction of the tooth-supporting tissues, severe periodontitis exerts a systemic impact on health and the patients run increased risk for atherosclerotic heart disease and diabetes. The complement system plays a major role in host defense against pathogens but has also been implicated in inflammatory pathology. Our studies indicate that the interaction of *P. gingivalis* with complement and leukocytes leads to amplification of inflammatory responses (e.g., increased leukocyte oxidative burst and proinflammatory cytokines) but paradoxically also to inhibition of *P. gingivalis* killing by neutrophils and macrophages. The latter involves a *P. gingivalis*-instigated crosstalk between the complement C5a receptor (C5aR; CD88) and Toll-like receptor 2 which impairs nitric oxide-dependent killing of this pathogen, which is exquisitely resistant to the oxidative burst. Our studies with specific C5aR antagonists and comparative studies in wild-type and C5aR-deficient mice indicate that the pharmacological blockade of the C5aR can be beneficial in periodontitis through inhibition of destructive inflammation and by facilitating the killing of *P. gingivalis*.

Redefining Eosinophil Crystalloid Granules as a Potential New Functional Unit in Extracellular Inflammatory Events

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Eosinophils are major effector cells in allergic inflammatory response. They are known to synthesize, store, and release a wide range of pro-inflammatory mediators. Eosinophils contain different populations of mediator-storage organelles, including small secretory vesicles as well as crystalloid granules. In cytolysis, eosinophil cell membrane loses its integrity and crystalloid granules are released to extracellular space. Potential function of crystalloid granules in extracellular space as it relates to inflammatory events remains widely unknown. We hypothesized that eosinophil crystalloid granules are equipped to function independently in extracellular space. Our findings indicate that both DNA and RNA localize to human and rabbit eosinophil crystalloid granules and that RNA seems to be synthesized in intra-granular space further suggesting the presence of functional transcription machinery inside the granules. Furthermore, we show here that crystalloid granules express functional membrane receptors for a cytokine, IFN-gamma, as well as G protein-coupled membrane receptors for a chemokine, eotaxin. Our findings indicate that these receptors function by activating signal-transducing pathways within granules leading to mediator release from granules to extra-granular space in a cell free environment. Taken together our findings define a new potential role for eosinophil crystalloid granules as independent extracellular functional units in inflammatory events and may reveal a novel target in modulating the inflammatory events.

A SAFE PROCEDURE[®] FOR MEASUREMENT OF S-NITROSOGlutATHIONE, THE CENTRAL METABOLITE IN S-NITROSOTHIOLS FORMATION AND BIOACTIVITY

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S-Nitrosoglutathione (GSNO) plays an important role in the transport and metabolism of nitric oxide (NO), and seems to be involved in many patho-physiological processes. Research and evaluation of GSNO in human diseases has long been hindered by the lack of analytical procedures provided with adequate sensitivity, specificity and reproducibility. All three requirements are now satisfied at the highest level by our patented protocol. The procedure is based on the innovative, unprecedented concept of using the commercial enzyme gamma-glutamyltransferase (GGT) coupled to the fast decomposition of its product CysGlyNO (CGNO) by copper ion (ancillary reaction), which give oxidized CysGly and NO. NO then is reacted with 4,5-diaminofluorescein (DAF-2) giving the triazole derivate, detectable by spectrofluorimetry (ex = 480 nm, em = 515 nm) or in black microplates (ex = 485±15 nm, em = 535±25 nm). The limit of quantitation (LOQc) of GSNO in PBS is 20 nM, the precision (CV) 5.5% at 300 nM concentration level, and the dynamic linear range 5-300 nmol/L, depending on the DAF-2 concentration. The method is environmental-safe (no mercury), and achieves detection limits two orders of magnitude lower than direct UV detection. These features make it suitable for investigating GSNO pathophysiology *in vitro* and *in vivo*, in experimental conditions encompassing animal, microbiology as well as plant studies.

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Targeting Bcl-2 Family Survival Proteins

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It has been now recognized and established that balance between anti-and pro-apoptotic proteins result in survival or death of cancer cells. Additionally, several studies have demonstrated that most cancer cell types express high levels of anti-apoptotic proteins. Among the anti-apoptotic proteins, Bcl-2, Bcl-XL, Bcl-w, Mcl-1, and A1/Bfl-1 are the main members. The anti-apoptotic proteins are inhibitors of Bax/Bak, either by direct binding to Bax/Bak or by counteracting the effects of Bax/Bak on the mitochondria. Among other pro-apoptotic proteins are members with BH3-only proteins with pro-apoptotic function. Overexpression of anti-apoptotic family proteins is a common feature in cancer and has been established for leukemia such as chronic lymphocytic leukemia (CLL). In addition, microenvironment-mediated signaling further enhances expression of these proteins (1). Because anti-apoptotic proteins bind to pro-apoptotic proteins through BH3 domain, efforts have been made to disrupt this interaction resulting in liberation of pro-apoptotic proteins and induction of cell death. Collectively these drugs are known as Bcl-2 antagonists or BH3 mimetics. These small molecule Bcl-2 antagonists are a new class of agents and include old drugs such as gossypol (2), its analogs (3) and new agent such as ABT-737 (4, 5). Using chronic lymphocytic leukemia as a model system, we elucidate mechanism of action of these BH3-mimetics and their current and future role as cancer therapeutics.

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Killing of cancer cells by CTL

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Immunological control of progressive tumors activation and expansion of tumor specific cytotoxic T lymphocytes (CTL) followed by an efficient effector phase in the tumor microenvironment. We established a real-time 3D matrix-based model of CTL function that allows the observation of active migration, interaction, dissociation and serial killing of CTL with target cells over up to 24 hours. Individual CTL-target cell contacts were variable in duration (min to hours), comprised a median lag-phase of 90 min until apoptosis of the target cell and were followed by CTL detachment and sequential killing of several target cells. Using this model, we presently address whether factors present in the tumor microenvironment interfere or enhance of anti-tumor CTL response and serial killing. CXCL12, a chemokine known produced by tumor cells, enhanced CTL migration, shortened interaction times with target cells and reduced the killing efficiency on a per-contact-basis which lead to near-!

complete abrogation of target cell killing at low CTL to target cell ratio. This effect was reversed by a CXCR4 antagonist, suggesting enhancing serial killing by targeting the CXCR4/CCL12 axis may improve anti-tumor immunotherapy.

PARP inhibitors: new tools to protect from inflammation

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Poly(ADP-ribosylation) is a post-translational modification of proteins which plays a crucial role in basic cellular processes, including DNA repair and replication, transcription and cell death. The biochemical reaction of poly(ADP-ribosylation) consists of the conversion of β -NAD⁺ into ADP-ribose, and the further formation of polymers of variable length and structure bound to nuclear protein acceptors. Polymer synthesis and degradation are performed respectively by poly(ADP-ribose) polymerase (PARP) and poly(ADP-ribose) glycohydrolase (PARG) enzymes. Although poly(ADP-ribosylation) is considered as an emergency reaction to DNA damage, high levels of PARP activation cause NAD depletion and consequent necrosis, thus having a pathogenetic role in many diseases. As for chemical compounds able to inhibit poly(ADP-ribosylation), since the use of nicotinamide and benzamide, potent derivatives have been developed and new molecules have been tested. Pharmacological inhibition of PARP enzymes proved to reverse the noxious effects of ROS, thus exerting a protective role towards a number of pathological conditions. Remarkably, pharmacological inactivation of poly(ADP-ribosylation) represents a novel therapeutic strategy to limit inflammation.

Anti-apoptotic effects of COX-2 inhibitors

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The cyclooxygenases (COXs) are a family of enzymes catalysing the rate-limiting step in prostaglandin biosynthesis. COX-2, the inducible isoform up-regulated during inflammation, was found constitutively expressed in different adherent tumors, where a poor prognosis have been associated. Consequently, the use of preferential/selective COX-2 inhibitors, alone or in combination with traditional chemotherapeutic agents, is currently under investigation for different cancer models, with the purpose to improve the efficiency of anti-cancer therapies.

In this study, we analyzed a possible role of COX-2 in survival/apoptosis on hematological cancer models. We selected the COX-2-positive acute myeloid leukemia U937 vs. the low level COX-2 expressing chronic myeloid leukemia K562 and investigated the effects of the two specific COX-2 inhibitors nimesulide and NS-398. We show that COX-2 inhibitors strongly prevent apoptosis induced by a panel of chemotherapeutic agents in U937 but not in K562 cells. Similarly, the selective COX-2 inhibitor celecoxib affects apoptosis only in U937 cells. Apoptosis prevention correlates with an up-regulation of the multidrug resistance protein MRP-1 and an increased drug efflux, as revealed by rhodamine 1,2,3 and doxorubicin efflux assays. Concomitantly, the up-regulation of the most abundant isoform of glutathione-S-transferase (GST-PI) is detected upon the treatment with the COX-2 inhibitors. Altogether, this suggests the over-expression of chemoresistance-mediating proteins as mediators of different converging anti-apoptotic mechanisms. Accordingly, COX-2 inhibitors do not affect apoptosis induced by physiological stimuli, as FAS or TNF, that do not require cell internalization. Similarly, they have no effects on apoptosis induced by hydrogen peroxide, which is not targeted by multidrug resistance channels and is known to be unaffected by GST over-expression.

Our findings show a novel paradoxical anti-apoptotic effect of COX-2 inhibitors on hematopoietic COX-2-positive cancer cells. This recommends cautions in the use of anti-inflammatory agents as chemoadjuvant as well as in counteracting side adverse effects of chemotherapy during the treatment of such tumors.

Glucocorticoides induce cell death in the retina

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Glucocorticoids are commonly used in the treatment of ocular pathologies associated with inflammation, vascular leakage, vessels abnormalities, and ocular neovascularization. Intravitreous injections of Triamcinolone Acetonide (TA) are used for the treatment of macular oedema, resulting in a dramatic macular thickness reduction, inconsistently correlated with long-term functional recovery. For the treatment of choroidal neovascularizations associated with Age-related Macular Degeneration (AMD) for instance, TA decreases the vascular leakage on the short-term, although its long-term effect on visual acuity gain remains controversial. We showed that glucocorticoids induce a specific and dose-dependent reduction in retinal cell viability, affecting essentially cells from the pigmented epithelium (RPE) and Müller glial cells (RMG). Caspase-dependent or independent apoptosis pathways were not detected in vivo or in vitro but cytoplasmic vacuolization was observed in both circumstances. Further analysis indicated that these cells die by an alternative cell death pathway called paraptosis.

We also show, for the first time, that glucocorticoids exert direct toxic effect on endothelial cells through caspase-independent mechanisms. When used in animal models of neovascularization TA induces endothelial cell death and decreases the amount of neovessels. These results have important implications on the therapeutic potential and safety use of glucocorticoids in human eyes. They also show the importance of considering cells death pathways other than caspases in the evaluation of these items.

Inflammation Dysregulates Notch Signaling in Endothelial Cells: Implication of Notch2 and Notch4 to endothelial dysfunction

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Although implicated in vascular morphogenesis and remodeling, regulation of the Notch pathway in vascular endothelial cells (EC) upon inflammation remains unclear. Here, we show that TNF drives specific regulation of the Notch pathway in vascular EC and smooth muscle cells leading to an induction of Notch2 and Dll1 and a decrease in Notch3 and -4 expression. Using two readouts we demonstrated a basal Notch/CBF1 activity in quiescent EC that decreases in response to TNF. Changes in Notch pathway were both cytokine and cell type specific. Notch transcriptional regulation was selectively mediated by at least the NFkB, JNK and the PI3K pathways. Next, we investigated the regulatory crosstalk between TNF signaling and Notch receptors and activity in cultured ECs and in transplant arteriosclerosis (TA). Decrease in Notch4 was consistently observed in TA and in ECs in response to TNF, TGF β and IL10, pointing out a functional relevance of Notch signaling in inflammatory processes. Notch4 and Hes1 knockdown enhances VCAM-1 expression and promotes EC apoptosis indicating that Notch4 functions were mediated by Hes1. Silencing Notch4 or Hes1 also drastically inhibits repair of endothelial injury suggesting that Notch4 and basal Notch activity are required to maintain EC quiescence and for optimal survival and repair. Thus, impaired Notch4 expression caused by inflammation in cardiac allograft vessels promotes EC dysfunction and TA (1). Little is known about the role of Notch2 expressed on endothelium. Here we show that a major consequence of Notch2 activation in ECs is the induction of a caspase-dependent apoptosis. We established the direct contribution of Notch2 signaling in the transcriptional regulation of several pro- and anti-apoptotic molecules. Both forced Notch2NICD expression and Notch2 silencing demonstrate interplay between Notch2 signaling and survivin expression in the control of EC apoptosis. Thus, dysregulated Notch2 signaling by TNF sensitizes ECs to apoptosis and survivin acts as effector of Notch signaling (2). Overall, we show that, in ECs, TNF drives a phenotypic switch where Notch4 is replaced by Notch2. Functionally, Notch2 signaling favors cell death while Notch4 activation was shown to be protective. (1) Quillard T. et al. by *Arteriosclerosis, Thrombosis, and Vascular Biology* 2008, Dec;28(12):2258-65. (2) Quillard T., et al. *PLOS ONE* 2009 4(12): e8244.

The second signal in antigen-presenting cells: Complementary JAK1 directs pro- and anti-inflammatory cytokine production induced by CD40L

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Aberrant expression of CD40 ligand (CD40L) represents a strong endogenous danger signal associated with chronic inflammatory disease. However, CD40 activation alone is unable to induce interleukin (IL)-12p70 secretion, whereas IL-6, IL-12p40, TNF- α and IL-10 are produced. We demonstrate that cytokine-induced Janus kinase (JAK)-1 is unique in mediating a complementary pro-inflammatory signal for the production of IL-12p70 whilst at the same time inhibiting IL-10 secretion in human APCs.

CD40- and JAK/STAT signals recruit complementary pathways: CD40 signals involve RelA and cRel for the induction of IL-12p70 and RelA for IL-10. In contrast, RelB inhibited IL-12p70 production. JAK1 complemented CD40-induced NF- κ B signals by phosphorylating STAT-1 and inducing IRF-1 and IRF-8 expression. siRNA inhibition of any of these signalling molecules (JAK1, STAT1, IRF1 and IRF8) abrogated IL-12p70 production. JAK1-dependent inhibition of IL-10 transcription was mediated either by STAT-1 directly binding the IL-10 promoter or by IRF-8 that binds to inhibitory IFN- γ -activated sequence (GAS/ISRE)-sites of the IL-10 promoter.

Our results demonstrate that specific inhibition of the “second signal” JAK1 will shift the balance of CD40-induced cytokine panels from IL-12p70 towards IL-10 in human APCs.

Hypoxia-inducible factor 1a is upregulated by Oncostatin M and participates in Oncostatin M signalling

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The interleukin-6-type cytokine oncostatin M (OSM) acts via the Jak/STAT pathway as well as via activation of MAP kinases and is known to critically regulate processes like liver development and regeneration, hematopoiesis, and angiogenesis which are also determined by hypoxia with the hypoxia-inducible transcription factor-1a (HIF1a) as a key component. Here we show that treatment of hepatocytes and hepatoma cells with OSM leads to an increased protein level of HIF1a under normoxic and hypoxic conditions. Further, the OSM-dependent HIF1a increase is mediated via Jak/STAT3- and the MEK/Erk1/2-pathway. OSM-mediated HIF1a upregulation did not result from an increase in HIF1a protein stability but from increased transcription from the *HIF1a* gene. In addition, we show that the OSM-induced *HIF1a* gene transcription and the resulting enhanced HIF1a protein levels are important for the OSM-dependent VEGF- and PAI1-gene induction associated with a number of diseases. In conclusion, HIF1a levels significantly increase after treatment of hepatocytes and hepatoma cells with OSM and HIF1a contributes to OSM downstream signalling events, pointing at a crosstalk between cytokine and hypoxia signalling in processes like liver development and regeneration.

NOVEL SIGNALING PATHWAYS INDUCED BY MELATONIN IN LEUKOCYTES: INFLAMMATORY IMPLICATIONS AND PHARMACOLOGICAL PERSPECTIVES

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Melatonin, the neuro-hormone implicated in the control of sleep, has recently attracted much interest as a regulator of immune/inflammatory response, after the discovery that leukocytes possess the machinery required to produce and to respond to melatonin. Melatonin is a modified triptophane with potent biological activity, exerted by stimulation of specific plasma membrane (MT1/MT2) receptors, by lower affinity intracellular enzymatic targets (quinone reductase, calmodulin), or through its strong antioxidant ability. Intriguingly, melatonin also promotes intracellular ROS in a fast (<1min) and transient (up to 5-6 hrs) way on a set of normal or tumor leukocytes. ROS production is independent from MT1/MT2 receptor interaction. In the search for the mechanism responsible for the pro-radical effect, we found that melatonin-induced ROS are produced by lipoxygenase (LOX), which is activated in a rapid, strong and transient way. LOX activation is accompanied by strong liberation of AA; inhibition of Ca²⁺-independent, but not Ca²⁺-dependent, phospholipase A2 (PLA2), prevents both melatonin-induced arachidonic acid and ROS production, whereas LOX inhibition only prevents ROS, indicating that PLA2 is upstream with respect to LOX, as occurs in many signaling pathways. Chlorpromazine, an inhibitor of melatonin-calmodulin interaction, inhibits both ROS and arachidonic acid production, thus possibly placing calmodulin at the origin of a melatonin-induced pro-radical pathway. Interestingly, it is known that Ca²⁺-independent PLA2 binds to calmodulin: our results are compatible with PLA2 being liberated by melatonin from a steady-state calmodulin sequestration, thus initiating an arachidonate signal transduction. These results delineate a novel molecular pathway through which melatonin may participate to the inflammatory response.

Flavonoids as anti-inflammatory compounds: Targeting signaling and molecules

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Phytochemical flavonoids have shown anti-inflammatory activity *in vitro* and *in vivo*. One of the important mechanisms is an inhibition of eicosanoid generating enzymes including phospholipase A2, cyclooxygenases, and lipoxygenases, thereby reducing the concentrations of prostanoids and leukotrienes. Recent studies have shown that certain flavonoids, especially flavone derivatives, modulate proinflammatory gene expression such as cyclooxygenase-2, inducible nitric oxide synthase, and several pivotal cytokines. Flavonoids have been considered to be reasonable candidates for new anti-inflammatory drugs due to these action mechanisms and significant *in vivo* activity. Although there are many cellular signaling pathways modulating inflammation processes, protein kinases play crucial roles in the regulation of multiple cell signaling pathways and inflammation. The inhibition of protein kinases has emerged as important targets for inflammation prevention and therapy. Accumulated data revealed that flavonoids exert anti-inflammatory effects through acting at protein kinase signaling pathways, more than as conventional hydrogen-donating antioxidants. Recent studies show that flavonoids can directly bind to some protein kinases including Akt/protein kinase B (Akt/PKB), Janus kinase (JAK), mitogen-activated protein kinase kinase 1 (MEK1), phosphoinositide 3-kinase (PI3K), and then alter their phosphorylation state to regulate multiple cell signaling pathways in inflammation processes. In this representation, the interactions of flavonoids and protein kinases, especially their direct binding and molecular modeling, will be reported. Moreover, the binding sites, selectivity, and the impact on cellular signaling and anti-inflammation of flavonoid-protein kinase interactions will be discussed.

Responses of cell targets to resveratrol ; involvement of common or separated mechanisms ?

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Resveratrol is produced in huge amount in vine in response to biotic or a-biotic stresses by activating plant defense signaling pathway leading to an anti-oxidative protection and a blockade of fungi infection. Similarly, in human resveratrol interferes with many physiological functions and pathological processes like ageing, infection, inflammation, proliferation, neurological processes or circulation.. In front of this diversity of effects is there any common signaling pathway affected by resveratrol ? The current knowledge brings resveratrol to light as a direct free radical scavenger molecule, as an indirect anti-oxidant or pro-oxidant activating process of regenerating glutathione enzymes or as a ligand-activating receptor based on pharmacological mechanisms. While resveratrol inhibits NFKB transcription factor and consequently abolishes oxidative stress. In contrast it activates NRF2 by promoting its nucleus translocation leading to gene activation of defense enzymes. On the other hand resveratrol stimulates NO production a vasorelaxant molecule by activating NitroOxideSynthase. Linked to inflammation, resveratrol inhibits COX2 responsible of proinflammatory arachidonate derived molecule PGE2 production. It also inhibits secretion of IL6, IL8. Towards cell proliferation, resveratrol activates cell death receptor through their recruitment in lipid membrane microdomains while it phosphorylates CDK1. However, some resveratrol analogues stop mitosis by blocking tubulin at the cochin binding site. The presentation will present new data in order to clarify on the involvement of common or separated mechanisms in responses of cell to resveratrol. In connection to anti-oxidation, apoptosis and inflammation.

A chemical genetic approach to the study of the TLR4 pathway: new chemical entities targeting selectively the CD14 and MD-2 receptors.

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In this communication we present the discovery and the biological characterization of new chemical entities derived from natural sugars targeting selectively the extracellular receptors of the Toll-like Receptor 4 (TLR4) inflammatory pathway. The main focus of an NIH-founded collaboration between the Universities of Milano (Italy) and Iowa (USA) is to gain information on the molecular basis of the TLR4 pathway using small molecules capable to interact selectively with the extracellular receptors LBP, CD14, MD-2 and TLR4. Positively charged monosaccharides with lipid chains have been developed by our group, that inhibit lipopolysaccharide (LPS) and lipid A-induced cytokine production in innate immunity cells. These molecules are also active in vivo in contrasting septic shock and other syndromes, such as neuropathic pain, caused by TLR4 activation. Biochemical studies indicated that these monosaccharides inhibit the TLR4 pathway by selectively antagonizing the endotoxin binding to the CD14 receptor.

The discovery of new lipodisaccharides containing negatively charged sulfate groups, active as mild agonists of TLR4, will be also described. These molecules selectively target the MD-2 receptor and are promising leads for the development of non-toxic vaccine adjuvant.

All molecules presented, besides being hit or lead compounds for the development of anti-sepsis ad anti-inflammatory agents, and immunotherapeutics, are also unique tools for investigating the structural and functional biology of the TLR4 pathway with the chemical genetic approach.

Interference between nitric oxide synthase and lipoxygenase pathways leads to reciprocal abrogation of the effects of melatonin and magnetic fields in leukocytes

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Magnetic fields (MF), both as static or alternate (50Hz) fields, in the range of 0.6-66 mT, or 0.08-0.4mT, respectively, elicit deep effect in leukocytes, especially when activated, leading to a strong reduction of apoptosis due to stimulation of a non-capacitative Ca²⁺ entry through plasma membrane, which promotes a pro-survival pathway. We found that this pathway involve production of NO, and is abrogated in the presence of NO synthase (NOS) inhibitors. Melatonin, the neuro-hormone involved in sleep control, and that is recently attracting much interest as a controller of leukocyte behaviour and inflammation, also reduce apoptosis induced by a variety of stimuli in leukocytes, by a mechanism involving activation of 5-lipoxygenase (LOX), whose product 5-HETE mediates the inactivation of the pro-apoptotic protein Bax. Surprisingly, when melatonin and MF are applied together to leukocytes induced to apoptosis, their anti-apoptotic effects do not synergize or sum, but are abrogated. LOX inhibition recovers MF anti-apoptotic effect, whereas NOS inhibition recovers melatonin anti-apoptotic effect, suggesting that LOX and NOS act as reciprocal inhibitors, acting at a downstream step. Interference between melatonin and MF effects are reported, since it is known that MF inhibit melatonin synthesis; the reciprocal effect instead is reported here for the first time, and provide an interesting field of investigation aimed at avoiding risk of MF exposure. Interference between LOX and NOS may help to dissect fine tuned regulation of the inflammatory response in terms of timing and mechanism of leukocyte recruitment, function and demise.

The legacy of hyperglycemia is associated with persistent gene-activating epigenetic marks on the NFkB-p65 gene

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It is now clearly evident that metabolic memory, whereby diabetic complications continue to develop and progress even in individuals who have returned to improved glycemic control after a period of transient hyperglycemia, could have long lasting effects. We present primary findings that transient hyperglycemia causes profound transcriptional changes in aortic endothelial cells. We have specifically hypothesized that ambient hyperglycemia caused gene-activating events of the NFkB-p65 promoter that were mediated by changes in epigenetic information (1). In this follow-up study we have identified two histone specific writing and erasing enzymes involved in the underlying regulation of gene expression during transient hyperglycemia and subsequent return to normoglycemia. Experimental evidence indicates that previous hyperglycemia is associated with persistent upregulation of NFkB-p65 gene expression, which leads to NFkB activation, and upregulation of NFkB dependent proteins such as MCP-1, which are implicated in diabetes - associated vascular injury. Gene expression is associated with specific increases in H3K4m1 but not H3K4m2 and H3K4m3 on the promoter region of the NFkB-p65 gene (2). In human endothelial cells we show that the histone methyltransferase Set7 specifically mono-methylates H3K4 and is recruited as a gene co-activator. Furthermore, glucose induced changes in H3K4m1 are related to the recruitment of this specific H3K4 methylase. Knockdown of Set7 in endothelial cells prevented glucose induced p65 upregulation via its ability to inhibit H3K4m1. We hypothesize that these molecular events represent an integrated response of the epigenome that lead to changes in expression of genes and proteins that regulate critical roles in the development and progression of vascular complications in diabetes. It is postulated that further understanding of these glucose induced epigenetic events and the identification of key enzymes involved could transform our understanding of the pathways implicated in diabetic vascular injury providing new strategies to reduce the burden of diabetic complications.

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The inhibition of TNF α -induced NF- κ B activation by marine natural products

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email: m.jaspars@abdn.ac.uk**

The number of identified natural compounds from marine sources has progressively increased especially in the field of anticancer research. The over-expressed activation of NF- κ B is closely linked to cancer development and inflammatory diseases. In order to find new NF- κ B inhibitors, isolation, purification and characterization of marine compounds extracted of various sponges have been carried out. Bioactivity results show that isolated compounds had an apoptotic effect on chronic myelogenous leukemia cells, inhibited both TNF α -induced NF- κ B-DNA binding as well as TNF α -induced I κ B α degradation and nuclear translocation of p50/p65. Interestingly, natural products inhibited IKK β kinase as well as the 26S proteasome proteolytic activity. The biochemical effect of astaxanthin, chalcones, cembratrienes, diterpenes, heteronemin, jasplakinolide, flavolactones, naphthopyrones and stellettin will be elucidated in this presentation.[1-3]

- [1] Schumacher M, Cerella C, Eifes S, Chateauvieux S, Morceau F, Jaspars M, et al. Heteronemin, a spongean sesterterpene, inhibits TNFalpha-induced NF-kappaB activation through proteasome inhibition and induces apoptotic cell death. *Biochem Pharmacol* 2010;79:610-22.
- [2] Folmer F, Jaspars M, Solano G, Cristofanon S, Henry E, Tabudravu J, et al. The inhibition of TNF-alpha-induced NF-kappaB activation by marine natural products. *Biochem Pharmacol* 2009;78:592-606.
- [3] Rateb ME, Houssen WE, Schumacher M, Harrison WT, Diederich M, Ebel R, et al. Bioactive diterpene derivatives from the marine sponge *Spongionella* sp. *J Nat Prod* 2009;72:1471-6.

Substituted 1,3-cyclopentadiones analogues differentially inhibit inflammation in vitro and collagen-induced arthritis in mice

Veera R. Konda, Anuradha Desai, Gary Darland, Brian J. Carroll, Jeffrey S. Bland, and Matthew L. Tripp

KinDex Therapeutics, LLC, Seattle, Washington, 98104, USA.

Email: vrkonda@metagenics.com

We previously discovered that META060 (tetrahydro-iso-alpha acids, a defined mixture of 3 chemically distinct substituted 1,3-cyclopentadiones; TH1, R=isopropyl; TH4, R= sec-butyl; TH5, R=isobutyl) mixture was a multi-target kinase inhibitor which reduced NF-kB signaling and inflammatory biomarkers in macrophages (Inflamm Res 2009; 58:1-6). To identify the efficacy of the individual analogues, we separated them using countercurrent chromatography and compared their efficacy to inhibit inflammation in lipopolysaccharide (LPS) activated RAW264.7 macrophages. We found that TH4 and TH5 showed similar efficacy, which was greater than that of TH1 in nitric oxide (NO) inhibition. To assess the effects on inflammatory signal transduction pathways, we analyzed TH1, TH4, and TH5 against several human protein kinases in cell-free enzyme assays. All three inhibited kinases involved in inflammatory signal transduction, including SyK, BTK, GSK3beta, and PI3K. TH4 and TH5 showed a lower IC₅₀ than TH1 on some kinase targets. These kinases are involved in Fc receptors and B cell receptor signaling, and are therapeutic targets in asthma, allergy and rheumatoid arthritis. Further studies focused on TH1 and TH5; both inhibited IL-1 beta activated PGE₂, cytokines, chemokines and matrix metalloproteinases in synovial fibroblasts/chondrocytes isolated from human rheumatoid arthritis subject. To determine the therapeutic potential of TH1 and TH5 in vivo, they were tested in the collagen-induced arthritis model. DBA/J mice were immunized with bovine type II collagen for 3 weeks. Mice demonstrating the onset of inflammation were randomized and therapy initiated. The joint swelling was measured for the next 2 weeks and joint damage was analyzed histologically to determine the extent of the disease. Both TH1 and TH5 dose-dependently reduced the arthritis index and joint degradation, with TH5 being more efficacious than TH1. These molecules may be of great value as an efficacious and safer alternative to treat chronic inflammation.

Impact of selective CDK inhibitors on functional status of cellular factors promoting survival of cancer cells

Józefa Węsierska-Gądek

Cell Cycle Regulation Group, Division: Institute of Cancer Research, Department of Medicine I, Medical University of Vienna, Borschkegasse 8a, 1090 Vienna, Austria. E-mail: Jozefa.Gadek-Wesierski@meduniwien.ac.at

A fine-tuned balance between pro-survival and apoptosis-promoting factors determines the clonal expansion of transformed cells as well as their susceptibility to therapy. In most cases, tumor cells divide rapidly due to the shortening of the cell cycle. Progression of the mammalian cell cycle is driven by transient activation of complexes consisting of cyclins and cyclin dependent kinases (CDKs). The escape from the proper control of the cell cycle by up-regulation of cyclins or aberrant activation of CDKs as well as by inactivation of cellular inhibitors of CDKs (CKI) leads to acceleration of cell divisions and malignant transformation. For these reasons the regulators of cell cycle have been considered as very promising therapeutic targets in human malignancies. It has been recognized that deficits in cellular CKIs, as frequently observed in cancers, could be compensated by synthetic compounds and provided a rationale for development of pharmacological inhibitors of CDKs. Tri-substituted purine derivatives such as roscovitine (ROSC) and olomoucine II (OLO), are selective CKI and affect CDKs involved in the regulation of both: cell cycle progression and transcriptional control. The inhibition of CDK7 and CDK9 has a serious impact on the activity of RNA Pol II. Unphosphorylated CTD is not able to recruit co-factors required for transcriptional elongation resulting in a global transcriptional block. Interestingly, the cellular RNA Pol II is also necessary for transcriptional regulation of virally encoded proteins. We observed that ROSC and OLOII inhibit CDK7 and RNA Pol II in HPV⁺-ve cancer cells resulting in the repression of virally-encoded oncoproteins promoting cell survival. Interestingly, both inhibitors affected the functional status of other cellular factors like NF-κB. In view of several modes of action the distinct small molecule CDK inhibitors are predestinated for therapy of virus-associated cancers.

In vitro testing for anti-inflammatory properties of compounds employing peripheral blood mononuclear cells freshly isolated from healthy donors

Katharina Kurz, Marcel Jenny, Sebastian Schroecksnadel, Harald Schennach^b, Florian Ueberall, Dietmar Fuchs.

Biocenter, Medical University, and ^bCentral Institute of Immunology and Transfusion Medicine, University Clinics, Innsbruck, Austria

Inflammation is crucially involved in a variety of diseases like autoimmune syndromes, cardiovascular and neurodegenerative disorders, cancer, sepsis and allograft rejection. Accordingly, immunosuppressive and anti-inflammatory medication is important to treat and prevent such disorders. Useful drugs usually exert most important effects on the T-cell/macrophage interplay. Thereby, Th1-type cytokine interferon-gamma (IFNg) is a most important pro-inflammatory mediator. For development and characterization of new anti-inflammatory drugs we investigate effects of compounds and biologicals on human peripheral blood mononuclear cells (PBMC) freshly isolated from whole blood from healthy donors and stimulated with mitogens. PBMC are stimulated with 10 µg/ml concentration of mitogen phytohaemagglutinin which was found optimal to detect potential suppressive effects. After incubation for 48h, supernatants are collected and measurement of neopterin formation by, e.g., ELISA and/or tryptophan degradation by HPLC is used as a convenient read-out, both biochemical effects are induced by IFNg. The test system reveals very reproducible results even between assays using blood of different donors. The combined study of effects on PBMC, a mixture of T-cells and macrophages, provides insight into signalling cascades especially those initiated by T cells. Monitoring biochemical effects like neopterin formation and tryptophan degradation appears to reveal more stable results in quantitative terms than monitoring cytokine production. Moreover, this strategy monitors the net effect of compounds on various pro- and anti-inflammatory cascades which are initiated during stimulated immune response *in vitro* and *in vivo*. Finally, both the read-out systems seem especially suited to test for anti-inflammatory effects of compounds, because enhanced production of neopterin and accelerated tryptophan degradation was found earlier to be closely related to the pathogenesis of various diseases in which inflammatory processes are involved such as cardiovascular diseases and neurodegenerative disorders, but also autoimmunity and cancer. Using this approach we demonstrated a dose-dependent effect to slow down activation cascades of PBMC for compounds like aspirin, resveratrol and vitamins C and E and also of specific plant extracts with supposed anti-inflammatory activity.

Inhibition and genetic deficiency of p38 MAPK induces the anti-inflammatory heme oxygenase-1 gene via the redox-regulated transcription factor Nrf2 in macrophages

Srivatsava Naidu¹, Sentot Santoso¹, Thomas Kietzmann², Stephan Immenschuh^{1,3}

1 Institute for Clinical Immunology and Transfusion Medicine, Justus-Liebig-University Giessen, Giessen, Germany; 2 Department of Biochemistry, University of Kaiserslautern, Kaiserslautern, Germany; 3 Institute for Transfusion Medicine, Hannover Medical School, Hannover, Germany

Heme oxygenase (HO)-1 is the inducible isoform of the first and rate-limiting enzyme of heme degradation. It has been shown in HO-1 knock out mice and in human genetic HO-1 deficiency that this enzyme has potent anti-inflammatory and immunomodulatory functions which have been ascribed to its products carbon monoxide and bilirubin. Although HO-1 has previously been shown to be induced by various stimuli via activation of the p38 MAPK signaling pathway, the role of this protein kinase for HO-1 gene regulation is largely unknown. In the present study it is demonstrated that pharmacological inhibitors of p38 and siRNA-dependent knock down of p38 α induced HO-1 expression in monocytic cells. Moreover, basal HO-1 gene expression levels were markedly higher in untreated murine embryonic fibroblasts (MEF) from p38 $\alpha^{-/-}$ mice as compared to that from wild type mice. Transfection studies with luciferase reporter gene constructs indicate that increased HO-1 gene expression via inhibition of p38 was mediated by the transcription factor NF-E2-related factor-2 (Nrf2), which is a central regulator of the cellular oxidative stress response. Accordingly, inhibitors of p38 induced binding of nuclear proteins to a Nrf2 target sequence of the HO-1 promoter, but did not affect HO-1 protein expression and promoter activity in Nrf2 $^{-/-}$ MEF. Genetic deficiency of p38 led to enhanced phosphorylation of ERK and increased cellular accumulation of reactive oxygen species (ROS). In addition, pharmacological blockage of ERK and scavenging of ROS with N-acetylcysteine reduced HO-1 gene expression in p38 $^{-/-}$ MEF, respectively. Taken together, it is demonstrated that pharmacological inhibition and genetic deficiency of p38 induce HO-1 gene expression via a Nrf2-dependent mechanism in monocytic cells and MEF. These findings not only give new insights into the complex signaling events of HO-1 regulation during the inflammatory response, but may also help to develop novel therapeutic approaches for the treatment of inflammatory diseases.

Workshop presentations

(in chronological order)

Wednesday January 27th:

Workshops: 14h00 – 15h00: Becton Dickinson 15h00 – 16h00: Promega	Posters: 14h00 – 16h00: Poster session (All posters)
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Thursday January 28th:

Workshops: 13h00 – 14h00: IBA 14h00 – 15h00: Polyplus-Transfection 15h00 – 16h00: AMS Biotechnology	Posters: 14h00 – 16h00: Poster session (Posters with even numbers: 2, 4, 6, ...)
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Friday January 29th:

Workshops: 14h00 – 15h00: Bio-Rad 15h00 – 16h00: GE Healthcare	Posters: 14h00 – 16h00: Poster session (Posters with odd numbers: 1, 3, 5, ...)
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Simplifying the study of signaling pathways in T cell subsets by flow cytometry using an optimized reagent system: The BD Phosflow T Cell Activation Kit

Lori Anderson, BA, ART, Applications Scientist

BD Biosciences, San Diego CA

Lori_Anderson@bd.com

Phosphorylation and dephosphorylation of intracellular proteins drives most cellular processes including cell growth and differentiation, cell proliferation, cell survival, and apoptosis. Many currently available tools for studying phosphorylated proteins, such as Western blot, require that populations of cells be lysed to gain access to intracellular protein targets. To study protein phosphorylation within cell subsets derived from heterogeneous cell populations, such as whole blood, requires cell sorting and/or purification of cell populations-of-interest prior to analysis of protein phosphorylation. This process can be time consuming and negatively impact experimental results. By combining BD Phosflow optimized reagents, cell surface markers, and routine multiparametric flow cytometry techniques, investigators can study intracellular protein phosphorylation in cell subsets without the need for physical cell sorting. The BD Phosflow T Cell Activation kit is an optimized reagent system that includes a T-cell-specific multicolor cocktail, and several phosphoprotein-specific monoclonal antibodies directed to targets in the JAK-STAT and MAP Kinase pathways. These pathways are involved in cytokine, growth factor and antigen-specific T cell signaling. This kit enables the study intracellular signaling of CD4+, CD8+, and other lymphocyte subsets from whole blood in a rapid and simple technique that can be used in the study of immune cell interactions, drug discovery, and the monitoring of disease processes.

HaloTag Technology: A Proteomics tool for Understanding Protein Function in a Biochemical and Cellular Environment – with a twist of NF-κB signaling pathway.

Presenter: Dr. Marie Stahl, Product Manager - Proteomics and Cellular Analysis

Company name: Promega Benelux BV

Promega has developed the HaloTag technology, which is based on the covalent binding of a protein fusion tag (HaloTag) to specific synthetic ligands. The ligands can carry different fluorescent dyes enabling protein labeling or are attached to solid support for protein immobilization. The specific and covalent nature of capture allows efficient washing and removal of non-specific interactions without loss of specific interactions.

To demonstrate the power of this technology for analysis of protein function, Promega has used several different model systems. Using NF-κB signaling pathway as model system, we will show how using HaloTag enables simultaneous analysis of protein migration and interactions with other proteins and DNA.

More information:

<http://www.promega.com/halotag>



Contact:

benelux@promega.com

***Strep-tag®*-based tools for cell biology: *Streptamer®* for reversible staining and isolation of cells applicable for cell therapy, and FasL-*Strep* for apoptosis research**

¹Lothar Germeroth, ¹Christian Stemberger, ¹Christine Piossek , ²Thomas Schmidt and ³Dirk H. Busch

¹Stage Cell Therapeutics GmbH, Göttingen, Germany, ²IBA GmbH, Göttingen, Germany, ³Institute for Medical Microbiology, Immunology and Hygiene, Technische Universität München, Munich, Germany; schmidt@iba-go.com

Streptamers are reversible MHC I-multimers for antigen-specific staining and isolation of CD8⁺ T-cells. With biotin *Streptamer* reagents can be completely dissociated from the stained cells, providing quasi untouched, fully functional antigen-specific cells. Especially therapeutic applications of *Streptamer*-isolated T-cells benefit twofold from the absence of isolation reagents. First, the effector function of CD8⁺ T-cells remains uncompromised when staining, isolation and dissociation is performed at 4°C and second, reversibly *Streptamer*-isolated cells have significant regulatory advantages for adoptive T-cell therapy. After validation of these MHC I-*Streptamer* advantages within a clinical trial targeting CMV-infections after stem cell transplantations, we have extended the reversible *Streptamer* approach to Fab-*Streptamers*, which can address any cell surface marker. Fab antibody fragments are genetically engineered to provide complete reversibility. Thus, Fab-*Streptamers* provide similar advantages compared to MHC I-*Streptamers* but extend the range of accessible target cells to virtually all therapeutically relevant cells including regulatory T-cells, stem cells and natural killer cells. Therefore, the combination of MHC I- and Fab-*Strep-tamers* provide a versatile technology platform for a rapid and economic access to clinical trials with a broad range of therapeutically relevant cells. An overview of the *Streptamer* technology will be presented and potential therapeutic applications will be discussed. Apoptosis plays a major role in the pathogenesis of cancer, autoimmune and neurological diseases. Due to a novel trimerization module, the T4 Foldon, conformational stabilization of the FasL-*Strep* homotrimer enables efficient and highly reproducible induction of apoptosis in human and murine cells without chemical crosslinking. FasL-*Strep* is also ideally suited for high-throughput screening and quantitative determination of anti-apoptotic agents.

Understanding and performing DNA and siRNA transfection

Géraldine Guérin-Peyrou, Scientific and Technical Support Specialist

Polyplus-Transfection

ggp@polyplus-transfection.com

Transfection is widely used as a tool in research. It consists in introducing nucleic acids into mammalian cells. If inadequate, transfection can become a limiting step for any project. Thus it is critical to understand the mechanism of transfection to choose the most appropriate method. This tutorial will present the various transfection mechanisms as well as currently available methods and reagents for DNA and siRNA transfection. We will explain why some methods are more effective at transporting and releasing DNA and mRNA while others are better for siRNA. In addition the critical parameters for transfection and their optimisation will be described. Our goal is to give a solid basis in transfection in order to obtain the best results in the shortest timeframe.

Oris™ Cell Migration Assays from amsbio: An Innovative Platform For Studying Cell Migration & Invasion

Alex Sim

AMS Biotechnology (amsbio), AlexS@amsbio.com

amsbio is proud to offer the Oris™ product line: an innovative line of 96-well, cell exclusion zone assays for performing cell migration and cell invasion experiments. The Oris™ assays utilize cell seeding stoppers to create a circular Detection Zone in the center of each well into which cell movement can occur. Data can be generated using multiple fluorescent stains and quantified using fluorescence microplate readers, inverted microscopes, or imaging instruments. The Oris™ assay platform allows various Extracellular Matrix coatings for cell migration, and has also been adapted for the study of cellular invasion within 3-dimensional extracellular matrices. Data demonstrating the robustness and versatility of the Oris™ assays and their advantages over other motility assay platforms will be presented.

The presentation will be the platform for the European launch of the NEW **Oris™ Pro Cell Migration Assay**; which features a dissolving, non-toxic biocompatible gel to create an annular monolayer of cells with a cell-free, central Detection Zone into which cell migration can occur. This format is specifically aimed at high throughput applications as it enables the use of automated liquid handling.

By allowing unlimited real time access to wells from cell seeding to data readout the presentation will demonstrate the capture and quantification of cell migration results using inverted microscopes and high content screening (HCS) and high content imaging (HCI) instruments.

Working across the European market since 1987, **amsbio** (formerly **AMS Biotechnology (Europe) Ltd**) is a global supplier of research products and custom services for biological & pharmaceutical research. We offer antibodies, peptides, DNA, RNA, proteins, tissue blocks, arrays, slides, assay kits, cell culture consumables, glycobiology reagents, apoptosis and DNA damage kits & reagents, cell migration / invasion assays and zymolyase.

Getting the Best Data from your Multiplex Immunoassays!

Dennis Buurman, Bioplex Specialist

Bio-Rad Laboratories - TechSupport.Belgium@bio-rad.com

Dennis_Buurman@bio-rad.com

Multiplex immunoassays with Luminex xMAP technology allows researchers to measure up to 100 biomarkers simultaneously and dramatically increase the amount of information obtained from irreplaceable or volume-limited samples such as mouse or rat serum. Major applications include cytokine profiling in health and disease conditions.

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High quality results are required that allow you to make good decisions on the direction of your research and that will stand close scrutiny for publication.... but first time! No-one wants to waste precious samples or valuable assays kits.

Bio-Rad Laboratories have been pioneers in the development of the Bio-Plex system including the recent introduction of more-robust magnetic bead assays and magnetic washing workflows. Come along and share in what we've learned over the years.

- The factors that most influence multiplex immunoassay workflows
- The importance of routine calibration and validation
- Critical parameters for successful data analysis
- Hints and tips from the experts that will improve your results



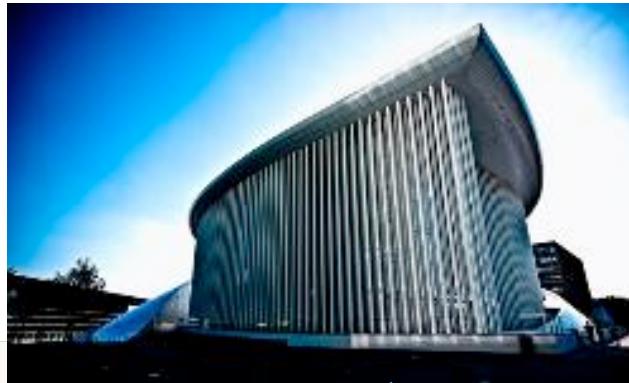
Innovative 2D DIGE technology for differential protein expression : examples in inflammation research.

Leon Kraakman, Product specialist 2D DIGE

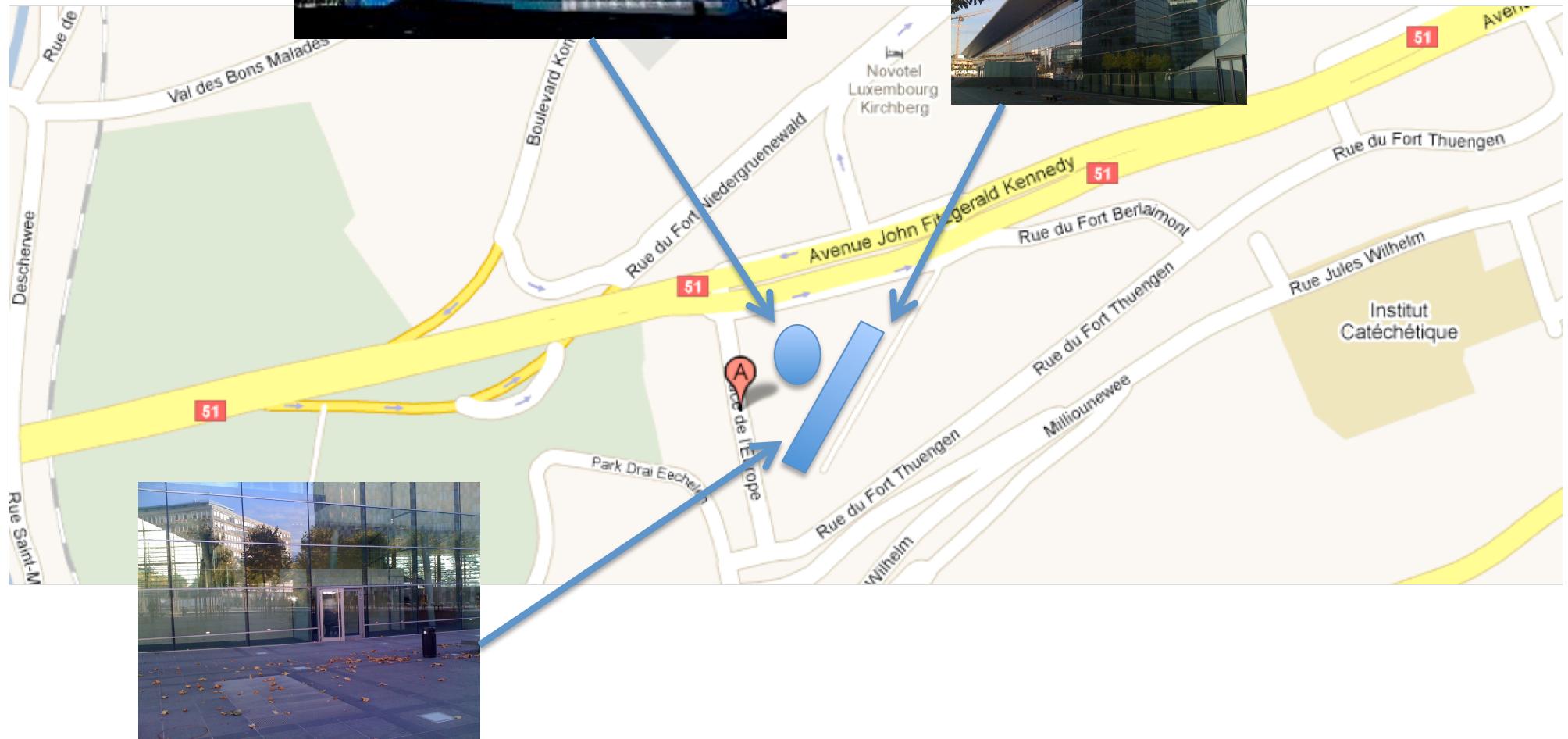
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2D DIGE is an innovative technology to compare the expression of proteins between control and disease state to identify proteins involved in the onset of the disease. 2D electrophoresis is a gel-based technology where proteins are separated based on pI value and size resulting in very high resolution separation of about 2000 proteins per gel. 2D DIGE is an innovative approach by pre-labelling the proteins with fluorescent dyes making it possible to perform multiplexed analysis comparing in one gel control and disease samples. 2D DIGE is unique as it can exclude technical variation and inherent biological variation by working with an internal standard and multiple biological replicates. More than 1500 publications have referred to the 2D DIGE technology. Examples of applications in inflammation research will be given.

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4. Hotel Campanile
5. City Hotel Gare
6. Hotel Alfa Mercure
7. Hotel Novotel Center
8. Hotel Le Royal

Timetable

Wednesday January 27th

20h30: Congress Center -> Hotels

Thursday January 28th

07h20: Hotels -> Congress Center
19h00: Congress Center -> Hotels
20h00: Hotels -> Le Royal (Gala)
23h30: Le Royal -> Hotels

Friday January 29th

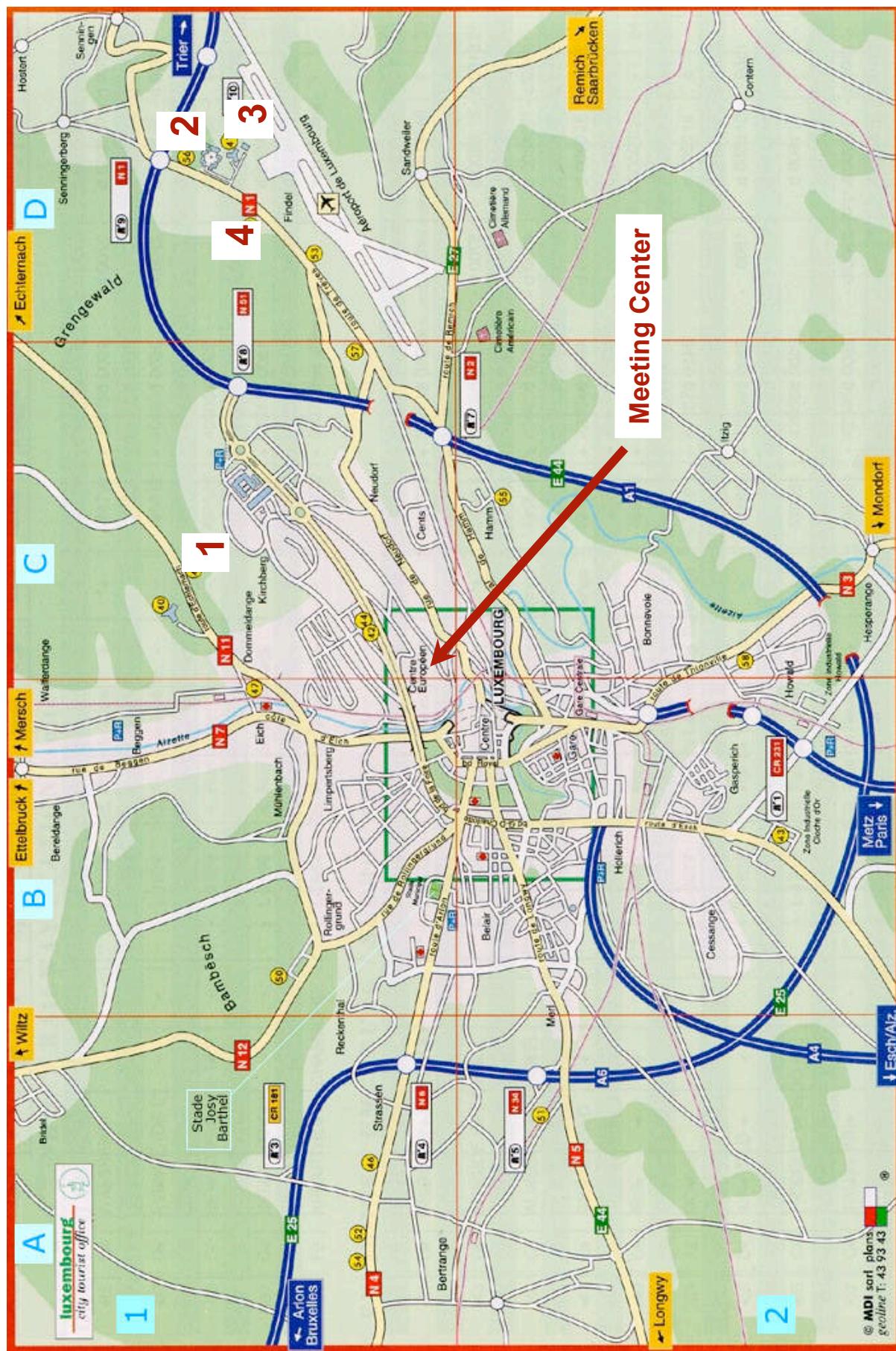
7h20: Hotels -> Congress Center
19h15: Congress Center -> Hotels

Saturday January 30th

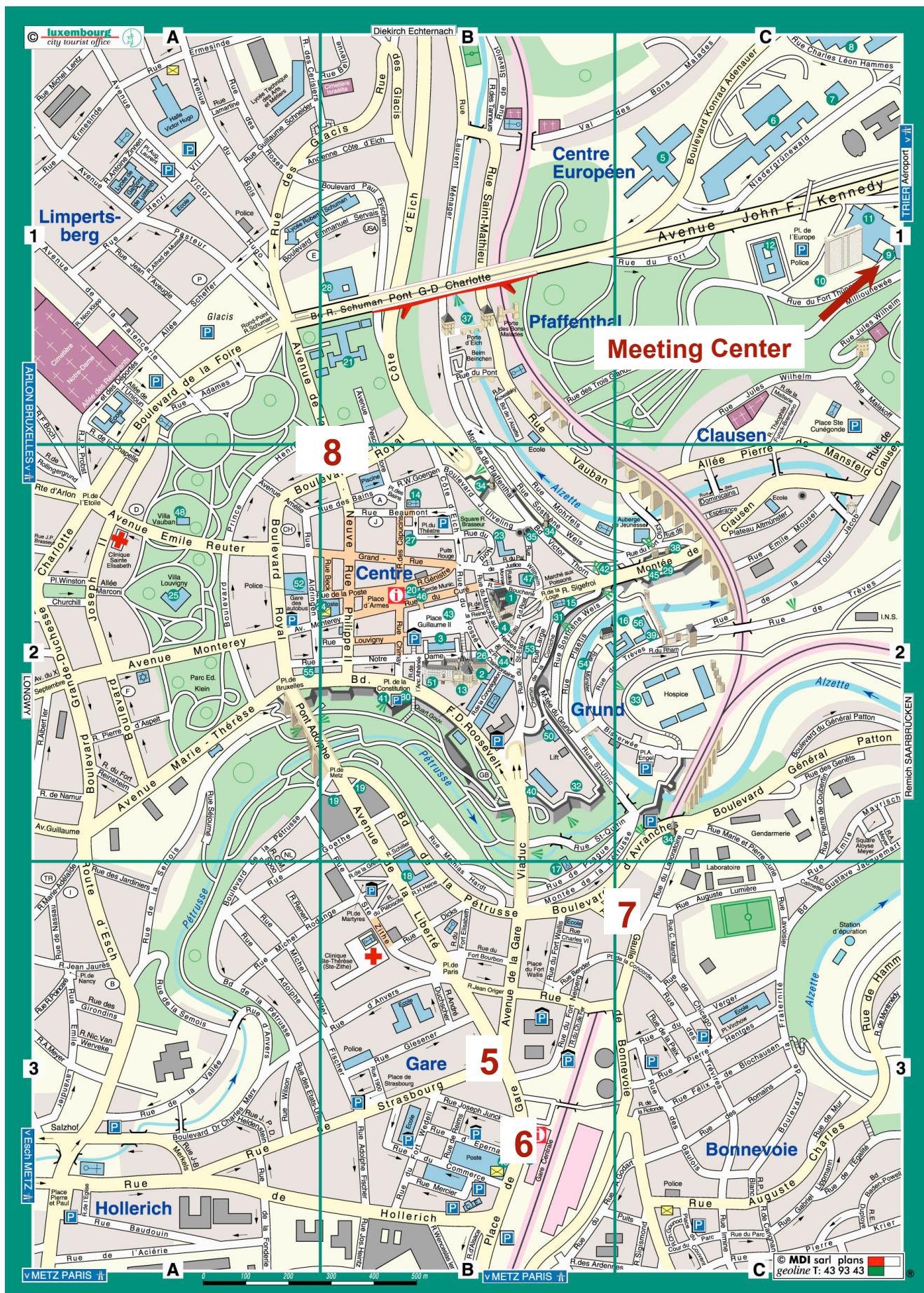
07h20: Hotels -> Congress Center
13h00: Congress Center -> Hotels
14h00: Congress Center -> Hotels



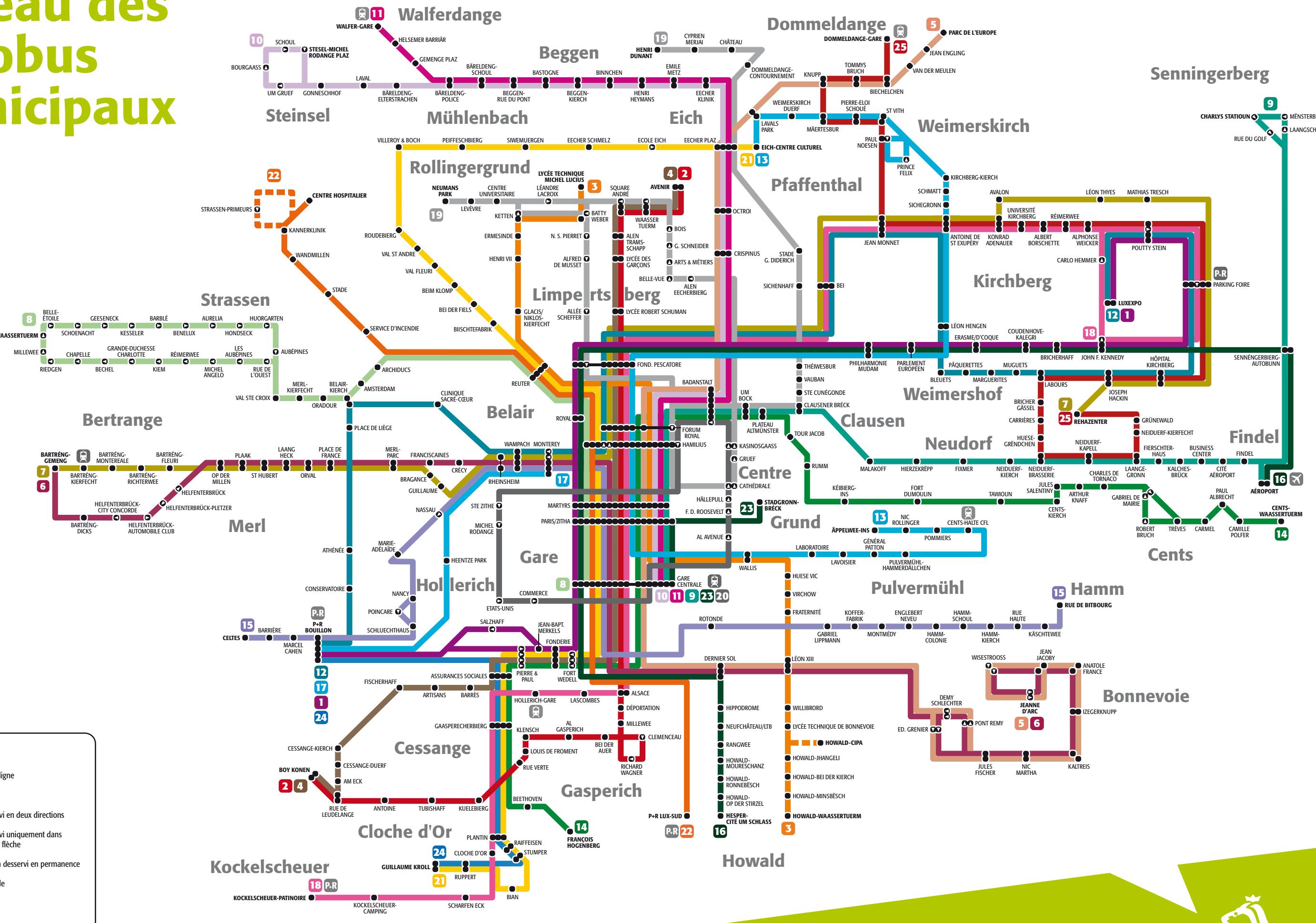
Map and bus stops



Map and bus stops (city center)

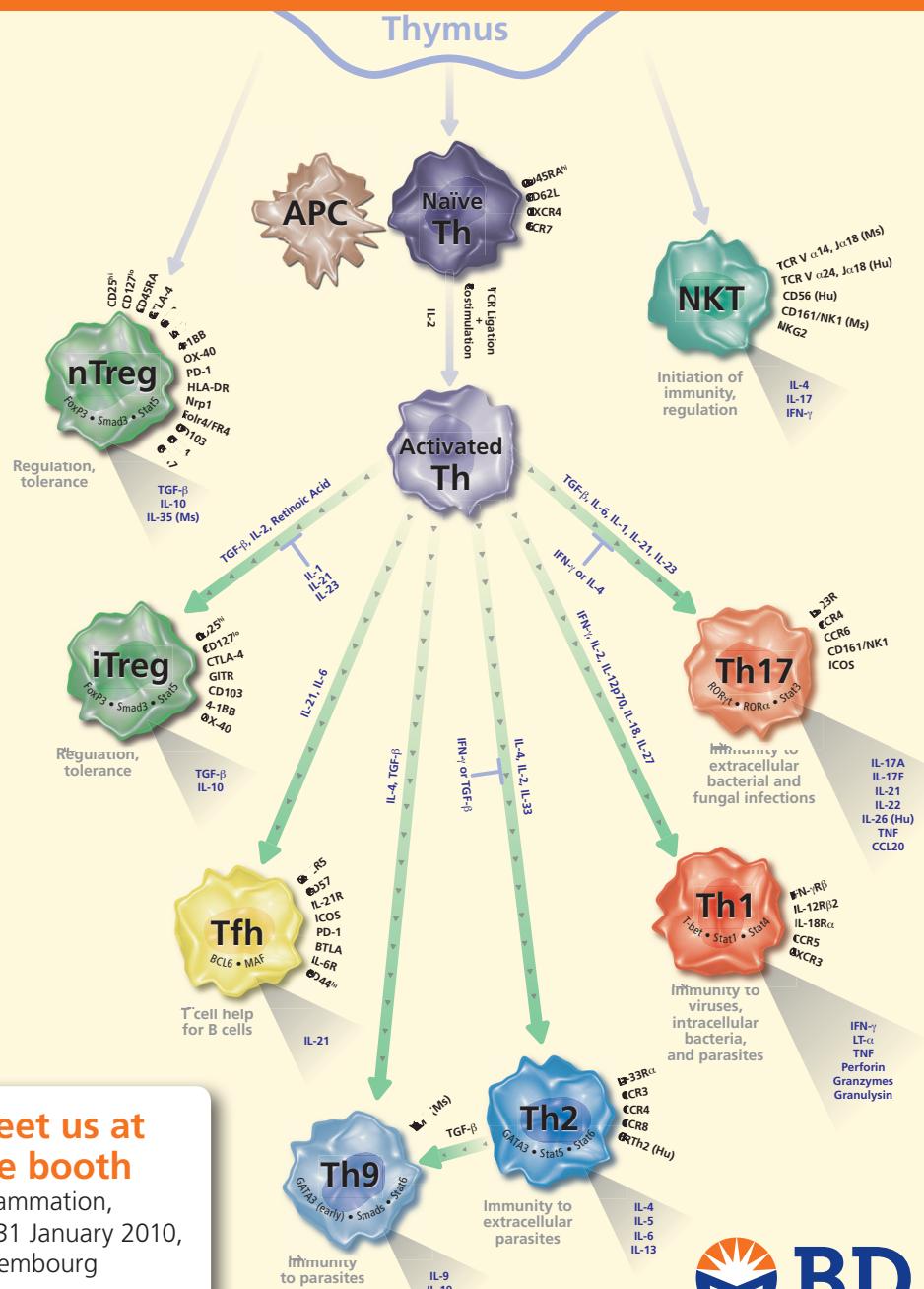


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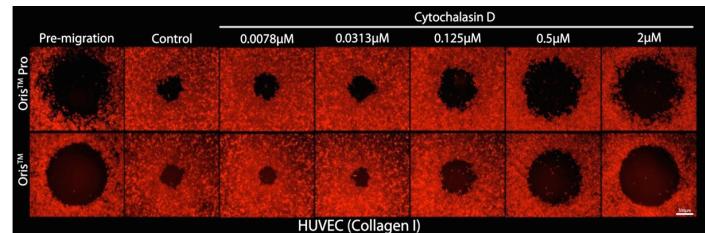
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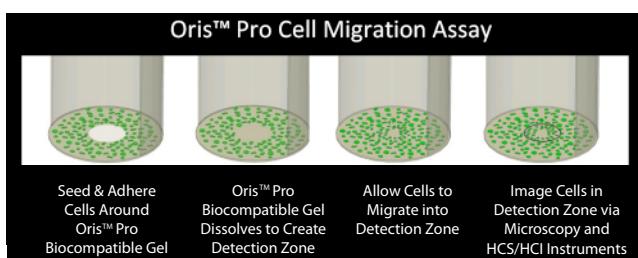
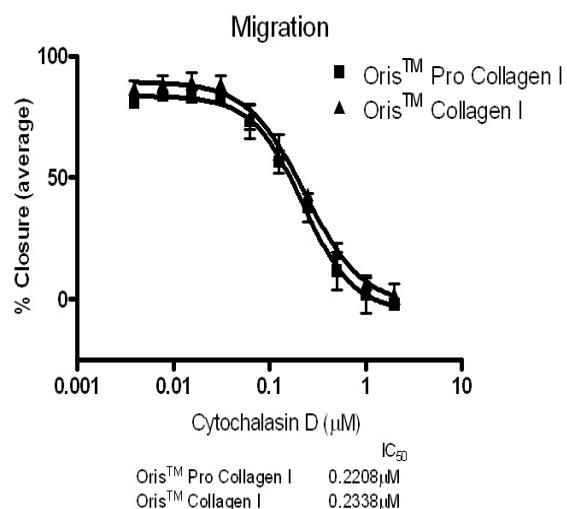
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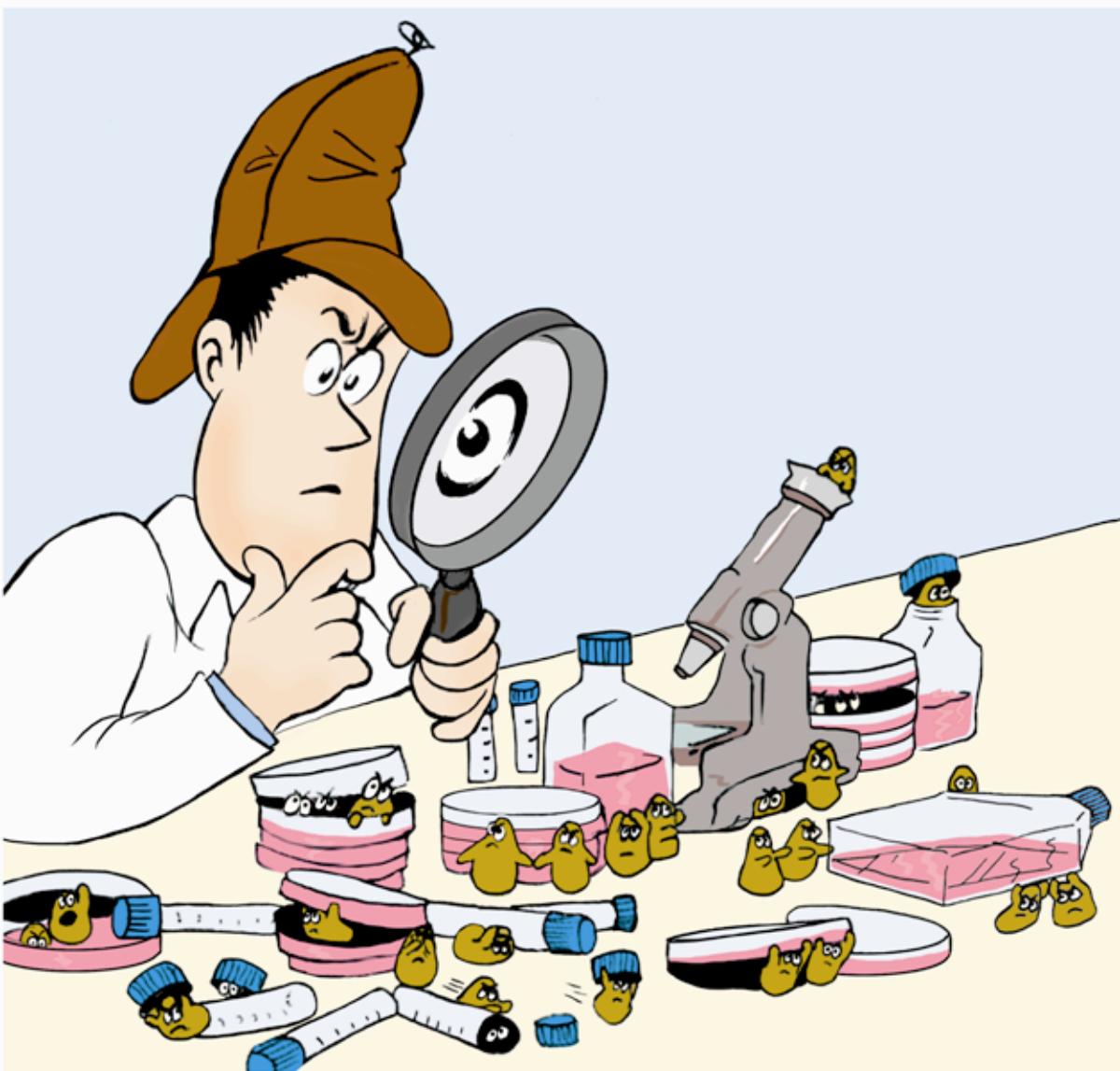
HUVECs were seeded onto an Oris™ Pro Collagen I plate. A dose-response titration was performed using the actin polymerization inhibitor, Cytochalasin D. Cells were treated for 18 hours, fixed, and stained for F-actin.



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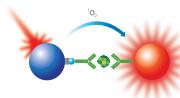
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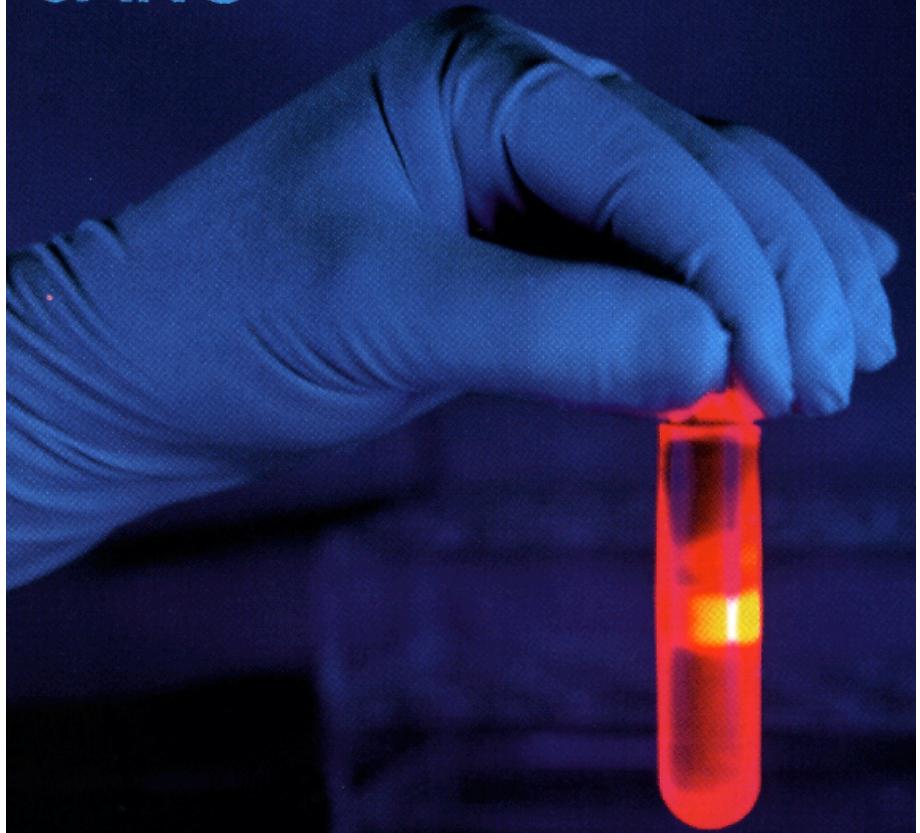
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Integrated cellular pathology - Systems biology of human disease

Contact : Marc Diederich

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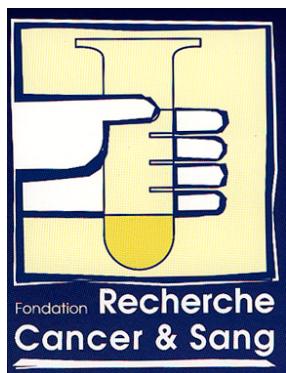
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Poster Presentations

All posters will be up from Wednesday January 27th until Friday January 29th:

Wednesday January 27th 14h00 – 16h00: All posters

Thursday January 28th 14h00 – 16h00: Posters with even numbers (2, 4, 6...)

Friday January 29th 14h00 – 16h00: Posters with odd numbers (1, 3, 5...)

On Friday January 29th 16h00, all poster presenters are requested to recover their posters (the boards will be taken away).

**Posters are classified by session
& then in alphabetical order (of the first author)**

Session I: Regulation of Cell Signaling Pathways in Inflammation

Session II: Inflammatory Mediators

Session III: Anti-inflammatory Compounds

Session IV: Virus Infections and Innate Immunity

Session V: Chronic Inflammatory Pathologies

Session VI: Inflammation and Cancer

Session VII: Cell death and Inflammation

NB: sessions were reorganized based on the number of submissions

Session I: Regulation of Cell Signaling Pathways in Inflammation

Apoptotic cell-derived S1P contributes to arginase II expression in murine macrophages by activating ERK5/CREB**Vera Barra¹, Andreas von Knethen¹, Andreas Weigert¹ and Bernhard Brüne¹**

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Apoptotic cell (AC)-derived factors alter the physiology of macrophages towards a regulatory phenotype that is characterized by an attenuated pro-inflammatory cytokine profile and reduced nitric oxide (NO) production. Impaired NO formation in response to ACs or AC-conditioned medium (CM) is facilitated by arginase II (ARG II) expression, which competes with inducible NO synthase for the common substrate L-arginine. Here we investigated the signaling pathway that allowed CM to up-regulate ARG II in RAW264.7 macrophages. A sphingolipid, further identified as sphingosine-1-phosphate (S1P), was involved. S1P signaled through S1P₂, since the antagonist JTE013 and siRNA knockdown of S1P₂ prevented ARG II up-regulation. Further, inhibition and knockdown of extracellular signal-regulated kinase 5 (ERK5) attenuated CM-mediated ARG II protein induction. Exploring ERK5-dependent transcriptional regulation, promoter deletion and luciferase reporter analysis of the murine ARG II promoter (mpARG II) suggested a cyclic adenosine monophosphate (cAMP) responsive element binding protein (CREB) to be implicated. This was confirmed by EMSA analysis and decoy-oligonucleotides scavenging CREB in RAW264.7 macrophages, thereby preventing it from binding to the promoter of its target genes and thus, blocking ARG II expression. We conclude that AC-derived S1P shapes an anti-inflammatory macrophage phenotype. Along this line, S1P contributes to ARG II induction via binding to S1P₂, thereby activating ERK5 and subsequently CREB.

Permanent inhibition of catalase (CAT) activity in peripheral white mononuclear cells (PWMC) of naïve Crohn's Disease (CD) patients depends on a down regulation of CAT-gene expression.

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Background: Oxidative stress (OxS) in PWMC depends on an increased production of H₂O₂. Although some H₂O₂ scavenger enzymes activity have been shown to be increased in CD, we reported that CAT enzyme is permanently inhibited in CD 1). The cause for such a CAT inhibition and its possible implications in cellular processes have never been reported and /or studied. In cancer cell lines, CAT enzyme has been shown to be more implicated in regulating cellular processes than in detoxifying H₂O₂. **Aim:** To analyze the quantity of CAT protein, its genetic expression and/or its regulation, in order to characterize the cause of the permanent inhibition of CAT activity in CD patients. Other genes related to OxS and genes previously reported to be differentially expressed in CD have been also analysed. **Methods:** Blood samples from healthy subjects (n=12, mean age 28.33 ± 4.2) and from patients at first flare of CD, before initiating any specific medication (n=12, mean age 30.17 ± 8.3) were obtained. PWMC were isolated by Ficoll-Histopaque centrifugation. The quantity of CAT protein was studied by western blotting (WB). Total RNA from leukocyte population was extracted according to manufacturer's protocol (LeukoLOCK™ Total RNA Isolation System; Ambion). mRNA expression was measured by Sequenom's MassARRAY® quantitative gene expression (QGE) analysis application (Sequenom TM). The genes analysed were: CAT, SOD1, SOD2, NOS2A, STAT1, NFKB1, PKCg, PKCzeta, PSKH1, PPID, ABCB1, ASK, FASR, FASLG, TERT, IL-2, IL23R. **Results:** WB results showed diminished levels of CAT in active and inactive CD patients compared to controls. RNA CAT expression levels in active CD patients were lower than healthy subjects (12.12 ± 10.23 vs 41.53 ± 25.86, data in aM). Other genes differentially expressed in CD are: SOD1, SOD2, STAT1, PSKH1 and PKCg. **Conclusion:** The inhibition of CAT activity depends on a down regulation of the RNA expression of Cat-gene which correlates with a low concentration of CAT protein. Despite SOD1 and SOD2 are increased in CD, CAT does not contribute to H₂O₂ detoxification. The inhibition of PKC γ , a CAT activity modulator, may contribute to the final CAT activity. The increased expression of STAT1 highlights the importance of IFN- γ in CD and its possible correlation with OxS regulation. The increased PSKH1 identifies the trafficking and processing of pre-mRNA as a target for CD pathogenesis.

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Glucocorticoids are potent modulators of LPS-induced Interferon signalling in macrophages**Anja M. Billing, Claude P. Muller**

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Lipopolysaccharide (LPS), the main cell wall component of gram-negative bacteria, is recognized by toll-like receptor 4 (TLR). TLR4 signalling is MyD88-dependent or – independent leading mainly to the activation of NF-kappaB or interferon regulatory factor 3 (IRF3), respectively. Glucocorticoids, as the end products of the hypothalamic-pituitary-adrenal (HPA) axis, are the most important endogenous inhibitors of inflammation. Synthetic GC are potent immunosuppressants, but the therapeutical efficiency in endotoxemia, sepsis, and septic shock is controversial. Here we investigated the effects of cortisol on LPS-activated monocyte-derived THP-1 macrophages by two dimensional difference in gel electrophoresis (2D DIGE) combined with matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry. Proteomic data was complemented by qRT-PCR, immunoblotting, and *in-silico* pathway/promoter analysis. We found 47 proteins to be modulated: 20 by cortisol, 11 by lipopolysaccharide (LPS), and 16 by cortisol and LPS. We identified new cortisol-sensitive proteins (HCLS1, MGN, and MX1) and new LPS-induced cortisol-suppressed variants of MX1, SYWC and IFIT3. The combined results of proteomics and mRNA expression studies clearly identified the LPS-activated IFN pathway as a complex and prominent target of the immune suppressive activity of cortisol. Suppression of the IFN pathway in sepsis may well be one of the key therapeutic activities of high dose of cortisol in this condition.

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NADPH oxidase - mediated production of reactive oxygen species induces inflammation and apoptosis in *Helicobacter pylori*-infected gastric epithelial cells

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Reactive oxygen species (ROS) are considered as an important regulator in the pathogenesis of *Helicobacter pylori* (*H. pylori*)-induced gastric ulceration and carcinogenesis. Apoptosis and inflammation linked to oxidative stress has been implicated in gastric cell injury. NADPH oxidase is a major source of reactive oxygen species (ROS) in phagocytic and non-phagocytic cells. Chemokine monocyte chemoattractant protein-1 (MCP-1) was relatively highly expressed in gastric epithelial cells infected with *H. pylori* in Korean isolates (HP99). The purpose of the present study is to investigate whether NADP oxidase mediates apoptosis and MCP-1 expression in *H. pylori*-infected in gastric epithelial AGS cells. AGS cells were cultured with *H. pylori* (HP99) at a ratio of 300:1. Apoptotic cell death was determined by cell viability, DNA fragmentation, and the levels of apoptotic protein Bax and p53 as well as anti-apoptotic protein Bcl-2. Expression of MCP-1 was determined in *H. pylori*-treated AGS cells as inflammatory index. As a result, inhibition of NADPH oxidase using a NADPH oxidase inhibitor, diphenyleneiodonium (DPI), suppressed *H. pylori*-induced apoptotic cell death and MCP-1 expression in parallel with the decrease in the levels of hydrogen peroxide in the medium. DPI inhibited *H. pylori*-induced decrease in anti-apoptotic Bcl-2 and increase in pro-apoptotic Bax in AGS cells. These results suggest that the activation of NADPH oxidase may induce apoptosis and MCP-1 expression which may be mediated by ROS in gastric epithelial cells.

Inhibitory mechanism of α -lipoic acid on cytokine expression in human gastric epithelial cells

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Helicobacter pylori (*H. pylori*), gram negative bacteria, induces the expression of proinflammatory cytokines including IL-8 in gastric epithelial cells. NF- κ B and JAK/STAT have been associated with the expression of proinflammatory genes. α -lipoic acid is a well known antioxidant, and shows anti-inflammatory effect. The present study aims to investigate whether α -lipoic acid inhibits signaling molecules such as JAK/STAT and NF- κ B in *H. pylori*-stimulated gastric epithelial AGS cells. The results show that *H. pylori* induces the expression of IL-8 and activation of NF- κ B and JAK/STAT, which was suppressed by treatment of α -lipoic acid in AGS cells. In conclusion, α -lipoic acid may inhibit the expression of pro-inflammatory cytokine by suppressing the activation of NF- κ B and JAK/STAT in gastric epithelial cells.

Long non-coding Heg RNA, TLR7, IFN-gamma and inhibition of autoantibodies.

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The aim of the present study was to elucidate the mechanism of action of lnc Heg RNA. Heg is a long non-coding RNA transcript (GenBank EU 137727) in blood mononuclear cells. Heg is negatively correlated with TSH receptor autoantibodies (TRAk) in patients with early, untreated Graves' disease and with CD14 mRNA in treated patients and in controls. Heg correlated positively with IFN-gamma (at least in normal subjects) and with TLR7 mRNA. The relationship between TRAk and Heg was increased by including Cdk1 mRNA (an index of cell cycle activity) in the analysis (TRAk vs. the ratio Heg/Cdk1, $r = -0.82$, $p < 0.001$). Cdk1 was positively related to TRAk. In treated patients both TRAk and Cdk1 decreased.

Increasing cytoplasmatic concentrations of Heg (exogenous Heg) decreased CD14 mRNA and increased TLR7 and IFN-gamma mRNA. IFN type 1 was not expressed during basal conditions but increased more than 100 fold with addition of exogenous Heg RNA. These responses were not specific in the sense that a fragment of the anti-sense Nucks mRNA also decreased CD14 mRNA and increased IFN mRNA. Thus the effect of exogenous Heg RNA was not dependent on the exact sequence. This finding suggests that endogenous Heg RNA may be an index of long non-coding RNA molecules, which escapes degradation in the nucleus. Heg may interact with TLR7 in the cytoplasma or in the endolysosome and increase the transcription rate of IFN-gamma mRNA and decrease CD14 mRNA (apoptosis?). Small increments in Heg in the cytoplasma by administration of exogenous Heg (fragment or stabilized) may inhibit autoimmune function. We are currently testing if exogenous Heg can be applied to inhibit early experimental autoimmune disease.

Cross-talk of androgen receptor with Akt and beta-catenin in prostate cancer?**Joanna Dulinska-Litewka, Dorota Gil and Piotr Laidler****Chair of Medical Biochemistry, Jagiellonian University Medical College, ul Kopernika 7, 31-034 Krakow, POLAND; email: mblitewk@cyf-kr.edu.pl**

Patients with advanced prostate cancer initially benefit from androgen-ablation therapy, which leads to temporary tumor remission due to apoptosis of androgen-sensitive tumor cells. However, recurrence of androgen-independent tumor is inevitable for most patients and renders the conventional hormone therapy ineffective. In androgen target cells, androgen receptor (AR) is predominantly localized in the cytoplasmic fraction in the absence of ligand while it translocates to the nucleus in the presence of the endogenous androgens and activates transcription of AR-responsive genes. Regulation of its function involves cross-talk with the other signaling pathways (PI3K/Akt), transcription factors and coregulatory proteins e.g. beta-catenin which plays a critical role in tumorigenesis. It's proposed that PI3K/Akt plays role in regulating AR activity through phosphorylation of AR at Ser(P)-213/210 but we observed that in differentiated prostate post-surgical tissues, only a small percentage of cells are positive for AR Ser(P)-213, which suggests that phosphorylation is not prevalent. Akt also phosphorylates beta-catenin at Ser(P)-552/675 induces bet-catenin accumulation in the nucleus and increases its transcriptional activity. Our results suggest that in prostate cancer cells AR plays the most important regulatory function. In addition interaction between AR and Akt in beta-catenin signaling pathway may directly contribute to cancer progression in effect of loss of androgen dependence. Silencing of AR with siRNA in LNCaP cell line significantly reduced proliferation (50-80%), nuclear beta-catenin, beta-catenin Ser(P)-675, activity of MMPs and in parallel significantly increased expression of E-cadherin and cell cycle inhibitors p21 and p27. Our data suggest that loss of E-cadherin can elevate the nuclear levels of beta-catenin in prostate cancer cells, which may directly contribute to invasiveness and more malignant tumor phenotype by augmenting AR activity during prostate cancer progression. Prolonged silencing of AR (over 72 hrs) in LNCaP cells results in increasing proliferation as well as expression of nuclear beta-catenin with appearance of beta-catenin Ser(P)-552/675. We noticed activation of the PI3K/Akt pathway, which has a survival role in prostate cancer by protecting cells from apoptosis. Silencing of Akt significantly reduced nuclear beta-catenin Ser(P)-675. We propose that Akt replaces AR role and contributes to the development of androgen-independent cancer progression. Activated PI3K/Akt can stabilize beta-catenin through inhibition of GSK-3 by its phosphorylation at Ser11 and Ser9 and/or direct phosphorylation of beta-catenin Ser(P)-675 resulting in its nuclear accumulation. This work was supported by MNiSzW grants: K/ZDS/001003 and K/ZDS/000455 - Jagiellonian University Medical Collage, Krakow – Poland.

Neurosphere-derived astrocytes as a model to study astrocyte behaviour during neuro-inflammation**Sebastien Gabel, Luc Grandbarbe, Tony Heurtaux, Eleonora Morga, Paul Heuschling.****Life Sciences Research Unit, Université du Luxembourg, 162a avenue de la Faïencerie, L-1511 Luxembourg, sebastien.gabel@uni.lu**

Brain inflammation, currently described in several neurodegenerative diseases like Alzheimer's disease (AD), has been recognized as a complex phenomenon with numerous related aspects. Although, microglial cells are considered as the main actors of the neuro-inflammation, several studies have recently highlighted the importance of astrocytes during these inflammatory events. Primary rodent astroglial-enriched cultures are the most popular model to study astroglial biology in vitro. From the original methods a great number of minor modifications have been incorporated into these protocols by different laboratories. These protocols result in cultures in which the astrocyte is the predominant cell type, but astrocytes never reach 100% of cells in these preparations. The proportion of microglial cells and the role they play in astroglial cultures are often underestimated. In this work, we clarify this complexity, by using novel approaches to prepare pure astrocyte cultures entirely devoid of microglia. The production of pure astrocyte cultures is based on promoting neural stem cell (NSC) differentiation into astrocytes. In the current study we obtained pure astrocyte cultures by growing NSC in the presence of 10% of serum, which promotes NSC differentiation into astrocytes. Microglia free astrocyte cultures obtained from primary mixed glial cell cultures, which are needed to validate the model of neurosphere derived astrocytes, are obtained by using the CD11b MicroBeads magnetic separation technique on mixed glial cells. From the CD11b column we isolate microglia by positive selection and astrocytes by depleting microglia. Microglial isolates by positive selection are >99% pure and free of astrocytes, while astrocytes collected by negative selection are 95–97% pure and completely devoid of microglia. Here, we show that neurosphere derived astrocytes and microglia free astrocyte culture, present the same behaviour in inflammatory conditions. Pure astrocytes are activated by TNF- α , IL1- β and IFN- γ , but do not respond to Lipopolysaccharide (LPS). In addition, we observe that inflammation induces an induction of SOX2, EGFR and CD133 expression on astrocytes.

Genes involved in endothelial NO production are regulated by NKX2-3 in inflammatory bowel disease

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The homeodomain transcription factor NKX2-3 is expressed in the smooth muscle and in microvascular endothelial cells within the lamina propria and submucosa of the intestine. Recently, NKX2-3 has been associated with inflammatory bowel disease (IBD), and its mRNA and protein expression levels have been found to be up-regulated in Crohn's disease (CD) patients. In this study, we used cDNA microarrays in a NKX2-3 knockdown B cell line and identified NKX2-3 regulated genes to be involved in endothelial NO production. High-density cDNA microarrays (Illumina Human HT12 v3 Beadchip) representing over 25,000 genes with >48,000 probes were used to determine genome-wide gene expression changes in siRNA-mediated NKX2-3 knockdown B cell line generated from a CD patient. Ingenuity Pathway Analysis (IPA) was used to identify gene networks. mRNA expression levels of argininosuccinate synthetase (ASS1), endothelial nitric oxide synthase (eNOS) and endothelin (EDN1) in comparison to GAPDH were examined by quantitative real-time RT-PCR using cDNA generated from 23 matched pairs of diseased and adjacent non-diseased intestinal tissue from 21 IBD patients (2 patients with 2 pairs from different locations). cDNA microarray analysis was conducted with NKX2-3 knockdown and control cells. Pathway analysis indicated that the most functional gene network impacted by NKX2-3 knockdown involved genes associated with NO production in endothelial cells, including KLF2, ASS1, and EDN1. The production of NO has been shown to regulate anti-inflammatory immune responses. ASS1 is involved in arginine synthesis from citrulline, while eNOS uses arginine as substrate for the synthesis of NO. EDN1 is a potent vasoconstrictor kept in delicate balance by NO. In 23 matched pairs of intestinal tissue from IBD patients, mRNA expression of ASS1 was found to be significantly increased by 13% in diseased vs. non-diseased samples ($p < 0.05$). On the other hand, we detected a significant down-regulation of mRNA expression levels for eNOS (10%) and EDN1 (24%) in diseased vs. non-diseased intestinal tissue ($p < 0.05$ each). Our results indicate a potential decrease in NO production in IBD as shown by down-regulation of eNOS and EDN1. Consistent with recent observations of elevated arginine levels in IBD, we found ASS1, the key enzyme in arginine synthesis, to be up-regulated in IBD. However, the role of high arginine levels in NO production is currently unclear. We speculate that the diminished generation of NO might be regulated by NKX2-3 in IBD and contributes to disruption of vascular homeostasis by imbalanced inflammatory responses.

NF-kB and beta-catenin pathway is essential for migration and invasion in human melanoma.

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Malignant transformation in melanoma is characterized by a phenotype “switch” from E to N-cadherin, which is also associated with invasiveness and progression of the tumor and is a major hallmark of Epithelial-Mesenchymal Transition (EMT). In carcinoma cells, EMT can be associated with tumor growth, invasion and metastasis. EMT can be promoted by various intrinsic signals (e.g. gene mutations) as well extrinsic (e.g. extracellular matrix proteins or growth factors). Crosstalk mechanism between growth factors and integrins, involves the integrin linked kinase (ILK), a serine-threonine protein kinase, which regulates many cellular processes, including growth, proliferation, survival, differentiation, migration, invasion and angiogenesis. ILK exerts control over a diverse set of downstream effectors, in particular, activates Akt by its phosphorylation at Ser 473 and inactivates glycogen synthase kinase 3 (GSK3) by its phosphorylation at Ser 9 in various cell types. One of their major roles is to regulate beta-catenin transcriptional activity. In addition Akt and GSK-3 has been reported to activates the NF-kB subunit p65, increasing the binding of the NF-kB complex to DNA or inactivates the NF-kB signaling, respectively. These results indicate that ILK/AKT/GSK-3 directly mediates not only beta-catenin but also NF-kB activation. The transcription factors, nuclear factor kappa B and beta-catenin were also found to contribute to EMT in a range of human cancers. We were able to show that silencing of ILK gene expression by siRNA process significantly inhibits N-cadherin expression. In addition NF-kB (p65) nuclear translocation and beta-catenin activation are completely abolished upon ILK silencing in melanoma cells. Studies were carried out on human melanoma cell lines. Secretion of matrix metalloproteinases was studied by zymography, and the invasive potential by using Boyden chambers. Expression of cell signaling proteins, was analyzed using Western Blot, siRNA transfection was done for ILK (Ambion). Our results indicate that, ILK signaling is essential for the induction and maintenance of EMT and inhibition of ILK may be a useful strategy for control of tumor invasion and metastasis not only in melanoma.

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Androgen regulation of motility in fibroblasts.

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Steroids influence cell growth, inflammation, wound healing, cardiovascular health, bone integrity, immunity and other processes. Much evidence from our and other laboratories shows that steroids rapidly activate various signaling effectors in target cells. This occurs through a direct interaction of classical steroid receptors with Src, the p85-regulatory subunit of PI3K and other signaling components. Activation of these pathways fosters cell cycle, prevents apoptosis and leads to cytoskeleton changes in reproductive as well as non reproductive cells.

The role of signaling activation in steroid action has been corroborated by findings showing that mouse embryo NIH3T3 fibroblasts express very low amount of classical androgen receptor (AR) that rapidly activates Rac1 upon stimulation with physiologic androgen concentration. Such an activation leads to cytoskeletal changes and cell motility of fibroblasts. The upstream events responsible for androgen induced Rac1 activation have been analyzed. Collected data indicate that rapid, non-genomic androgen action promotes cell spreading and invasiveness.

NF-kappaB activation by DNA double-strand breaks is MDC1 and 53BP1 dependent.

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Cancer therapies such as ionizing irradiation (IR) and DNA topoisomerase I and II inhibitors, rely on the genotoxic effect of DNA double-strand breaks (DSB) to eradicate tumors. ATM kinase, rapidly activated by DSB, orchestrates different aspects of the DNA Damage Response: cell cycle arrest, DSB repair and transcription factors activation. Both NF-kappaB and p53 are activated by DSB, both modify the balance between pro- and anti-apoptotic signals and thereof affect cell survival and the outcome of the cancer treatment.

In this work, the importance of MDC1 protein (Mediator DNA damage checkpoint 1) and of the nuclear foci in the DNA damage signalling to NF-kappaB is studied. As expected, MDC1 reduction by siRNAs resulted in a smaller number of nuclear foci after IR and Camptothecin treatments. We observed that reduced MDC1 level leads to a reduction of NF-kappaB nuclear translocation after both treatments. NF-kappaB transactivation potential was assessed with two reporter systems; either a transiently transfected kappaB-Luc reporter plasmid or a stably integrated kappaB-GFP reporter. In both cases, we noticed a large reduction of the transcriptional activity in cells treated with siRNA-MDC1. The reduction was however less important than the one observed in cells treated with an siRNA-ATM. Mefs WT and KO for MDC1 were tested for NF-kappaB activation by IR and Camptothecin. However, as Mefs WT did not activate NF-kappaB following DNA damage we could not use this model. SiRNA for 53BP1, a protein that is recruited later in the nuclear foci, also reduces drastically NF-kappaB activation after DNA damaging treatments.

In summary, our data indicate that the disruption of nuclear foci amplification and maturation by siRNA-MDC1/53BP1 has an impact on NF-kappaB activation after both IR and Camptothecin treatments contrarily to the disruption of NBS/Mre/Rad50 complex that affects only IR signalling.

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Inhibition of p38 MAPK during cellular activation modulate gene expression of head kidney leukocytes isolated from Atlantic salmon (*Salmo salar*) fed soy bean oil or fish oil based diets

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Head kidney leukocytes isolated from Atlantic salmons fed either a diet based on fish oil (FO) or soy bean oil (VO) were used in order to evaluate if different lipid sources could contribute to cellular activation of salmon innate immune system. A specific inhibitor of p38 MAPK, SB202190, was used to investigate the effect of lipopolysaccharide (LPS) signalling in the head kidney leukocytes. The results show that LPS signals through the p38 MAPK pathway and up regulate *IL-1β*, *TNF-α*, *Cox2* expression in leukocytes isolated from fish fed either diet. The p38 MAPK inhibitor, SB202190, reduced the LPS induced expression of these genes in both dietary groups. In LPS stimulated leukocytes isolated from VO fed fish, SB202190 showed a dose dependent inhibitory effect on *IL-1β*, *TNF-α* and *Cox2* expression, which was not observed in leukocytes isolated from FO fed fish. Furthermore, there was a stronger mean induction of *Cox2* in LPS stimulated leukocytes isolated from the VO group compared to LPS stimulated leukocytes isolated from the FO group. This may indicate that *Cox2* expression can be modulated by dietary fatty acids which may have consequences for the fish's ability to handle infections and stress. LPS stimulation of salmon head kidney leukocytes increased the induction of *CD83*, a dendrite cell marker, but significantly inhibited the expression of *p38MAPK* itself, indicating a p38 MAPK feedback loop. The inhibitor SB202190 alone reduced *hsp27* expression and increased *CD83* expression in salmon head kidney leukocytes, indicating that signalling through p38 MAPK is ligand dependent and affect gene expression differently.

Key words: Atlantic salmon head kidney leukocytes, cellular activation, p38 MAPK, IL-1β, TNF-α, Cox2, hsp27, hsp70, CD83

Inhibitory mechanism of lycopene and docosahexaenoic acid on IL-6 expression in pancreatic acinar cells

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Cholecystokinin (CCK) analogue cerulein causes pathophysiological, morphological, and biochemical events similar to those shown in human pancreatitis. Redox-sensitive transcription factor NF- κ B has a critical role in the pathogenesis of cerulein-induced acute pancreatitis by regulating the expression of pro-inflammatory genes in the pancreas. Lycopene and omega-3 fatty acid docosahexaenoic acid (DHA) have anti-inflammatory effect in various cells. In the present study, we examine the effects of lycopene and DHA on NF- κ B activation and IL-6 expression in cerulein-treated pancreatic acinar cells. The cerulein induced IL-6 expression and NF- κ B activation, which were inhibited by lycopene and DHA in pancreatic acinar cells. In conclusion, lycopene and omega-3 fatty acids may be beneficial for prevention or treatment of pancreatic inflammation by inhibiting expression of inflammatory cytokine and suppressing NF- κ B activation in pancreatic acinar cells.

Effects of Exercise Training on Redox and Inflammatory Signaling in Human Peripheral Blood Mononuclear Cells**Si-Young Kim¹, Young-Soo Lee², and Young-Joon Surh¹**

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There has been considerable accumulation of evidence supporting that exhaustive exercise causes oxidative stress, which may lead to various disorders as well as inflammation. However, it is considered that regular exercise can attenuate oxidative stress and inflammation. In the present study, we investigated the effects of one-bout exhaustive exercise and long-term regular exercise on oxidative stress and inflammatory responses in human peripheral blood mononuclear cells (PBMCs). In the first experiment, twenty volunteers who participated in the guided exercise program were subjected to progressive exercise until they were exhausted on the treadmill followed by resting conditions. Isolated human PBMCs were collected immediately following exercise and after 1 h recovery. One-bout exhaustive exercise induced expression of glutamate-cysteine ligase catalytic subunit (GCLC), interleukin 1 β (IL-1 β), and cyclooxygenase 2 (COX-2) as well as nuclear factor kappa B (NF- κ B) DNA binding activity and phosphorylation of both IKK α and I κ B α in PBMCs. Initial induction of the above oxidative signaling and pro-inflammatory response fully returned to the basal level during 1 h of recovery. In a follow-up experiment, twenty men who participated in the exercise training of different two intensities (40% and 80% of VO₂max) for 4 times per week for 12 weeks were randomly allocated to low- and high-intensity exercise groups (n=10; each group). After 12 weeks, the expression of IL-1 β , p-IKK α , and p-I κ B α in the high-intensity exercise group was significantly decreased in comparison with control and low-intensity exercise groups. On the other hand, there were not significant differences in NF- κ B DNA binding activity and COX-2 expression among all groups, and the levels of glutathione and GCLC in high-intensity exercise group were statistically higher than those in the control group. Taking all these findings into account, although the one-bout exhaustive exercise transiently induced NF- κ B signaling and subsequent inflammatory response, regular exercise may potentiate cellular antioxidant and anti-inflammatory capabilities.

15-Deoxy- $\Delta^{12,14}$ -prostaglandin J₂ inhibits STAT3 signaling and induces apoptosis in human mammary cancer cells**Su-Jung Kim¹, Do-Hee Kim¹, Hye-Kyung Na² and Young-Joon Surh^{1*}****¹National Research Laboratory of Molecular Carcinogenesis and Chemoprevention, College of Pharmacy, Seoul National University, Seoul 151-742, South Korea****²Department of Food & Nutrition, College of Human Ecology, Sungshin Women's University, Seoul 136-742, South Korea****E-mail : nynna79@naver.com; surh@plaza.snu.ac.kr**

Constitutive activation of the signal transducers and activators of transcription 3 (STAT3) occurs frequently in many cancerous and transformed cells. Activated STAT3 contributes to its oncogenic potential by inducing cell proliferation and inhibiting apoptosis. We explore the possibility that 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂ (15d-PGJ₂) could exert an anti-tumorigenic effect by targeting STAT3 in *Ras*-transformed human mammary epithelial (MCF-10A) cells. When MCF-10A-*Ras* cells were treated with 15d-PGJ₂, they underwent apoptotic death as determined by activation of caspases (caspases 3 and 9) and cleavage of poly(ADP-ribose)polymerase (PARP). 15d-PGJ₂ inhibited translocation to the nucleus, DNA binding and dimerization of STAT3. In contrast to 15d-PGJ₂, 9,10-dihydro-15d-PGJ₂, a non-electrophilic analog of 15d-PGJ₂, failed to inhibit STAT3 activation. Moreover, we found that biotinylated 15d-PGJ₂ directly bound to STAT3 protein. Taken together, these results suggest that 15d-PGJ₂ has an anti-tumor activity through inhibition of STAT3 in *Ras*-transformed cells.

Hydrogen peroxide-induced IL-8 expression is mediated by STAT3 activation in gastric epithelial AGS cells

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Reactive oxygen species (ROS) are involved in the pathogenesis of gastric inflammation. IL-8 is a potent mediator of the inflammatory response by activating and recruiting neutrophils to the site of infection. ROS activates STAT3 and induces IL-8 expression in various cells. The present study aims to investigate whether hydrogen peroxide directly turns on the transcription of IL-8 in gastric epithelial AGS cells and whether STAT3 activation mediates IL-8 expression in AGS cells exposed to hydrogen peroxide. As a result, hydrogen peroxide induced the expression of IL-8 and the phosphorylation of STAT3 in a time-dependent manner. The expression of IL-8 was dose-dependently inhibited by treatment of antioxidant N-acetyl-L-cysteine (NAC) in AGS cells. In conclusion, hydrogen peroxide-induced IL-8 expression is mediated by STAT3 activation in gastric epithelial cells.

Effect of N-acetylcysteine on Down Syndrome Candidate Region-1 Protein-mediated cytokine expression**Je Won Ko¹, Sung Hee Jang², Joo Weon Lim¹, and Hyeyoung Kim¹**

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The *Down syndrome candidate region-1* gene (*DSCR1*) is situated close to the Down Syndrome Critical Region (*DSCR1*), which contains genes responsible for many features of Down Syndrome. *DSCR1* modulates IL-1 receptor- mediated signaling pathways to IL-8 expression in HEK (Human Embryonic Kidney) 293 cells. IL-8 expression is induced by oxidant-sensitive transcription factor NF-κB. In the present study, we investigated the effect of antioxidant N-acetyl-L-cysteine (NAC) on *DSCR1*-mediated IL-8 expression in HEK 293 cells. As a result, transfection of *DSCR1* gene to HEK293 cells induced IL-8 expression in the cells, which was augmented by treatment of IL-1β. NAC significantly inhibited IL-8 expression in the IL-1β-treated cells regardless of transfection of *DSCR1*. In conclusion, antioxidant NAC may be beneficial for prevention or treatment of the disease associated with *DSCR1* gene.

TGF-beta and IL-10-mediated resolution of inflammatory reaction is delayed in MMP-9-deficient mice**Elzbieta Kolaczkowska¹, Bernd Arnold², Ghislain Opdenakker³****¹Department of Evolutionary Immunobiology, Jagiellonian University Krakow, Poland,****²German Cancer Research Center, Heidelberg, Germany;****³Rega Institute for Medical Research, University of Leuven, Belgium**

Metalloproteinase 9 (MMP-9, gelatinase B) belongs to Zn²⁺-endopeptidases of the matrix metalloproteinase (MMP) family and its substrates include components of basement membranes and extracellular matrix (e.g. collagens). Therefore the role of MMP-9 in inflammation is generally associated with the onset of the reaction and migration of leukocytes into the inflammatory focus. Indeed, we have shown that in the MMP-9-deficient mice less neutrophils accumulated in peritoneum during early (6 h) zymosan peritonitis. However, we detected that during late peritonitis (24 h) there were more neutrophils in peritoneum of the MMP-9-deficient mice than the wild-type mice and this was due to their impaired apoptosis and compensatory activity of other protease(s). The aim of the current study was to verify if also other anti-inflammatory mechanisms are MMP-9-dependent. For this acute zymosan peritonitis was induced in MMP-9-deficient and wild-type mice and expression (on gene and protein levels; RQ-PCR/ELISA) of anti-inflammatory TGF-β and IL-10 was verified during the peak (6 h) and the termination phase of the reaction (24 h). In contrast to the wild-type controls, in the MMP-9 knockouts the expression of both mediators was significantly attenuated at 6 h while it was significantly augmented at 24 h. Thus the same phenomenon was observed as in the case of neutrophil accumulation. Therefore overall the data suggest that the genetic lack of MMP-9 limits early infiltration of neutrophils but it is not driven by anti-inflammatory mediators. However, the late neutrophil influx paralleled with the high expression of TGF-β/IL-10 which indicates that the anti-inflammatory response is delayed in MMP-9-deficient mice.

NOD2 Interactome.

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The mammalian innate immune system has evolved to detect pathogens-associated molecular patterns (PAMPS). Recognition of PAMPS involves membrane-spanning proteins like the Toll-like receptors (TLR) and cytosolic proteins such as the recently identified NOD-like receptors (NLR). This family is comprised 23 members with important functions in immune sensing in humans, as highlighted by severe inflammatory disorders linked to polymorphisms in some NLR members. Best studied are mutations in Nod2 that are linked to Crohn's disease (CD) and Blau syndrome. Nod2 was shown to sense the bacterial peptidoglycan subunit muramyl-dipeptide (MDP) and to subsequently mediate inflammatory responses in cells by activating the NF-kappa B and MAPKs signalling pathways. This project is consisting in the identification of new Nod2 partners and in the evaluation of their role in the Nod2 signalling pathways. We chose two strategies to select new Nod2 interactants. First, we performed a yeast two hybrid assay using the human ORFeome 15.1 and the protein Nod2 WT or mutated (deletion or CD mutants) as bait. The second proteomic strategy consisted in the purification of Nod2-containing complexes in stably Nod2-expressing cells after MDP treatment or infection by *Listeria monocytogenes* followed by Nod2 partners identification by mass spectrometry. Through the proteomic approach, we obtained several potentially interesting partners: cytoskeleton proteins, scaffold proteins, proteins shuttling between the cytosol and nucleus and interfering with transcription, membrane receptor as well as bacterial proteins from *L. monocytogenes*. By these two complementary approaches, we hope to bring new insights concerning the molecular mechanisms underlying the loss of function of CD mutants and the MDP recognition. This work could open onto new NOD2-dependent host defense mechanisms and/or new strategies used by *L. monocytogenes* to escape from the host immune response.

Glutamine deficiency induces IL-8 expression in gastric epithelial AGS cells

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Glutamine is a conditionally essential amino acid in the state of illness or injury. It regulates the expressions of many genes related to metabolism and signal transduction, and shows anti-inflammatory effects in various cells. Glutamine deficiency induces the expression of pro-inflammatory chemokines including IL-8 through activation of nuclear factor-kappa B (NF- κ B) in some cells. Glutamine provides a precursor for the synthesis of glutathione (GSH), the major endogenous antioxidant, which protects cells from oxidative injury. In the present study, we investigated the inflammatory signaling induced by glutamine deficiency in relation to IL-8 expression in gastric epithelial AGS cells. As a result, glutamine deficiency induced IL-8 expression by activating mitogen-activated protein kinases and redox sensitive transcription factors NF- κ B and AP-1 in AGS cells. Glutamine deficiency-induced IL-8 expression was suppressed by GSH. In conclusion, glutamine deficiency may induce IL-8 expression which is mediated with the activation of MAPK, NF- κ B and AP-1 in gastric epithelial cells.

Isoangustone A blunted high glucose-triggered renal inflammation and fibrosis through disrupting NF-kappaB signaling**Jing Li, Young-Hee Kang****Department of Food Science and Nutrition, Hallym University, Chuncheon, Korea****E-mail: yhkang@hallym.ac.kr**

Development of diabetic nephropathy is associated with hypereglycemia-linked renal inflammation. Increased levels of some proinflammatory factors such as intercellular adhesion molecule-1 (ICAM-1) and monocyte chemoattractant protein-1 (MCP-1) have been found in diabetic patients with nephropathy. The present study was to test whether Isoangustone A (IsoA), a phenolic compound found in licorice, can inhibit proinflammatory factors promoted by high ambient glucose (HG) in human mesangial cells (HRMC) through blocking nuclear translocation of NF-kappaB. Serum starved HRMC were cultured in media containing 5.5 mM glucose plus 27.5 mM mannitol as an osmotic control or 33 mM glucose for 3 days in the presence of 1-20 micromole IsoA. Exposure of HRMC to HG caused a marked increase in ICAM-1 expression, which was dose-dependently suppressed by IsoA. In addition, IsoA appeared to retard HG-triggered MCP-1 mRNA upregulation. Such effects were mediated most likely through disturbing NF-kB signaling. Furthermore, exposure of cells to HG caused marked increases in collagen secretion and CTGF expression, which was dose-dependently reversed by IsoA at the transcriptional levels. IsoA boosted HG-plummeted type matrix metalloproteinase-1 (MT-1 MMP) expression and dampened HG-elevated tissue inhibitor of MMP-2 (TIMP-2) expression, facilitating the degradation of mesangial matrix. The results demonstrate that the bioactive IsoA diminished mesangial inflammation and matrix accumulation in response to ambient HG through retarding NF-kappaB signaling transduction. Therefore, IsoA may be a potential therapeutic agent for diabetes-associated renal inflammation and fibrosis. This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MEST)" (The Regional Research Universities Program/Medical & Bio-Materials Research Center)

Signaling Pathways involved in the Poly-L-arginine - induced IL-6 and IL-8 Release in Cultured Human Bronchial Epithelial Cells, 16HBE14o-**J.F. T. Liang, A.W.M. Chow, W.C.Y. Yip and W.H. Ko****School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, Shatin, Hong Kong. E-mail: whko@cuhk.edu.hk**

Eosinophil-derived cationic proteins, particularly major basic protein (MBP), are implicated in the pathogenesis of bronchial asthma. It is established that MBP actions could be mimicked by synthetic poly cations poly-L-arginine. Previous data of our lab confirmed that poly-L-arginine may contribute to neutrophilic airway inflammation by inducing the release of pro-inflammatory cytokines IL-6 and IL-8, which are known to modulate the recruitment, activation and survival of neutrophils, in a human bronchial epithelial cell (16HBE14o-) model.

The mitogen-activated protein (MAP) kinase family and NF κ B signaling pathways are central in mediating certain cytokines expression. In this study, we examined the involvement of MAP kinases and NF κ B pathways in poly-L-arginine induced release of IL-6 and IL-8 in 16HBE14o- cells. Co-incubation studies showed that SB202190, a specific inhibitor of p38 MAP kinase and BAY 11-7085, an inhibitor of NF κ B pathways, dramatically reduced poly-L-arginine - stimulated IL-6 and IL-8 production. However, PD98059, inhibitor of extracellular-signal-regulated kinase [ERK] pathway, did not alter IL-6 or IL-8 levels in poly-L-arginine - stimulated cells. Activation of P38 MAPK pathway was further confirmed by Western blotting, which showed that phosphorylated P38 MAPK was elevated in poly-L-arginine treated cells. Immunofluorescent assay demonstrated translocation of NF κ B from the cell cytoplasm to the nucleus and confirmed NF κ B activation.

These data indicate a role for P38 pathways and NF κ B pathways in poly-L-arginine induced release of IL-6 and IL-8 in human bronchial epithelial cells. These mechanisms may be potential pharmacotherapeutical targets for inhibition of the MBP mediated airway inflammation.

Role of protease activated receptor-2 on *Helicobacter pylori*-induced IL-8 expression and apoptosis in gastric epithelial cells**Joo Weon Lim and Hyeyoung Kim**

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Hallmarks of *Helicobacter pylori* (*H. pylori*)-induced gastric injury are inflammation and apoptosis. Protease activated receptor-2 (PAR-2), a novel family of G protein-coupled receptors, is activated by serine proteases such as trypsin and tryptase. PAR-2 is present in gastric epithelial cells and activated by *Helicobacter pylori* (*H. pylori*) infection. Previously, we demonstrated the presence of trypsinogen 1 and 2 which may be converted and activated to trypsin in *H. pylori*-infected gastric epithelial AGS cells. The present study aims to the role of PAR-2 in *H. pylori*-induced expression of pro-inflammatory cytokine IL-8 and apoptosis in gastric epithelial AGS cells. As a result, *H. pylori* induced both activation and expression of PAR-2 in AGS cells. IL-8 expression and apoptosis (determined by DNA fragmentation and cell viability) were induced by *H. pylori* in AGS cells, which were inhibited by transfection with antisense (AS) oligonucleotide (ODN) for PAR-2. *H. pylori* induced activation of mitogen-activated protein kinases (MAPKs) including extracellular signal-regulated kinases (ERKs), p38 kinase, and c-Jun NH₂-terminal protein kinases (JNKs). *H. pylori*-induced MAPKs were suppressed in the cells transfected with AS ODN for PAR-2. In conclusion, the activation of MAPKs, IL-8 expression and apoptotic cell death are mediated by the activation of PAR-2 in *H. pylori*-infected gastric epithelial cells. Inhibition of PAR-2 may be beneficial for the treatment and prevention of gastric damage caused by *H. pylori* infection.

Identification of disease-associated DNA methylation in intestinal tissues from patients with inflammatory bowel disease

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Inflammatory bowel disease (IBD) is a complex disease with a wide variety of clinical presentations. Crohn's disease (CD) and ulcerative colitis (UC) are the major subtypes of IBD. Overwhelming evidence supports the theory that IBD is caused by genetic predisposition of multiple genes and an abnormal interaction with environmental factors. With genome-wide association studies, currently more than 32 genes/loci have been identified for IBD susceptibility genes. However, the risk conferred by all the genetic risk factors discovered so far can only account for approximately 20% of the genetic risk. This suggests that other factors, including possible epigenetic factors, are involved in IBD pathogenesis. In this study, we have explored the role of DNA methylation in IBD pathogenesis. Fifty-four intestinal tissues (26 diseased and 26 matched normal) from 26 IBD patients (9 CD and 17 UC) used in this study were collected at the time of surgery and then classified by a pathologist as diseased and paired with normal tissue for each IBD patient. Genomic DNA was isolated and unmethylated C in the genome was converted to T by bisulfite treatment. The converted genomic DNA was used for methylation profiling using GoldenGate™ BeadArray (1505 CpG sites of 807 genes). The microarray data were then validated by the PCR based-RFLP method. After initial identification of a training set (14 non-diseased and 14 diseased CpG sites) and subsequent validation with a testing set (12 non-diseased and 12 diseased CpG sites), we identified 7 CpG sites that are differentially methylated in intestinal tissues of IBD patients ($p < 0.05$, mean β value difference across the samples 0.05-0.19). These CpG sites included AATK_P709_R, BGN_P333_R, GABRA5_P862_R, MAPK10_E26_F, SERPINA5_P156_F, STAT5A_P704_R, and TNFRSF1A_P678_F. Of these methylated genes, STAT5A, SERPINA5, and BGN are of particular interest. We further studied DNA methylation in CD and UC patients. We observed that 25 methylated CpG sites exhibited significantly differential methylation in diseased intestinal tissues of CD patients ($p < 0.02$ and β value difference > 0.1), and 13 methylated CpG sites exhibited significant differential methylation in UC patients ($p < 0.02$ and β value difference > 0.1). These results indicate that IBD-associated DNA methylation is disease subtype-specific. We used IPA pathway analysis to examine the pathways and gene networks in which these IBD-associated genes are involved. The genes that were preferentially methylated in CD patients highlight an interesting network of 4 CD associated methylation genes, FGF2, NOTCH4, S100A4, and SPARC, and a glucocorticoid receptor (NR3C1)-related gene network for several genes preferentially methylated genes in UC patients.

IKK α is required for maintaining skin homeostasis and preventing skin tumor development

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IKK α is required for the formation of the embryonic epidermis. Loss of IKK α prevents keratinocytes from terminal differentiation and promotes keratinocyte proliferation *in vivo* and *in vitro*. Furthermore, downregulation, altered localization, mutations, and/or loss of heterozygosity (LOH) of *Ikk α* have been reported in squamous cell carcinoma (SCC) of the skin, lungs, esophagus, and head and neck in humans, highlighting the importance of IKK α in human cancers. It has long been known that excessive mitotic activity due to H-Ras can block keratinocyte differentiation and cause skin cancer. It is not clear whether there are any innate surveillants that are able to ensure that keratinocytes undergo terminal differentiation, preventing the disease. IKK α induces keratinocyte terminal differentiation and its reduction promotes skin tumor development. However, its intrinsic function in skin cancer is unknown. Thus, our lab generated *Ikk α* conditional knockout mice, deleted IKK α in keratinocytes in mice by using keratinocyte specific Cre mice, and then examined the effect of IKK α loss on skin development and maintenance, and skin tumorigenesis. We found that mice with IKK α deletion in keratinocytes developed a thickened epidermis and spontaneous squamous cell-like carcinomas. Inactivation of epidermal growth factor receptor (EGFR) or reintroduction of IKK α inhibited excessive mitosis, induced terminal differentiation, and prevented skin cancer through repressing an EGFR-driven autocrine loop. We also identified that IKK α repressed expression of several EGFR ligands at their transcription level, thereby inhibiting the pathway of EGFR and Ras. Thus, IKK α serves as an innate surveillant. These findings shed light on therapeutic targets for preventing IKK α -related skin cancer development. We are currently investigating how inflammation effects IKK α defect-mediated skin tumor development.

Downregulation of ITGB4 expression on airway epithelial cells inhibit the Th1 inflammation response

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Objective: Adhesion molecule integrin beta4 (ITGB4) play an important role in constitutive adhesion of airway epithelium. The preliminary study of our group found that loss of expression of ITGB4 and typical Th2 inflammation bias has been observed in the asthmatic airways. The airway epithelial cells have been demonstrated as a kind of accessory antigen presentation cells (APC) to activate T cells which may play an important role in the development of allergic airway inflammation of asthma. The purpose of this study is to find the connection of decreased expression of ITGB4 with adaptive immune response of airway epithelial cells.

Method: We silence ITGB4 expression in bronchial epithelial cells by small interference RNA and studied effects on antigen presentation ability. T cells proliferation and cytokine production were also investigated after co-culturing with ITGB4 silence epithelial cells.

Result: Small interference RNA silencing of ITGB4 resulted in impaired antigen presentation ability. ITGB4 silent epithelial cells inhibited Th1 cells activation, proliferation and secretion of Th1 cytokine IFN-gamma, while secretion of Th2 cytokine was unaffected.

Conclusion: Down regulation of ITGB4 expression on airway epithelial cells may impair the antigen presentation ability and inhibits the Th1 inflammation response in allergic asthma.

Modulation of chronic microglial inflammation through Liver X Receptor activity.

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Alzheimer's Disease (AD) is a neurodegenerative disorder. AD is characterized by the formation of extracellular senile plaques generated by the aggregation of the β -amyloid (A β) peptide. The presence of these aggregates causes chronic microglial activation, which induces an inflammatory state and a subsequent neuronal loss in AD patients. The oligomeric A β conformation (olA β) is known to be the most neurotoxic peptide form. Moreover, we have recently shown that this form is also the most potent inflammatory activator. Microglia are the resident tissue-macrophages of the brain. It is known that activated macrophages can differentiate towards a continuous spectrum of phenotypes of which the two extreme states are M1 for the pro-inflammatory state and M2 for the anti-inflammatory state. Our team and others have described these two phenotypes in microglial cells. Moreover, we have shown that olA β induces the pro-inflammatory M1 state. The Liver X Receptors (LXRs) are members of the nuclear receptor family. Two isoforms of LXR exist: LXR α and LXR β . LXRs are known to regulate inflammatory responses. In fact, activation of LXRs by specific agonists is able to repress pro-inflammatory gene expression in activated microglia. The aim of this project is to study whether activated LXR is able to shift microglia from an olA β -induced M1 state towards the anti-inflammatory M2 state.

Molecular and Morphological Characterization of Piecemeal Degranulation in Human Neutrophil Azurophilic Granules

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Mediators pre-stored in neutrophil azurophilic granules are central to the acute inflammatory response and tissue degradation and damage through their proteolytic activity. Different granule populations mobilize and release their content via distinct and hierarchical molecular mechanisms. The molecular mechanisms by which mediators pre-stored in azurophilic granules are mobilized and released to the extracellular space remain largely unknown. We used a number of complementary techniques including; confocal laser scanning microscopy, subcellular fractionation, flow cytometric analyses, Western blot analyses and electron microscopy to examine the ultrastructural and molecular nature of mediator release in neutrophil azurophilic granules. We found that following IL-8 activation, neutrophil azurophilic granules undergo piecemeal degranulation (selective mediator release) leading to altered granule content. Piecemeal degranulation of azurophilic granules is characterized by budding of small secretory vesicles and consequent reduction in granule density. Furthermore, budding of small secretory vesicles and selective mediator mobilization and release from azurophilic granules is associated with reduced localization of CD63, Hck and β-arrestin-1 to granule membranes and also cell surface upregulation of these molecules. Our study is first to identify piecemeal degranulation as a potential underlying mechanism of mediator release from neutrophil azurophilic granules and supports the involvement of CD63, Hck, and β-arrestin-1 in this process.

Modulatory actions of estrogen in transactivations of SXR-mediated liver X receptor response element (LXRE) and CAR-mediated phenobarbital response element (PBRU) in human hepatoma cell line, HepG2

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Inflammatory response involves transcriptional regulation of nuclear receptors that play important functions in mediating lipid and drug metabolism in the kidney and liver. For example, expression of liver X receptor (LXR) and liver X receptor response element (LXRE)-driven transcription of several LXR target genes were reported to be regulated by inflammatory agents such as cytokines. And an orphan nuclear constitutive androstane receptor (CAR) that modulate phenobarbital response element (PBRU) of cytochrome P450 genes is also involved during inflammation. This study reports modulatory actions of estrogen in transactivations of SXR-mediated LXRE and CAR-mediated PBRU in HepG2 cells. HepG2 cells were transiently transfected with either (LXRE)₃-tk-luciferase reporter or PBRU2C1luciferase reporter plasmids. When cells were expressed exogenously with estrogen receptor (hER-alpha) and steroid and xenobiotic receptor (SXR), treatment with rifampicin and corticosterone promoted SXR-mediated transactivation of LXRE reporter gene 13-fold and 30-fold respectively, as compared with ethanol or 17-beta-estradiol treatment. However, combined treatment with estrogen plus rifampicin or corticosterone resulted in less than 50% in mean values of transactivation by rifampicin and corticosterone alone. These results may suggest that ligand-bound SXR can transactivate LXRE and estrogen has repressive effects via ER. The CAR-mediated PBRU transactivation was not influenced by Mox-estrol in the absence of transfected hER-alpha, but transfection with hER-alpha slightly stimulated CAR-mediated PBRU2C1luciferase both in the absence and in the presence of the CAR agonist, TCPOBOP. More interestingly, the potentiation by estrogen receptor was significantly repressed by Mox-estrol in the agonist-bound CAR transactivation of PBRU. Thus, estrogen may play modulatory roles in transactivation of LXRE and PBRU of nuclear receptor target genes.

Notch Inhibition in Astrocytes: Implication for Inflammation and Microarray Analysis

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In the injured CNS, astrocytes appear as a key component of reactive gliosis. Reactive astrocytosis is defined as a hypertrophy and often hyperplasia of these cells that is associated with an increased synthesis of GFAP and cytokines. The Notch pathway is implicated in many aspects of the CNS development and functions. More recently, the Notch pathway has been identified to be involved in inflammatory events of the CNS. In an attempt to understand the implication of this pathway on astrocytes, we have characterized the Jagged-Notch-Hes pathway under inflammatory conditions. LPS exposure induced an upregulation of Jagged1 expression on astrocytes. To address the role of Jagged1 in the modulation of inflammation, we used a siRNA mediated silencing of Jagged1 (siRNA J1). siRNA J1 repressed the mRNA expression of genes known as hallmarks of the gliosis. On activated astrocytes, the inhibition of Jagged1 had anti-inflammatory effects and resulted in a decrease of LPS-induced proinflammatory cytokines as well as the iNOS expression. Moreover, Jagged1 inhibition modulated the NF-κB and JAK/STAT pathways. In the current study, Agilent whole rat genome oligonucleotide microarrays (41000K) were used to examine alterations in gene expression of astrocytes treated with siRNA J1. DNA microarray data filtration by intensity signal, x-fold change and p-value, revealed 3978 genes varying for the comparison ctr/siRNA J1 and 3577 genes for LPS/LPS+siRNA J1. Several clusters of genes involved in various cellular processes such as immune response, cell signaling, cell adhesion and many others were altered by the inhibition of the Notch pathway. Previous results showing a modulation of the NF-κB and JAK/STAT pathways could be confirmed in the microarray study. Interestingly, other pathways showed important variations due to Jagged1 inhibition (ex: IGF-1 -, EGF -, chemokine - signaling), highlighting possible crosstalks between these signaling pathways and the Notch pathway.

Induction of atherogenic changes in vascular endothelial cells by radiation: role of the receptor for advanced glycation endproducts**Jens Pietzsch**

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Exposure to ionizing radiation is a prominent factor associated with increased cardiovascular morbidity and mortality. The aim of this study was to assess acute effects of moderate dose X-ray radiation (2 to 10 Gy) on atherogenic parameters in arterial (HAEC) and microvascular (HDMEC) endothelial cells. Three days after irradiation, activation of NF- κ B, functional expression of the receptor for advanced glycation endproducts (RAGE), production of cytokines (TNFalpha, IL-6, MCP-1), leukocyte adhesion, and endothelial cell injury (thrombomodulin (TM)) was assessed in all cells compared to sham-irradiated controls. Irradiation of EC resulted in a dose-dependent augmentation of all parameters studied. When irradiated EC were treated with RAGE ligands (glycated LDL, recombinant human S100 calcium binding proteins/calgranulins, cell debris from irradiated leukocytes), both production of cytokines and leukocyte adhesion was further upregulated. Moreover, a dose-dependent induction of EC injury (TM deficiency) could be observed after treatment with RAGE ligands. The effects modulated by RAGE were attenuated in the presence of molar excess of soluble RAGE (sRAGE). Taken together, the data suggest that after exposure to ionizing radiation the concerted action of autocrine and paracrine activation loops and, furthermore, multiligand ligation of the pattern recognition receptor RAGE contributes to a highly inflammatory and atherogenic state in vascular endothelial cells.

Molecular and functional links between the IKK complex, proteasomes and the CSN signalosome**Johannes A. Schmid , Hannah Neumeier and Lukas Orel.****Center for Biomolecular Medicine and Pharmacology, Dept. of Vascular Biology,
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A variety of signaling pathways regulating inflammation, cell development and cell survival require NF-kappa B transcription factors, which are normally inactive due to binding to inhibitors, such as I-kappa B alpha. The canonical activation pathway of NF-kappa B is initiated by phosphorylation of the inhibitor by an I-kappa B kinase (IKK) complex triggering ubiquitination of I-kappa B alpha by SCF-type E3-ligase complexes and rapid degradation by 26S-proteasomes. The ubiquitination machinery is regulated by the COP9 signalosome (CSN), a complex of eight proteins homologous to 19S proteasome regulator subunits. We show that IKK-complexes interact with a functional super-complex consisting of SCF, CSN and 26S-proteasomes, providing an explanation for the rapid signaling-induced degradation of I-kappa B alpha. We found that IKK's phosphorylate not only I-kappa B alpha, but also the CSN-subunit Csn5/JAB1 and that this subunit serves as negative regulator of basal NF-kappa B activity (Orel et al., 2009). The inhibitory effect requires a functional Csn5-MPN+ metalloprotease domain, which is responsible for cleaving ubiquitin-like Nedd8-modifications. Upon activation of cells with TNF-alpha, CSN dissociates from IKK's allowing full and rapid activation of the NF-kappa B pathway by the concerted action of interacting protein complexes. Furthermore, we have evidence that IKK molecules phosphorylate subunits of 26S proteasomes and influence proteasomal activity. All together our observations imply multifaceted mutual regulatory processes between protein complexes involved in NF-kappa activation, ubiquitination and proteasomal degradation.

Ref: Orel L., Neumeier H., Hochrainer K., Binder B.R. and Schmid J.A. Crosstalk between the NF-kappaB activating IKK-complex and the CSN signalosome. *J. Cell. Mol. Med.* (2009) in press; published online July 28th 2009.

The role of TAK1 signaling in epithelial cells in maintaining intestinal homeostasis

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Disruption of the intestinal barrier results in overproduction of proinflammatory mediators, which are associated with inflammatory bowel disease. Given that TGF- β -activated kinase 1 (TAK1) is an upstream signaling molecule of the NF- κ B-pathway, it is suspected that TAK1 might regulate epithelial growth, differentiation and apoptosis. To investigate the impact of TAK1 in the intestinal epithelium, we generated two transgenic mouse lines which overexpress functional active TAK1 (TAK1wt) or the inactive dominant negative variant of TAK1 (TAK1dn) specific in intestinal epithelial cells (IECs). RT-PCR and western blot analysis of sorted IECs exhibited the specific upregulation of TAK1wt and TAK1dn expression on RNA and protein level. In addition, gene expression analysis of IECs isolated from TAK1wt and TAK1dn demonstrated distinct expression profiles in comparison to non transgenic IECs. However, under normal conditions no spontaneously intestinal inflammation was observed. In future experiments we set out to investigate the role of TAK1 in acute and chronic colitis as well as in intestinal infections.

Association of NF-kB and Bax expression with apoptosis in varicose veins of aging women**Helle Evi Simovart¹, Marina Aunapuu¹, Jüri Lieberg², Andres Arend¹****¹Department of Anatomy, University of Tartu, Estonia****²Surgery Clinic, University of Tartu, Estonia****Department of Anatomy, University of Tartu, 19 Ravila St., Tartu 50411, Estonia****e-mail:helle-evi.simovart@ut.ee**

The aetiology and pathophysiology of varicose vein disorders still remain unclear. NF-kB is implicated in multiple physiological and pathological processes, including cell proliferation and differentiation, inflammatory and immune response, cell survival and apoptosis. Thus changes in NF-kB expression, apoptosis and apoptosis associated proteins in veins of patients may contribute to an increased risk of varicosities. The aim of the study was to investigate the expression of NF-kB and Bcl-2 associated protein x (Bax) together with apoptosis (Ao) of endothelial cells (EC) and smooth muscle cells (SMC) in varicose veins of aging women. Women (n=30) undergoing the excision of varicose veins were divided into 3 groups: younger than 35 years (I), 35-50 years (II), older than 50 years (III). Apoptosis was determined using TUNEL method, NF-kB and Bax expressions were investigated immunohistochemically. Apoptotic cell percentages were counted in the EC and in SMC. Remarkable changes took place in the varicose veins of group II, in which NF-kB expression had the lowest level in all layers of the vein wall. Especially low NF-kB level was found in the media. NF-kB level rose again in all layers of the vein wall in group III. In group I NF-kB level was significantly higher in the adventitia. In group II Bax level, on the contrary, increased in all layers of the vein wall and the most intensive expression of Bax was found in the media. The percentage of apoptotic EC of varicose veins increased in groups II and III, but the percentage of apoptotic SMC was higher in group II.

In conclusion, the results demonstrate that changes of NF-kB and Bax expressions in the wall of varicose veins were different in middle-aged women, but were approximately at the same level in young and older women. EC and SMC apoptosis increased with advancing age.

Expression of CD44 on lymphocytes during experimental inflammatory response of bovine mammary gland**Petr Slama****Department of Animal Morphology, Physiology and Genetics, Mendel University in Brno, Zemedelska 1, 613 00 Brno, Czech Republic, petr.slama@mendelu.cz**

CD44 is a proteoglycan that is expressed by most cell types (leukocytes, fibroblasts, neurons and myeloid cells). CD44 plays a role in leukocyte trafficking to extra lymphoid sites of inflammation or as a non-specific accessory adhesion molecule¹. The aim of this study was to inquire development over time of the surface expression of CD44 on lymphocytes during an inflammatory response of bovine mammary gland induced by lipopolysaccharide (LPS) and muramyl dipeptide (MDP). Intramammary application of LPS and MDP resulted in a significant increase in the proportion of CD44-positive lymphocytes after 24 hours. During resolution of inflammatory response, there was observed a decrease in the proportion of CD44-positive lymphocytes. The results suggest that the cell surface receptor CD44 play an important role in inflammatory response of bovine mammary gland to bacteria and their toxins and components.

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The human paracaspase MALT1 is regulated by an intracellular serpin

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MALT1 is a caspase-like cysteine dependent protease that plays a key role in immunity and inflammation. Antigen receptor activation on T and B cells has recently been shown to induce the MALT1-mediated cleavage and inactivation of the ubiquitin-editing protein A20 (also known as TNFAIP3), which is known to have anti-inflammatory as well as tumor suppressor activities. Inhibition of MALT1 paracaspase activity therefore offers interesting potential in the treatment of autoimmune diseases or certain tumors.

To understand the molecular mechanisms that regulate MALT1 protease activity, we focused on their potential regulation by serpins. Serpins are serine- and cysteine- dependent protease inhibitors, and inhibition of caspases or metacaspases by specific serpins has already been demonstrated before. For example, the Cowpox virus serpin CrmA is an efficient inhibitor of caspase-1 and 8, and AtSerpin1 inhibits Arabidopsis metacaspase-9.

A screening of human intracellular (B-clade) serpins with a cleavage site fitting the substrate preference of MALT1 revealed a single serpin with MALT1 inhibitory potential. The serpin forms reduction-sensitive high-molecular weight complexes with proteolytically active MALT1 and inhibits MALT1-induced NF- κ B activation and A20 cleavage. These data provide novel insight in the molecular regulation of MALT1.

Increased inflammatory response in diabetic placenta may depend on histamine concentration and expression of bradykinin receptors.

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BACKGROUND: Increased histamine content and bradykinin levels were reported within diabetic placenta. Both compounds are well known mediators of the inflammatory reactions. Bradykinin induces mast cell degranulation, leading to increase in the local histamine concentration. Bradykinin B1 receptor shows strong involvement in the inflammatory response, whereas the B2 receptor mediates most of the effects induced by kinins. The aim of this study was to examine comparatively correlation between placental histamine concentration and placental bradykinin receptors B1 and B2 expression in diabetes class C versus normal pregnancy. **MATERIAL AND METHODS:** Sixteen diabetic placentae were compared with 16 normal placentae (Group I and II, respectively). Histamine concentrations in placental cuts were estimated fluorimetrically. Expression of B1 and B2 was examined in immunostained paraffin sections by quantitative morphometry in the areas matched in mean vascular density. **RESULTS:** Histamine concentration in Group I was significantly increased compared to Group II (387 ± 25.3 versus 239 ± 14.3 ng/g of wet weight \pm SEM). Mean expression of the B1 was augmented in diabetes and reached 289.8% of the value for Group II ($p<0.05$). The differences in mean expression of B2 receptors were non significant. **CONCLUSION:** Increased amounts of histamine in placental tissue in diabetes producing pro-inflammatory conditions may change vascular properties to some degree by influence on bradykinin receptors expression and vice versa (i.e. bradykinin may affect action of histamine). Pro-inflammatory reactions mediated via B1 should be expected rather than changed vasomotor reactivity related to B2. Angiogenic properties of histamine and kinins should also be considered.

MageD2, a newly identified ATM substrate, regulates the DNA damage induced transcriptional response

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Cellular responses to DNA damage are crucial for maintaining cell homeostasis and preventing the development of cancer. Of the many types of DNA damage that exist, the most dangerous is DNA double strand break (DSB). Ataxia Telangiectasia mutated (ATM) is a serine-threonine kinase, present in the nucleus that is activated by DNA double-strand breaks (DSBs). This kinase phosphorylates a number of proteins (including p53, Chk2, BRCA1,...) involved in cell cycle checkpoint control (G1/S, intra-S and G2/M), apoptosis, transcription responses and DNA repair.

In order to identify new ATM partners, we have performed a yeast 2 hybrid experiment with two ATM fragments and a HeLa cDNA library. We have identified 18 proteins and one was selected to verify its interaction with ATM in an eukaryotic environment and investigate its potential role in DSB cellular response.

MageD2 is a member of the MAGE (Melanoma Antigen) genes family and is expressed in various normal adult human tissues. This protein has a MAGE homology domain and a hydrophobic domain. The functions of the MAGE genes are partially unknown but it would seem that the MAGE genes are important for cell survival, cell cycle progression and apoptosis.

According to the known ATM target consensus sequence “SQ”, we have identified four ATM putative phosphorylated sites within the primary structure of mage D2. *In vitro* kinase assays have allowed us to establish that mageD2 is a novel substrate of ATM kinase, and that 3 serines are phosphorylated. The importance of these phosphorylations in the DNA Damage Signaling Pathway is investigated.

Cigarette smoke and alpha,beta-unsaturated aldehydes elicit VEGF release through the p38 MAPK pathway in human airway smooth muscle cells and lung fibroblasts

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The angiogenic factor vascular endothelial growth factor (VEGF) is elevated in the sputum of chronic obstructive pulmonary disease (COPD) smokers as well as in the asymptomatic smokers and correlate with smoking pack years. Consistent with this notion is our observation that inhalation of cigarette smoke for 3 days elevated VEGF in broncho-alveolar lavage fluid (BALF) of mice. In order to investigate the mechanism through which cigarette smoke elicits VEGF release we challenged cultured human airway smooth muscle cells (ASMC) and normal human lung fibroblasts (NHLF) with aqueous cigarette smoke extract (CSE). VEGF release was characteristically elevated by sub-toxic concentrations of CSE in both ASMC and NHLF, in a concentration-dependent fashion. In both ASMC and NHLF cultures, CSE-evoked VEGF release was mimicked by acrolein, crotonaldehyde and 4-hydroxy-2-nonenal (4-HNE) at concentration range of 10-100 uM, and fully prevented by a scavenger of alpha,beta-unsaturated aldehydes, N-acetylcysteine (NAC, 0.3 mM). CSE-evoked VEGF release was accompanied by a rapid and lasting p38 MAPK and ERK1/2 phosphorylation in ASMC which was blocked by NAC and mimicked by acrolein. VEGF release elicited by both acrolein and CSE was abolished by pharmacological inhibition of p38 MAPK signaling but not by selective inhibitors of ERK1/2 or PI3K. Taken together, our data indicate that alpha,beta-unsaturated aldehydes of endogenous (4-HNE) or exogenous (smoke) origin, evoke VEGF release from pulmonary resident cells via p38 phosphorylation pathway. Given the pivotal role of VEGF as angiogenic factor, our results shed light on the mechanisms through which cigarette smoke can initiate vascular remodeling in the lung.

Key words : COPD, Airway smooth muscle cells, fibroblasts, VEGF, p38, acrolein.

Activation of human CTNNAL1 gene promoter by LEF-1 and AP-2 α in human bronchial epithelial cells**Yang Xiang, Xiao-Qun Qin*, Yu-Rong Tan, Chi Liu, Fei Qu, and Hui-Jun Liu****Dept of Physiology, Xiangya School of Medicine, Central South University, Changsha 410078, Hunan, China - *E-mail xiaoqun1988@xysm.net**

Adhesion molecules play vital roles in airway hyperresponsiveness or airway inflammation. Our previous study indicated an alpha-catenin-related protein, catenin alpha-like 1 (CTNNAL1) was downregulated in asthma patients and animal model. We also observed that the expression of CTNNAL1 was increased with the acute ozone stress. CTNNAL1 contribute to the wound repair and proliferation of human bronchial epithelial cells (BECs). In the present study, we determined molecular mechanisms of CTNNAL1 regulation in human BECs. 8 oligonucleotide probes corresponding to various regions of the CTNNAL1 promoter were used in EMSA (electrophoretic mobilityshift assays). 5 were found to have an enhanced mobility shift with extracts from BECs. On the basis of the assay of mutated probes and antibody supershift, they were verified as LEF-1, AP-2 α and CREB. Next, ChIP (chromatin immunoprecipitation) assay was used to observe the interaction between these transcription factors and CTNNAL1 promoter. Only AP-2 α and LEF-1 show the binding on CTNNAL1 promoter. By site-directed mutagenesis of putative transcription-factor-binding sites within pGL3/FR/luc, we observed a reduction in human CTNNAL1 promoter activity of mutants of both AP-2 α and LEF-1 sites. The time courses of AP-2 α and LEF-1 activation, followed by CTNNAL1 expression were also examined. It was shown that ozone stress can activate the AP-2 α and LEF-1 within one hour, ozone-inducible CTNNAL1 expression and AP-2 α and LEF-1 binding activity correlated during a 16 hour time course. Our data suggest that a robust transcriptional CTNNAL1 up-regulation occurs during acute ozone-induced stress and is mediated at least in part by ozone-induced recruitment of LEF-1 and AP-2 α to the human CTNNAL1 promoter.

Effect of prostaglandin E₂ on monocyte chemoattractant protein-1 expression in *Helicobacter pylori*-infected gastric epithelial AGS cells

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Monocyte chemoattractant protein (MCP-1) plays an important role in the recruitment of monocytes to the site of tissue injury. We previously demonstrated that *Helicobacter pylori* (*H. pylori*) in Korean isolates induces the expression of cyclooxygenase-2 (COX-2) and MCP-1 which is mediated by the activation of AP-1 and NF- κ B in gastric epithelial AGS cells. Prostaglandin E₂ (PGE₂) is a product of COX-2 in various cells. The present study aims to investigate the role of PGE₂ on *H. pylori* -induced MCP-1 expression in AGS cells. As a result, *H. pylori* induced expression of MCP-1. Treatment of PGE₂ enhanced the *H. pylori*-induced MCP-1 expression. The expression of MCP-1 up-regulated by *H. pylori* and PGE₂ was inhibited by H-89, an inhibitor of protein kinase A (PKA). The results indicate that PGE₂ modulates *H. pylori*-induced MCP-1 expression in a PKA-dependent manner. DNA-binding activity for cAMP response element binding protein (CREB), which is reported to be mediated by protein kinase A, was increased by *H. pylori* and PGE₂. Furthermore, PGE₂ augmented *H. pylori*-induced activation of AP-1 and NF- κ B in AGS cells. In conclusion, PGE mediates inflammatory signaling including NF- κ B, AP-1 and CREB and induction of MCP-1 in a PKA-dependent manner in *H. pylori*-infected gastric epithelial cells.

Selective regulation of nuclear orphan receptors 4A by adenosine receptor subtypes in activated mast cells

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Nuclear orphan receptors 4A (NR4A) belong to the superfamily of hormone receptors and comprise NR4A1 (Nur77), NR4A2 (Nurr1) and NR4A3 (NOR-1). These early responsive genes have been associated to transcriptional activation of multiple genes involved in inflammation, apoptosis and cell cycle control. In this study, we established a link between NR4As and adenosine, a paradoxical inflammatory molecule that can contribute to persistence of inflammation or mediate inflammatory shutdown. Transcriptomics screening of the human mast cell-line HMC-1 revealed a sharp induction of transcriptionally active NR4A2 and NR4A3 by the adenosine analogue NECA. A2AAR silencing exaggerated this effect, suggesting that upregulation of these factors is mediated by other AR subtypes (A2B and A3) and that A2AAR activation counteracts NR4A2 and NR4A3 induction. A similar effect was observed by the concomitant treatment with NECA and the A2AAR selective antagonist SCH-58261. Interestingly, upregulation of these factors correlated with ERK1/2 phosphorylation (but not with CREB activation) and could be partially reverted by PKC or MEK1/2 inhibition. Furthermore, selective A2AAR activation with CGS-21680 decreased both PMA/ionomycin-induced ERK1/2 phosphorylation and upregulation of functional nuclear orphan receptors 4A. Taken together, these results establish a novel PKC/ERK/nuclear orphan receptors 4A axis for adenosinergic signalling in mast cells, which can be modulated by A2AAR activation, not only in the context of adenosine but of other mast cell activating stimuli as well.

Session II: Inflammatory Mediators

Lipoic acid prevents wound healing inhibition induced by dietary n-3 fatty acids in rats**Andres Arend and Marina Aunapuu****Department of Anatomy, University of Tartu, Biomedicum, Ravila 19, Tartu 50411, Estonia. andresarend@ut.ee**

Applying rat liver wound healing model we have previously shown the suppressive effect of a n-3 PUFA-rich diet on the wound healing process, where the proliferation of the connective tissue was suppressed together with increased level of lipid peroxidation, the peroxidizability of lipids and decreased content of 2-series prostaglandins (PGs). It is known that any injury of tissue is followed by a normal inflammatory response and several products of lipid peroxidation, as chemo-attractants, are able to disturb the normal migration of neutrophils and monocytes as well the proliferation of fibroblasts. As lipoic acid (LA) when provided exogenously in pharmacological doses, may serve as an antioxidant the present study was performed to test the influence of LA on the n-3 PUFA-induced inhibition of connective tissue proliferation in the rat liver wound. The experiments were performed on male albino Wistar rats. The first group of animals served as a control-group kept on the standard diet low in unsaturated fatty acids. The second group of animals was on a diet where 10% of sunflower oil was added to the standard pellets. The third group of rats was fed with standard pellets with 10% addition of cod liver oil (containing over 25% of n-3 PUFAs). The animals were fed with above-mentioned diets for 8 weeks. Then, 10 days before the operation, 0.1% of lipoic acid (DL-6,8-thioctic acid) was added to all diets and feeding was continued for six days postoperatively. The test model used was a liver thermic wound of a standard size. LA prevented the suppression of connective tissue proliferation in the healing wound induced by n-3 PUFAs, avoided the increase in peroxidation of lipids, reduced peroxidizability of lipids and modulated the decrease in PGE2 and PGF2alpha content. LA also improved glutathione redox status (GSSG/GSH) both in the liver tissue and in the liver wound. It seems that the elevation of the level of reduced glutathione (GSH) in liver might have an impact on the protective effect of LA. It can be concluded that the protective effect of LA on the suppression of wound healing induced by n-3 PUFAs includes the prevention of increased lipid peroxidation and the maintenance of a suitable spectrum and/or level of 2-series PGs.

TNF-alpha induced release of pro-inflammatory proteins by cystic fibrosis IB3-1 cells encapsulated in alginate microbeads

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The inflammatory processes activated in cystic fibrosis are complex and need the development of specific systems to possibly study the mechanism of bacterial activation of cystic fibrosis cells, as well as the effect of the secreted chemokines on target cell populations. To this aim, we have developed an ionic alginate microencapsulation procedure for the entrapment and manipulation of IB3-1 cystic fibrosis cells. This microencapsulation procedure does not alter viability and the secretomic profile of encapsulated IB3-1 cells. Most of the analyzed proteins (members of the interleukin family, chemokines, growth factors and soluble forms of adhesion molecules), using Bio-plex technology, were secreted both by the free and encapsulated cells, even if in a different extent. In order to determine the biotechnological applications of this procedure, encapsulated and free IB3-1 cells were treated with TNF-alpha and after 24 hours the culture media from both cell populations were collected. As expected, TNF-alpha induced a sharp increase in the secretion of interleukins, chemokines and growth factors. Of great interest was the evidence that induction of IL-6 and IL-8 occurs also by encapsulated IB3-1 cells. In conclusion, the encapsulation of secreting cells in alginate microbeads represents a promising strategy for biotechnology applications in tissue engineering and biomedicine.

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**DEVELOPMENT OF NEW ALPHALISA NO-WASH IMMUNOASSAY KITS FOR
SENSITIVE, RAPID AND EFFICIENT QUANTIFICATION OF CYTOKINES**

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PERKINELMER INC. BIO-DISCOVERY, MONTRÉAL, QUÉBEC, CANADA.

Enzyme-linked Immunosorbent Assay (ELISA) is the most widely adopted method for detection and quantification of cytokines and other biomarkers. This traditional technology offers good selectivity, sensitivity and assay versatility; however, it has certain disadvantages such as limited dynamic range and low throughput due to the numerous wash steps. In addition, ELISA is not well suited for the use of medium or low affinity antibodies. In contrast, chemiluminescent bead-based AlphaLISA® assays do not face these limitations. AlphaLISA dynamic range is generally between 3 to 4.5 log units and the absence of wash steps allows performing these assays in high throughput mode. Assay development is simple and fast, and hands-on time as well as total assay time is significantly reduced. AlphaLISA assays are easy to miniaturize and automate enabling both Research and High Throughput Screening (HTS) laboratories to efficiently quantify analytes of interest. PerkinElmer has developed several AlphaLISA assays for the detection and quantification of cytokines in cell culture supernatants and serum samples. Experiments showing Lower Detection Limit (LDL) and dynamic range will be presented. The performance of the assays is excellent, including a large dynamic range, high sensitivity, accuracy and precision. The overall results confirm the user-friendly AlphaLISA technology as a new generation of tools available for immunoassays.

The important role of molecular chaperones (HSPAs/HSP70s) in oral cavity and oral inflammatory diseases

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Heat shock proteins of the 70kDa family (HSPAs/HSP70s) are major molecular chaperones and cytokines of most cells and microbes, extracellular and interstitial fluids, blood, synovial fluids and secretory body fluids like saliva. The induction of human HSPAs plays an important role at cellular level under most stress conditions; whereas microbial HSPAs improve microbial tolerance to environmental changes, and improve virulence and resistance against antimicrobial peptides. Extracellular HSPAs reveal cytoprotective properties and are involved in numerous physiological and pathological events, including modulation of cytokine release and immunity. Accordingly, HSPAs play a role in the maintenance of pulpal health, and the repair of injured dental hard tissues. HSPAs also play a role in stress adaptation of periodontal tissues, and in the maintenance of periodontal and mucosal health including defense against microbes, prevention of mucosal allergic reactions, and facilitation of healing of ulcers and wounds. Despite their advantageous effects maintaining health of several oral tissues, HSPAs are likely to play a role in the disadvantageous amplification of pulpal inflammatory response to bacteria, and in the formation of several periapical inflammatory lesions. HSPAs may also induce gingivitis under certain conditions, and play a role in the progression of periodontal bone defects. HSPAs may also play a role in atopic – type allergic reactions, autoimmune disorders, and haptenation in certain cases. Based on the above data, it can be assumed that HSPAs play an important role in oral defense under healthy conditions; however, their role is somewhat “Janus-faced” under pathological conditions.

Role of CXC chemokines in innate immune response of Teleost fish

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A large array of chemokines is known in mammals, some of which are important chemoattractants induced during the pro-inflammatory phase of inflammation. Others are constitutively expressed in the lymphoid organs and in the central nervous system, where they are crucial for development. To date a fair number of CXC chemokines (CXCL) were discovered in Teleost fish, but for the majority orthology with any particular mammalian CXCL is difficult to establish. Current discovery of a new carp CXCL (CXCL8) motivated us to study the profile of CXCL and CXC receptor (CXCR) expression during the immune response of carp (*Cyprinus carpio L.*). Two *in vivo* models of inflammation were used: zymosan-induced peritonitis (Z) and hyperosmotic shock (HI). Gene expression of CXCL (CXCL8, CXCa, CXCb) and their receptors (CXCR1, CXCR2) was measured by RQ-PCR. Hyperosmotic treatment induced mild disruption of the integrity of the gill epithelia, and was related to rapid granulocyte migration. Immediately, after HI treatment substantial up-regulation of expression of the CXCL8 gene was observed in gills, while only small effects were detected for CXCa and CXCb. Expression of CXCR1, but not CXCR2, was up-regulated in the HI group 3-24 h after treatment. Z-injection induced acute peritonitis, manifested by massive influx of phagocytes. Expression of CXCL8, CXCa and CXCR1 was quickly and significantly up-regulated in peritoneal leukocytes. Increase of expression of CXCb and CXCR2 was recorded in peritoneal leukocytes during the later phase of inflammation. The expression profile of CXC_s and their receptors in carp leukocytes suggests that CXCL8, CXCa and CXCR1 are connected with the acute inflammatory response with quick granulocyte migration, while CXCb and CXCR2 are related to the later resolution phase.

Mobility of 2-oxoglutarate in plant in response to C- and –N metabolism under cadmium stress

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In plant leaf, 2-oxoglutarate is present at relatively low levels when compared to other organic acids. It has been shown that 2-oxoglutarate levels depend upon metabolic fluxes related to C- and N- metabolism, although it is not known whether 2-oxoglutarate fluctuations reflect a change in a given enzymatic activity. Indeed, 2-oxoglutarate levels are dependent on inorganic and organic N content and their related metabolism as well as nitrate and sugar levels. This has been studied in nitrate accumulation decrease and NR activity decrease in tomato leaves that led to an increase in 2-oxoglutarate content under conditions where glutamine levels stayed low. In tomato plants, leaf 2-oxoglutarate levels responded to N starvation when glutamine levels were low, nitrate was absent, NR activity was reduced, and sugar root allocation had increased. Sugar provided to detached tomato leaves induced a dramatic rapid increase in 2-oxoglutarate levels without any subsequent change in NR activity. Taken together, such data indicate that plant cell 2-oxoglutarate levels can reflect C/N status, and therefore this organic acid could play a signalling role in the co-ordination of C and N metabolism.

β -oxidation modulates metabolic competition between eicosapentaenoic acid and arachidonic acid regulating prostaglandin E₂ synthesis in rat hepatocyte-Kupffer cells

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The ability of n-3 PUFA to competitively inhibit the use of arachidonic acid (AA) for membrane phospholipids synthesis and prostaglandin E₂ (PGE₂) production has been well demonstrated in single cell models. In the present study, metabolic competition between AA and eicosapentaenoic acid (EPA) for PGE₂ synthesis in a rat hepatocyte-Kupffer cell (HPC/KC) co-culture system was investigated when the cellular oxidation capacity was enhanced by exogenous L-carnitine. Here we demonstrate that in absence of L-carnitine, 1) β -oxidation rates of EPA and AA were comparable in HPC and in KC; 2) AA was preferentially incorporated into glycerolipids rather than EPA; 3) addition of EPA significantly decreased AA-dependent PGE₂ synthesis in HPC and cyclooxygenase-2 (COX-2) expression in co-cultured HPC/KC. However, the addition of L-carnitine 1) significantly increased β -oxidation of EPA in HPC, but only marginally elevated the oxidation of AA in HPC and the oxidation of both fatty acids in KC; 2) decreased the esterification, but did not alter the preferential incorporation of AA into glycerolipids; 3) alleviated the significant competitive inhibition of AA-dependent PGE₂ synthesis and COX-2 expression by EPA. Taken together, the results strongly suggest that L-carnitine affects competition between AA and EPA in PG synthesis in liver cells by enhancing oxidation of EPA in HPC. This implies that the beneficial effects of n-3 PUFA, especially EPA, are affected by cellular oxidation capacity.

Keywords: L-carnitine; eicosapentaenoic acid; arachidonic acid; prostaglandin E₂; β -oxidation; hepatocyte-Kupffer cell co-culture

Endothelial ICAM-1 protein expression is regulated by cytosolic phospholipase A_{2a} via both NFkB and CREB transcription factors**Nurit Hadad, Liron Tuval, Vered Elgazar-Carmom and Levy Rachel****Infectious Disease and Immunology Laboratory, Clinical Biochemistry Department, Faculty of health Sciences, Soroka Medical University Center and Ben-Gurion University of the Negev, Beer Sheva 84105, Israel.**

The regulated expression of intercellular adhesion molecule-1 (ICAM-1) plays an important role in inflammatory process and in immune responses. The present study aimed to determine the *in vivo* involvement of cytosolic phospholipase A_{2a} (cPLA_{2a}) in ICAM-1 over-expression during inflammation and to elucidate cPLA_{2a} specific role in signal events leading to ICAM-1 upregulation in endothelial cells. Elevated cPLA_{2a} and ICAM-1 protein expression were detected in inflamed paws of mice with collagen induced arthritis and in periepididymal adipose tissue from mice fed a high fat diet. Intravenous injection of 2 mg/kg oligoantisense against cPLA_{2a} (AS) that reduced the elevated cPLA_{2a} protein expression also decreased ICAM-1 over-expression suggesting a key role of cPLA_{2a} in ICAM-1 up-regulation during inflammation. Preincubation of endothelial ECV-304 cells with 1mM AS prevented cPLA_{2a} and ICAM-1 up-regulation induced by TNFa and inhibited their adherence to phagocyte like-PLB cells. NADPH oxidase inhibitors, DPI or apocinine, inhibited cPLA_{2a} activation, suggesting that the NADPH oxidase acts upstream to cPLA_{2a}. Attenuating cPLA_{2a} activation by AS or DPI prevented the induction of COX2 and the production of PGE₂ that were essential for ICAM-1 up-regulation by TNFa. AS or DPI inhibited the phosphorylation of both the redox sensitive p65 NFkB on serine 536 and PKA-dependent CREB on serine 133. Our results are the first to show that CREB activation is involved in ICAM-1 up-regulation and suggest that cPLA_{2a} activated by NADPH oxidase, is required for both NFkB phosphorylation by an undefined kinase and CREB activation by PGE₂ mediated PKA.

Specific enhancement of TLR2 activity in chronically activated astrocytes

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Activated glial cells secrete inflammatory factors that have been implicated in several neurological diseases. In CNS inflammation, microglia and astrocytes interact with respect to the regulation of local innate immune reactions. However, up to now the knowledge about microglia-astrocyte interaction under repeated inflammatory stress is relatively scarce.

Therefore, in this work, we investigated activation of primary astrocytes under sequential stimulation conditions. For a first stimulus of primary astrocytes, we used conditioned medium of the microglia-like cell line BV-2, which has been activated with LPS. Microglia are a major source of cytokines such as IL-1 β and TNF α which are potent activators of astrocytes. Alternatively a more defined proinflammatory cytokine mix (CCM) which contained TNF α , IL-1 β and IFN γ was applied as primary stimulus. In both cases, activated astrocytes expressed higher levels of several genes linked to inflammation. In particular, expression of some pattern recognition receptors (PRRs) was upregulated significantly. This altered phenotype may be important in chronic inflammation and leads to the question whether pre-activated astrocytes react in a different way to a secondary stimulus which targets PRRs or cytokine receptors. In this work, we focus on Toll-like receptor 2 (TLR2) and its ligands as a secondary stimulus. As readouts we used NF- κ B activation as receptor-proximal signal and cytokine (IL-6) secretion as more distal event. We found reactivity of astrocytes towards a secondary stimulus was increased. Pre-stimulated astrocytes reacted more readily to a secondary stimulus compared to non-prestimulated cells. This finding might be crucial for the understanding of CNS host response under chronic stress conditions and its pharmacological modulation.

TNF-alpha is involved in the release of the acute phase protein PTX3 in lung co-cultures exposed to particulate matter from wood combustion and traffic.**Jan I Herseth^{1,2}, Vivi Volden², Per E Schwarze¹, Anette Kocbach Bølling¹**

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Exposure to ambient particulate matter has been associated with cardiopulmonary morbidity and mortality, and inflammation is thought to play a role in the pulmonary and cardiovascular effects induced by particles. Long pentraxin 3 (PTX3) is a newly discovered acute phase protein produced by cells involved in innate immunity as well as by bronchial and alveolar epithelial cells. Through interaction with for instance pathogens, growth factors and the complement factor C1q, PTX3 may be involved in tuning of the inflammatory response. It has also been suggested as a new marker of innate immunity and inflammation, reflecting tissue and vascular bed responses. The pro-inflammatory cytokines IL-1 and TNF-alpha have both been found to induce release of PTX3. In this study, we used a contact co-culture of monocytic and pneumocytic cell lines to determine the release of PTX3 induced by wood smoke particles (WSP) and different types of traffic derived particles (TDP) after 12, 40 and 64 h of exposure to 40 micrograms/cm². In addition, anti-TNF-alpha and IL-1 receptor antagonist were used to investigate the influence of these two pro-inflammatory cytokines on the release of PTX3. All particle samples induced a significantly increased release of PTX3 from the co-culture after 40 and 64 h of exposure, but TDP were generally more potent inducers of PTX3. TNF-alpha, but not IL-1, was involved in the release of PTX3 induced by WSP, whereas both these cytokines seemed to be involved in the PTX3 release induced by TDP. The role of PTX3 in particle induced inflammation has not been studied in any detail, and this is the first study investigating the release of PTX3 induced by ambient particulate matter in an *in vitro* model system. Although the role of PTX3 in particle toxicology needs further elaboration PTX3 may become an interesting marker for particle-induced inflammation.

Determination of TNF- α in inflamed radicular cysts

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TNF- α is pleiotropic cytokine that is considered a primary modifier of inflammatory and immune reaction in response to various diseases. It induces stimulation of osteoclastic bone resorption and helps fibroblast to release collagenase and participated also in inflammation. Based on these multiple effects we investigated its values in 33 radicular cysts obtained from patients with radicular cysts in maxillofacial region. The cysts were surgically enucleated under local anaesthesia. After this mucoperiostal flap was raised and cystic fluid was aspirated from non-ruptured cysts, then aspirate is immediately centrifuged to remove cells and supernatant was stored at -70°C until use in ELISA assay. TNF- α was analyzed in respect to clinical symptoms, cystic diameter, localization based on radiographic and histological examinations. In addition, each section was stained with hematoxylin-eosin and by immunohistochemistry using Anti-CD3, anti-CD20 and anti-CD68 monoclonal antibodies to access presence of inflammatory cells in pericytic tissues. These results show increase in TNF- α concentration in radicular cysts based on several clinical parameters including cysts volume, the protein concentration in cystic fluid as well as on histological findings including cyst wall thickness, count and subtype of inflammatory cells and degree of vascularization analyzed in cyst wall (Spearman correlation, $p < 0.05$). We believe that determination of TNF- α simultaneous with other clinical parameters can help in classification, earlier and better diagnosis of these cysts in routine practice.

Glutathione S-transferase polymorphism and the association with cytokine markers in Korean smokers

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Inflammation is the body's first line of defense against injury or infection or even wider array of chronic illnesses. Long-term cigarette smoking is associated with activation of a cascade of inflammatory response that leads to tissue injury and dysfunction. Glutathione S-transferases (GST) are a family of enzymes that detoxify xenobiotics such as carcinogens present in cigarette smoke and has been shown to exert different effect based on the phenotype of this enzyme. In this study, we tried to compare the level of cytokines between 48 smokers and 197 non-smokers and to examine whether these effects are dependent on their differences in GST polymorphism. The levels of TNF- α and interleukin (IL-6) were compared and we found that smokers had decreased level of TNF- α and increased level of IL-6 ($p<0.001$). When the inflammation markers were tested for relationship with antioxidant nutrient status (total radical trapping antioxidant potential, lipid peroxidation, vitamin C level) there were no significant relationship except negative association between the level of vitamin C and IL-6 in non-smokers. These result were again tested based on their GST genotype (GSTM1, GSTT1), but, found no significant differences. We observed that IL-6 inflammation marker is elevated in smokers, but, did not find any significant link regarding GST polymorphism. It may be possible that we need a bigger sample size for detecting any differences and for drawing a clinically significant conclusion.

Mono-2-ethylhexylphthalate (MEHP) activates both pro- and anti-inflammatory signals**Anette Kocbach Bølling, Mónica S Korsnes, Jørn A Holme, Rune Becher**

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Phthalates are plasticizers that are used in a wide range of commercial products. Since they are not chemically bound to the plastic they leak out to the environment, causing human exposure through inhalation, diet and skin. Epidemiological studies have associated indoor exposure to phthalates with increased incidences and severity of asthma in children and adults. Although airway inflammation has been suggested as a possible mechanism for these effects, the underlying cellular mechanisms for phthalate-induced effects are still largely unknown.

Since macrophages are known to be involved in the pathogenesis of asthma, we used the murine macrophage cell line RAW264.7 to investigate possible signalling pathways activated during exposure to mono-2-ethylhexylphthalate (MEHP). Exposure to 0.1-1.0 mM MEHP increased the formation of reactive oxygen species (ROS), whereas concentrations from 0.3mM induced a significant release of TNF-alpha as well as a necrotic cell death. By using the p38 specific inhibitor SB202190, we found that the mitogen activated kinase p38 appeared to be involved in the release of TNF-alpha. MEHP induced activation of p38 was confirmed by Western blotting showing increased levels of phospho-p38 after 30 min exposure to 0.1 and 0.5 mM MEHP. The levels of phospho-Akt were also increased at this time point, suggesting a concomitant activation of the phosphoinositide 3-kinase (PI3K)/Akt pathway. This pathway is known to be involved in innate immunity through regulation of cytokine production, and PI3K was recently found to limit the TNF-alpha production in monocytes. However, the role of the PI3K/Akt pathway in the MEHP-induced release of TNF-alpha needs further investigation.

Expression of the Luteinizing Hormone receptor in the spongius tissue

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Recent works show that the inflammatory cytokines implication in hypothalamo- hypophysocorticosuprarenal and gonad axis have consequence on ACTH, corticoids and LH secretion, explaining the gestational disorders. The proportion of men with serum LH > 6.0 U/l and serum testosterone > 9.8 nmol/l, i.e. of men with subclinical hypogonadism, increases significantly between 40 and 70 years. As the proportion of men with erection disturbances increases simultaneously, it is possible that the elevated LH concentrations are involved in the generation of the erection disturbances. The precondition for this is the expression of LH receptor in the spongius tissue. In the present study, the expression of the LH receptor in the male mouse spongius tissue was studied to see, if LH effects are possible in the spongius tissue. Balb/c mice were used as donors of normal penis spongius tissue and testis tissue. Immunocytochemistry, Western blotting and quantitative RT-PCR reactions were used for the detection oh the LH receptor. Positive immunoreaction for the LH receptor was found in the mouse penis spongius tissue using immunocytochemistry. Western blotting experiments demonstrated the presence of LH antigen at Mr = 97. 4 and 78 kD. Quantitative RT- PCR reactions confirmed the expression of LH receptor in the spongius tissue. Our results suggest that LH receptor is expressed in the male spongius tissue of the mice and thus the elevated LH levels of the aging men with subclinical hypogonadism may affect the spongius tissue.

De novo generation of cytokine-responsive astrocytes from murine embryonic stem cells

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Astrocytes are the most abundant glial cell type in the brain. They are involved both in the maintenance of physiological homeostasis and in inflammatory responses. Research into the inflammatory response of these cells would benefit from the availability of astrocytes cultures free of other glial contaminants. One approach to obtaining a reliable and reproducible cell culture system is differentiation of the cells from embryonic stem cells (ESC). We present here a method to generate highly astroglial-enriched cultures from ESC and characterise them for inflammatory signalling responses. During the differentiation of both, embryonic and neural stem cells, astroglial markers (GFAP, S100b, A2B5, CD44) were upregulated while the transient neural markers nestin, NCAM, and bIII-tubulin were downregulated in the final cultures. The presence of astroglial markers in about 90% of the cells of the culture and the absence of any microglial markers and of the neuronal markers NCAM and bIII-Tubulin indicate a specific differentiation process. We demonstrated inflammatory signalling capability of our astrocytes by nuclear factor kB translocation into the nucleus and an increased release of IL-6 and NO into the supernatant upon stimulation with a proinflammatory cytokine mix. ESC-derived astrocytes and neural stem cell-derived astrocytes showed the same response pattern as astrocytes isolated from mouse brain.

Serum amyloid A localization in human coronary artery endothelial cells in response to proinflammatory stimuli

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Greatly increased levels of acute phase protein serum amyloid A (SAA) have been reported during acute and chronic inflammation. They predict the risk of coronary artery disease and even mortality in humans. SAA is an active player in atherogenesis, although its mechanism of action is still unclear. In physiological concentrations, SAA plays a protective role in bacterial and viral infections. The objectives of this study are to understand the role of SAA in human coronary artery endothelial cells (HCAEC) and to determine its cellular localization. Our broad hypothesis is that secreted versus intracellular SAA have different functions that could lead to either protective or degradatory responses. The mechanism of how SAA works lead us to address the presence of SAA candidate cellular receptors. The identification of FPRL1/ALX, TANIS, CLA1, RAGE, TLR2 and TLR4 was confirmed in HCAEC and HUVEC using RT-PCR. Their mRNA expression was similar in interleukin-1beta or SAA-stimulated cells as compared to background controls, with the exception of TLR2, which was greatly increased in stimulated cells. TLR2 could be one of the major SAA receptors responsible for its role in promoting atherosclerosis. Immunofluorescence, cell fractionation studies and Western blots indicated prominent intracellular localization of SAA in HCAEC in the cytoplasm and in the nuclear fraction. Association of SAA was also found with microtubules and the membrane/vesicular fraction. Especially interesting is the fact that HCAEC do not secrete SAA in response to proinflammatory stimuli, as is the case for hepatocytes. In conclusion, the majority of SAA synthesized in HCAEC stays within the cells and could play a variety of different functions based on the compartments it is located in.

15-Deoxy- $\Delta^{12,14}$ -prostaglandin J₂ Induces 15-Hydroxyprostaglandin Dehydrogenase Expression in Human Breast Cancer Cells

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It has been reported that prostaglandin E₂ (PGE₂), one of the major products of cyclooxygenase-2 (COX-2), promotes cancer progression. 15-Hydroxyprostaglandin dehydrogenase (15-PGDH), a key enzyme for degradation of PGE₂, was initially found to be down-regulated in colon cancer. In the present study, we have found that 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂ (15d-PGJ₂), one of the terminal products of COX-2, upregulates 15-PGDH expression in human breast cancer (MDA-MB-231) cells. 15d-PGJ₂-induced 15-PGDH expression correlated with suppression of MDA-MB-231 cell migration. Of the several candidates of 15-PGDH regulators, Ets was considered as a major determinant responsible for the 15-PGDH-inducing effects of 15d-PGJ₂ as assessed by the luciferase assay by use of deletion constructs of 15-PGDH promoter. Elk-1, one of the Ets family members, was activated by treatment of 15d-PGJ₂. Taken together, these findings suggest that 15d-PGJ₂ may exert an anti-carcinogenic effect by upregulating the expression of 15-PGDH, a putative tumor suppressor.

Intestinal model of Inflammation in primary cells**S. B. Lobo¹, M. Denyer¹, F. A. Javid²**

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The gastrointestinal tract contains an enormous mucosal surface, which is continuously exposed to antigens hence making it susceptible to an inflammatory response. Such response targets potential pathogens by direct activation of the mucosal immune cells, however, in newborns the continuous inflammation attacks the intestine which leads to induction of necrotising enterocolitis. The present study aims at developing an "*in-vitro*" intestinal model of inflammation to assist in understanding the complex interplay of pro-inflammatory mediators during the immune response in neonates. Segments (1.5cm length) from the ileum were obtained from SD rat neonates (1-4 days old) and exposed to 0.25% trypsin/EDTA for 30min. Following trituration and subsequent centrifugation for 5min at 450xg, cells were suspended in DMEM-Hepes supplemented with 10% FCS, 2.5% Penicillin/Streptomycin, 2.5% L-Glutamine, and 0.2% Amphotericin B. Cell suspension were transferred to culture flaks and incubated at 37°C. Once confluent, the cell preparation media was replaced by FCS-free media, and treated with 0, 10, 50, and 100µg/ml of LPS. IL-8 and nitric oxide (NO) response were subsequently measured. In separate studies cell proliferation, cell viability, and cell adhesion were analysed. Additionally, the phenotypic properties of the intestinal muscle cells were also investigated via immunocytochemistry. Initial studies demonstrated that LPS treatments induced a significant increase in the release of IL-8 and NO compared to controls. The effect of LPS treatments on cell dynamics demonstrated small changes in cell viability and adhesion, whereas an increase in cell proliferation was observed. Immunocytochemistry studies indicated that LPS treatment caused a decrease in the expression of actin fibers with impaired distribution compared to controls. In the present model key aspects of intestinal inflammation were replicated "*in-vitro*" including the activation of pro-inflammatory mediators, the loss in enteric innervations and subsequent tissue hyperplasia. Thus, this model may be used as a tool to investigate the anti-inflammatory properties of candidate drugs targeting functional GI diseases.

Cytokine patterns in patients with neuropsychiatric diseases as determined in CSF and peripheral blood**Maxeiner H¹, Kurfiss S², Brettschneider J², Bechter K¹, Lehmensiek V², Tumani H².****¹Dept.of Psychiatry II and ²Dept. of Neurology, University of Ulm**

Background: Cytokines are mediators and regulators of inflammatory processes. We investigated neuropsychiatric patients with acute inflammation of the CNS (non-bacterial meningitis (N-MEN)), assumed chronic low level neuroinflammation (schizophrenic SCH-S and affective spectrum disorders PSYCH-A) and tension headache (CEPH) of unknown origin. Cytokines determined were proinflammatory cytokine IL-1beta, T cell derived IL-2, the proinflammatory IL-8/CXCL8, antiinflammatory IL-10 and IL-17. Purpose: to differentiate chronic and acute neuropsychiatric diseases by cytokine profiles and to evaluate cytokine levels in the CSF and peripheral blood to characterize the two distinct compartments.

Methods: In total 10 N-MEN, 10 CEPH, 15 PSYCH-A and 16 PSYCH-S patients were included. Peripheral blood and CSF was taken at the same time. CSF parameters were evaluated by conventional methods, cytokines determined on a sensitive commercial multiplex immunoassay platform (Mesoscale) by a modified protocol. Statistics: Mann-Whitney-U-Test and Kruskal-Wallis H-Test. **Results:** Serum and CSF levels: Highest levels of IL1b, IL-2, IL-8 and IL-17 were observed in the CSF of N-MEN, whereas in serum, levels were not or slightly elevated. Significant ($p<0.05$) tested in CSF for N-MEM versus CEPH,PSYCH-A,PSYCH-S for IL-8 and versus PSYCH-A,PSYCH-S for IL-17. IL-10, CSF levels were also highest in N-MEM, and significant also versus CEPH,PSYCH-A,PSYCH-S, but IL-10 in serum was even higher than in CSF, although not significant as compared to all other patient groups. We observed in all cytokine (except IL-2) significant differences either in serum or in CSF or both between the diseases; but more frequent CSF than in the serum.

Conclusions: Investigation of cytokine levels in CSF is more sensitive than in serum to detect significant differences between neuropsychiatric diseases, establishing the CSF as a distinct compartment for cytokines. Cytokine patterns are disease specific and may be used for therapy monitoring. Elevated IL-1beta, IL-2 and IL-8 CSF/serum ratios in N-MEM may indicate an intrathecal synthesis of these cytokines.

Analysis of in vitro ubiquitination and sumoylation of small heterodimer partner (SHP) and effects of the double point mutation in its putative ubiquitination sites on transcriptional modulation involved in bile acid synthesis

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Bile acids act as signaling molecules and inflammatory agents that modulate nuclear receptors and signaling pathways that regulate metabolic homeostasis. Several lines of evidence established that small heterodimer partner (SHP) plays an important role in maintaining cholesterol homeostasis by bile acid mediated feedback inhibition of cholesterol 7alpha hydroxylase (CYP7A1) gene. Recent studies demonstrated that SHP is a target of ubiquitin-proteosomal degradation with K122 and K123 being major ubiquitination sites. Individual mutation on each of these ubiquitination sites (K122R, K123R) altered the repression activity of SHP. This study further examined in vitro ubiquitinations of different regions of SHP fragments (1-92, 92-160, 160-260) and sumoylation of SHP proteins. When in vitro translated GST-SHP-wild type protein was incubated with HA-ubiquitin, high molecular weight forms of ubiquitinated GST-SHP were detected. Whereas both 1-92 and 160-260 regions decreased in ubiquitinated forms, the 92-160 region of the GST-SHP fragment showed significant increase and even higher than SHP wild type in ubiquitination. However, SHP did not show any sumoylation although discrete sumoylated bands occurred in liver receptor homolog-1 (LRH-1), a nuclear receptor protein known to be regulated by sumoylation. This study also examined the effects of double point mutation in putative ubiquitination sites of SHP (K122/123R) on transactivation of *hCYP7A1* gene and GAL4-TATA-luciferase reporter in hepatoma cell lines. Whereas K123R mutation increased the repressive activity of SHP on *hCYP7A1* transactivation in HepG2 cells, double point mutation of K122/123R decreased its repression activity of *hCYP7A1* transactivation in HepG2 and did not differ from wild type in transactivation of GAL4-TATA-luciferase reporter in H1c1c7 cells. These results suggest that major ubiquitinations within the 92-160 region of the SHP can be influenced by conformation of the protein, and the double point mutation of K122/123R may affect both the stability and the activity of SHP in transcriptional modulation.

Evidence for enhanced pro-inflammatory cytokine response to endotoxemia in the adult, neonatally flutamide-primed, male rat.

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It is accepted that a sexual dimorphism exists in the mammals' immune function. In fact, evidences suggest that while estrogens enhance the immune response, androgens inhibit it and gonadectomy alters this response. Exposure to androgens early in life development is essential for masculinization of the male phenotype. Male fetuses exposed to anti-androgens during peri-natal life are permanently demasculinized in their morphology and physiology. The discovery of androgen-active chemicals in the environment has placed increased emphasis on describing the effects of both natural and environmental androgens and anti-androgens on the physiology of the male individual. Thus, the aim of the present study was to explore in the male rat the effect of neonatal flutamide (F) treatment, a selective androgen receptor blocker, on peripheral TNFa levels during the acute phase response of endotoxemia at adult age. Five day-old male Sprague-Dawley rats were s.c. treated with a single injection of a small volume of either sterile corn oil alone (control animals, C) or containing 1.75 mg F. After weaning, rats were individually housed and kept with free access to Purina chow diet and water until experimentation. On the experimental day (age 100 days), animals were submitted to ketamine anesthesia and the right jugular vein was exposed, in a room with sterile atmosphere. Thereafter, rats were bled before (sample time zero) and at several hours (1-4 h) after i.v. injection of 25 micrograms/Kg BW of bacterial lipopolysaccharide (LPS, Sigma-Aldrich; dissolved in a small volume of sterile physiological NaCl solution as vehicle). The volume of blood collected at every time was immediately replaced by the same volume of red blood cells resuspended in heparinized-sterile vehicle. Due to the small blood volume of samples, only plasma tumor necrosis factor alpha (TNFa) concentrations were measured (ELISA) in samples taken from C and F rats. Our data indicated that no group differences were found in circulating TNFa concentrations when evaluated in samples from the time zero (n=9 rats per group). On the other hand, significantly ($p<0.05$ vs. C values) higher TNFa plasma concentrations were found in the F group at 1 h post-LPS. Thereafter, circulating TNFa levels remained (samples taken at 2 and 3 h post-LPS) elevated (albeit not significantly) over C values in F rats. Finally, TNFa values on time 4 h post-LPS recovered sample time zero-values in both groups examined. The area under the curve of circulating TNFa concentrations during the study resulted significantly ($p<0.05$) higher in F than in C rats. Our study adds new data sustaining a suppressive role of endogenous androgens on pro-inflammatory cytokine release in plasma during acute endotoxemia and, strongly supports a sex steroid basis for immunological sexual dimorphism in mammals.

4-OHE₂ Induces HO-1 Expression Through Activation of Nrf2 Signaling: Implications for Human Mammary Carcinogenesis

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Estrogen undergoes oxidative metabolism by CYP1B1 to form 4-hydroxyestradiol (4-OHE₂), a putative carcinogenic metabolite of estrogen. Further oxidation of this catechol estrogen to quinone through redox cycling produces reactive oxygen species (ROS) which can either cause DNA damage or stimulate abnormal cell proliferation. Our previous study showed that 4-OHE₂-induced ROS production contributed to neoplastic transformation of human breast epithelial (MCF-10A) cells (S.-A. Park et al., Cancer Res., 2009). In this study, 4-OHE₂ increased heme oxygenase-1 (HO-1) expression by activating antioxidant response element (ARE)-mediated Nrf2 signaling. By utilizing human HO-1 promoter reporter plasmids and the ChIP assay, we have identified that 4-OHE₂ transcriptionally activated the upstream ARE-rich enhancer region of the human HO-1 promoter. Induction of HO-1 expression by 4-OHE₂ was attenuated by a pharmacological inhibitor of PI3K/Akt as well as siRNA *Nrf2* gene knock down. Furthermore, 4-OHE₂-induced expression of HO-1 was abolished by the antioxidant *N*-acetyl-L-cysteine (NAC) and trolox. Inhibition of HO-1 expression and activity by siRNA knock-down of HO-1 gene and chemical inhibition of HO-1 enzymatic activity by zinc protoporphyrin IX (ZnPP) abrogated 4-OHE₂-induced cell migration. Moreover, ZnPP suppressed 4-OHE₂-induced matrix metalloproteinase-9 (MMP-9) mRNA expression. A recent report has revealed that HO-1 renders tumor cells resistant to photodynamic therapy-mediated cytotoxicity. Although a role of HO-1 in 4-OHE₂-induced hormonal carcinogenesis has not been fully clarified yet, the upregulation of this stress-responsive enzyme may provide cancer cells with survival advantage. In this context, HO-1 may hence represent a novel target for chemoprevention and treatment of breast cancer.

PPAR gamma activation diminishes thymic stromal lymphopoietin mRNA level in Caco-2 cells differentiated into enterocytes

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Thymic stromal lymphopoietin (TSLP) – a cytokine secreted constitutively from various epithelia – is considered as a very important agent in the crosstalk between epithelial and dendritic cells, driving the balance towards the mucosal homeostasis. Peroxisome proliferator-activated receptors γ ligands are also believed to calm down inflammation in the gut. The aim of this study was to investigate the influence of PPAR γ activation on the production of TSLP in human colon tumour derived Caco-2 cells. In the high density culture these cells undergo spontaneous differentiation into the intestinal enterocytes. Caco-2 cells were cultured in DMEM supplemented with 10% FCS or in Q286 epithelial cells serum free medium (PAA, Germany). The cells were seeded on permeable membranes of cell culture inserts (LD, 0.4 μ m) at initial density of 2.5×10^5 and were differentiated for 6 days. At that time the expression of sucrase-isomaltase, a marker of mature enterocytes, increased about 100-fold although TSLP and PPAR γ mRNA level were not significantly changed. TSLP and PPAR γ transcripts were also present in cells cultured at low density on plates. Since natural ligands of PPAR γ (conjugated linoleic acid, CLA) and its partner receptor – RXRa (dietary retinoids) – are components of normal diet, enterocytes are constantly exposed to its action. To mimic this physiological situation Caco-2 cells were stimulated with natural (conjugated linoleic acid, CLA mixed isomers, 1 μ M) and synthetic (ciglitazone, 1 μ M) PPAR γ ligands in the presence or absence of 9-cis retinoic acid (9-cis-RA 1 μ M). After 3 or 18 hours the total cellular RNA was isolated and mRNA levels of the selected genes were evaluated by RT-PCR. The analysis was performed on cells seeded in low density on plates (undifferentiated) and on cells cultured for 6 days on inserts (differentiated cells). The experiments demonstrated that only the mixture of PPAR γ ligand and 9-cis-RA reduced the amount of TSLP transcripts to almost undetectable level in differentiated cells. This effect was not observed when these agents were used separately. In undifferentiated Caco-2 cells cultured on plates in low density activation of PPAR γ and its co-receptor did not change the level of TSLP mRNA. Caco-2 cells were frequently treated as the model of functional enterocytes. Our results point out the importance of cell culture conditions in analysing the regulation of inflammatory gene expression in the *in vitro* studies.

Disturbance of IL-2 signaling in regulatory T cells improves abnormal cognitive function in mice

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Lymphocyte functions are strongly dependent on cell surface receptor signaling. Recently it was shown that alkylating agents in the dose 100 fold lower than cytostatic one are capable to disturb signal passage through various cell surface receptors, such as IL-2R, TNFR and Fas. Molecular mechanisms of the receptor blockage were investigated on model of TNF signaling. We succeeded in demonstration that low concentrations of alkylating agent melphalan (Mp) protect murine fibroblastoid cells against TNF-a-induced cytotoxicity. The protection did not depend on *de novo* protein synthesis. Moreover, no increase in NF-kB in nuclear extracts of Mp-treated cells was observed. At the same time, 1-hour treatment with Mp markedly reduced NF-kB activity in nuclear extracts of the cells challenged with TNF-a. These data support the suggestion that specific alkylation of components in the cytoplasm or cell membrane by Mp interferes with surface receptor signaling pathway. Among a large variety of T cell subsets only regulatory T cells (Tregs) constitutively express high affinity receptor for IL-2, the cytokine, which is the factor of their growth and survival. Surplus accumulation of Tregs is known to be at the bottom of many morbid conditions among them being neuropsychiatric diseases. In particular, Tregs may inhibit Th1 cells, including brain autoimmune lymphocytes, controlling the local microglial response. Malfunction of these cells leads to the appearance of neurodegenerative foci. The animal model of Treg accumulation has been used. BALB/c mice were chronically treated with dexamethasone and IL-2. Spatial learning/memory was assessed in the Morris Water Maze Behavioral Test. It was shown that the pharmacologically-induced Treg accumulation leads to cognitive and behavioral abnormalities, which may be prevented by Mp administration. Indeed, already on the 2nd day of training the percentage of Mp-treated mice, which were able to find a submerged platform, was significantly higher ($p=0.04$; Wilcoxon paired test) than in untreated animals after Treg induction. In conclusion, disturbance of IL-2 signaling with alkylating agents is a new safe method of Treg elimination.

Anti-inflammatory properties of *Lactobacillus acidophilus* and *Bifidobacterium longum* in a murine model of colitis

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There is some evidence that probiotics may be protective against intestinal bowel disease diseases, depending on the strain and/or mixture of probiotics, however the underlying mechanisms are still largely unknown. A regulation of *lamina propria* lymphocytes (LPLs) function by probiotics in colitis has been indicated, but data are lacking on possible involvement of intraepithelial lymphocytes (IELs). The aim of this study was to investigate whether a probiotic mixture prevented gut inflammatory disease and the role of both IELs and LPLs in such protection, using a murine model of colitis. BALB/c mice received orally a mixture of *Lactobacillus acidophilus* Bar 13 and *Bifidobacterium longum* Bar 33, daily for 3 weeks. Colitis was induced by intrarectal administration of trinitrobenzene sulfonic acid (TNBS), 48 h before the end of the experimental period. An increase of *L. acidophilus* and *B. longum* were found in the stools after 1, 2 and 3 weeks of probiotic treatment. The probiotic mixture was able to prevent the TNBS induced intestinal inflammation and ulcerations. While no differences were observed in LPLs, in IELs a reduction in CD4+, an increase in gammadelta T cells, and an expansion of regulatory T cells, both Treg (CD4+CD25+Foxp3+) and Tr1 (CD4+IL-10+) cells, were found associated with the protection elicited by the probiotics. A significant increase of apoptosis of both CD4+ and CD8+ in IELs was induced by the probiotics, whereas no differences were observed in the spleen. In addition, the probiotic mixture was able on one side to inhibit the TNF-alpha, MCP-1, IL-12 and IFN-gamma increase induced by TNBS, and on the other side to up-regulate IL-10 production. Our results indicate that the *L. acidophilus* and *B. longum* mixture was able to prevent the TNBS induced colitis and suggest the importance of the IELs population in such protection.

Non-crystalline silica nanoparticles trigger IL-1beta release in LPS-primed macrophages – possible role of the NALP3 inflammasome**Wiggo J Sandberg; Marit Låg, Per Schwarze, and Magne Refsnes****Norwegian Institute of Public Health, Department of Air Pollution and Noise, P.box 4404, Nydalen, 0403 Oslo, Norway. Correspondence: wisa@fhi.no**

Silicon dioxide (SiO_2) is abundant in nature as crystalline quartz particles, and is known to cause chronic inflammation and lung fibrosis (silicosis) upon long-term exposure. Lung macrophages are known to release the pro-inflammatory cytokine IL-1beta after phagocytosis of micrometer-sized crystalline silica particles. The induction of IL-1b release occurs by activation of the intracellular NALP3 receptor, a cytoplasmic pattern recognition receptor known to recognize crystalline structures. Upon activation, NALP3 assembles a protein complex (inflammasome) that activates caspase-1, which in turn cleaves pro-IL-1b to its mature form. NALP3-deficient mice have decreased pulmonary inflammation and fibrosis after silica challenge, suggesting an important role of NALP3 in silica-induced pathology. Due to increasing utilization, a critical question is whether silica particles of nano-size have the same pathogenic potential. The mouse macrophage RAW cell line and primary rat lung macrophages were primed with LPS for 3 hours, and then exposed to increasing concentrations of nano-sized silica particles (30 nm, 50 nm and 100 nm) and crystalline silica (MinUsil; median diameter 5 micrometer) for 6 hours, and then analyzed for IL-1beta release by ELISA. A marked dose-dependent IL-1b response was observed for all particles in both cell systems. The IL-1b release both by the silica NPs and MinUsil was blocked by 10 microM of the Caspase-1 inhibitor zYVAD-fmk, and by the actin inhibitor cytochalasin D, suggesting an uptake-dependent, inflammasome-mediated mechanism. In conclusion, non-crystalline silica particles of nano-size markedly induce IL-1b release from cultured macrophages that may involve an inflammasome mechanism, as previously reported for large-sized crystalline silica particles. Further experiments are in progress to confirm NALP3 engagement by non-crystalline silica NPs. Moreover, to address whether the nano-size alone is important for this pathogenic mechanism, other non-crystalline nanoparticles and their micro-sized counterparts are now included in this study.

Session II

Poster II, 26

C. elegans as a model organism for inflammatory neurodegenerative diseases

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Neuroinflammatory processes are etiologically important in neurodegenerative diseases such as Alzheimer's disease (AD). Proinflammatory cytokines promote AD, whereas anti-inflammatory therapeutics may delay the onset of the disease. The level of mitochondria-derived reactive oxygen species (mtROS), induced by proinflammatory cytokines, is a central pathogenic determinant in AD and several other neurodegenerative diseases. MtROS create oxidative damage upon ambient neurons, which is closely associated with neuronal damage. Consistently, reduction of mtROS suppresses neuroinflammatory processes. To study basic pathogenetic mechanisms in inflammatory neurodegenerative diseases, we have established *C. elegans* as a new model system for analysing the impact of aging and pharmacological intervention on the formation of mtROS, structural and functional neurodegeneration, and changes in lifespan. To mimic neuroinflammatory processes, we increased mtROS formation by suppression of the *C. elegans* APE1 ortholog *exo-3*, a central component of the mtDNA repair system. To evaluate reduction of mtROS as a therapeutic option, animals were treated with FCCP, an uncoupler of the mitochondrial electron transport chain. Generation of ROS increased steadily during aging of wild-type *C. elegans* (WT), and to a greater extent in *exo-3* RNAi-treated animals. Sites of ROS generation colocalized with the neuronal system. FCCP significantly reduced ROS formation in both groups. Neuronal damage was not detected in WT up to the age of 12 days, whereas *exo-3* RNAi induced structural disintegrity by the age of 6 days. FCCP did not affect neuronal structure in WT and clearly reduced neuronal damage in animals treated with *exo-3* RNAi. To assess loss of neuronal function, different parameters of motility were analyzed. *Exo-3* RNAi significantly reduced head and whole animal motility. FCCP did not affect WT and significantly increased motility in animals with *exo-3* suppression. Mean and maximum lifespan were significantly reduced by *exo-3* RNAi, whereas FCCP significantly increased lifespan in WT and *exo-3* RNAi-treated *C. elegans*. These data demonstrate for the first time that levels of mtROS formation are closely linked to structural and functional neurodegeneration and lifespan in *C. elegans*. Accumulation of mtROS may explain the reported association between aging, rising prevalence of neurological diseases and persistent neuroinflammatory events. Thus, reduction of mtROS production may represent a therapeutic option for the treatment of inflammatory neurodegenerative conditions.

2-MBQ and 2,6-DMBQ exert apoptosis through different regulation of redox state-apoptosis signaling pathway in HT-29 colorectal cancer cells.

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2-MBQ (2-methoxy-1,4-benzoquinone) and 2,6-DMBQ (2,6-dimethoxy-1,4-benzoquinone) have been known to anti-carcinogenic components in fermented wheat germ. In this study, we have examined the effects of 2-MBQ and 2,6-DMBQ on apoptosis through different regulation of redox signaling pathway in HT-29 colorectal cancer cells. Our results showed that the 2-MBQ and 2,6-DMBQ have the potent anti-carcinogenic effects in HT-29 cell and *in vivo* xenograft models. 2-MBQ and 2,6-DMBQ treatment increased ROS generation which was correlated with decrease of cell viability. 2-MBQ and 2,6-DMBQ (10 and 50 μ M) treatment increased an apoptosis of HT-29 cells. In 2,6-DMBQ treated group (30 μ g/g/day) tumor volume was reduced by 44% when compared to tumor group *in vivo* xenograft model. To investigate the mechanisms of apoptosis by 2-MBQ and 2,6-DMBQ in HT-29 cells, the expression of redox state-apoptosis proteins such as p53, p-ASK1, pro-caspase, cleaved-PARP and COX-2 were analyzed. The 2-MBQ treatment increased p53 expression accompanied with a marked increase in pro-caspase and cleaved-PARP expression, and the 2,6-DMBQ treatment increased p-ASK1 expression. Both 2-MBQ and 2,6-DMBQ inhibited the expression of COX-2. These results suggest that the regulation of redox state may be important in anti-carcinogenic effects of 2-MBQ and 2,6-DMBQ through different controlling mechanism upon p53 and p-ASK1 expression. Moreover, apoptosis by regulation of redox state may be closely related to COX-2.

Inflammation and osteoarthritis: Impact of cytokines on the chondrocyte glycobiology

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In osteoarthritis, the onset of inflammatory processes has been recently recognised as a decisive factor for the degradation of cartilage tissue. Despite the significance of glycoproteins for extracellular matrix assembly in cartilage, little is known about the regulation of the chondrocyte glycophenotype under inflammatory conditions. The present study aimed to assess the impact of pro-inflammatory cytokines interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α) on the glycophenotype of primary human chondrocytes. Particular focus was set on the sialylation of chondrocyte glycoprotein N-glycans and O-glycans. The transcription of 11 sialyltransferase (SiaT) genes was quantified using real-time RT-PCR assays, whereas N-glycan and O-glycan analysis was performed using LC-ESI-MS. We found that primary human chondrocytes predominantly express α -2,6-specific SiaTs and accordingly, α -2,6-linked sialic acid residues in glycoprotein N-glycans. Importantly, a considerable shift towards α -2,3-linked sialic acids and α -2,3-specific SiaT mRNA levels occurred in primary chondrocytes treated with IL-1 β or TNF- α . In general, diminished *ST6Gal1* and increased *ST3Gal4* mRNA levels appear to be responsible for this shift towards enhanced α -2,3-sialylation under inflammatory conditions. Similarly, downregulated *ST6GalNAc3* and increased *ST3Gal1* mRNA levels resulted in increased α -2,3-sialylation of O-glycans. Given the involvement of sialylated glycoproteins in many biological processes including cell-matrix interactions, the presented findings may shed light on their significance for cartilage homeostasis and on their function as trigger molecules for cellular signaling pathways in osteoarthritis.

Role of the PGE₂ and EP receptors in the regulation of the mast cell activation induced by osmotic changes**Ivonne Torres A.¹, Fernando de Mora P.², Margarita Martín A.¹, César Picado¹**

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Mast cells are known for their critical roles in asthma and others, allergic and inflammatory diseases. Their versatility and is attributable to their capability to produce a wide array of regulatory and proinflammatory mediators. Exercise produces AHR in some asthma patients. The mechanism for exercise-induced bronchoconstriction (EIB) has been suggested to be related to an increased airway fluid osmolarity. This may activate mast cells which subsequently release mediators that act on bronchial smooth muscle leading to bronchoconstriction. In parallel protective substances such as PGE₂ are probably also released and could explain the observed refractory period in patients with EIB. The aim of this study was to characterize the effect of PGE₂ in human mast cells under hyperosmolar stimulation. Cells from the human mast cells line LAD2 were osmotically stimulated with mannitol. Mast cell activity was determined by β -hexosaminidase (β -Hex) release assay. Mast cells degranulation, cytokine production and PGE₂ secretion were measured. LAD2 cells expressed EP2/EP3/EP4 receptors. Interestingly, PGE₂ inhibited degranulation induced by mannitol in a concentration-dependent manner. Using selective EP receptors antagonists we found that PGE₂ can either potentiate or suppress mannitol-induced degranulation. Osmotic changes induced release of PGE₂ and cytokine production. PGE₂ modulates mannitol-induced hyperosmolar stimulation of human mast cells through interaction with EP receptors. The concentration of PGE₂ may be critical to trigger an activating or inhibitory response in the human mast cell line, LAD2.

Hippocampal gene network analysis to determine the effects of antioxidant treatment in an experimental model of accelerated senescence

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Senescence-accelerated mouse prone 8 (SAMP8) is widely used as an animal model of accelerated aging. The accelerated aging in this model is known to be partially retarded by the administration of an antioxidant agent. To clarify the molecular events that occur during accelerated aging and the molecular mechanism underlying the effects of antioxidants on the aging process, we performed a comprehensive gene expression analysis of the hippocampus of SAMP8 mice and normal control animals (SAMR1) with/without the administration of an antioxidant agent, i.e., coral calcium hydride (CCH), by using a DNA microarray. A large difference in gene expression was observed between the SAMR1 and SAMP8 models. The most significant difference was upregulation of genes related to the inflammatory and other immune response in SAMP8, although some immune response-related genes were downregulated. The difference observed in hippocampal gene expression in the SAMP8 model by the effect of CCH administration was much less than the difference between the SAMR1 and SAMP8 models. An intriguing effect of CCH administration was upregulation of the genes related to inflammation. Our result suggests that CCH may be effective to some extent as an antioxidant agent and may retard the accelerated senescence observed in SAMP8 through the regulation of genes related to the inflammatory response.

Intracellular Serum Amyloid A is colocalized with E.coli within mouse urothelial cells

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Serum amyloid A (SAA) is an acute phase protein involved in homeostasis of inflammation. SAA is necessary in host defense and has beneficial properties in protection against viral and bacterial infections. In salmon, E.coli content is correlated with higher SAA. It is also used as a sensitive marker during bacterial infection (E.coli) of reindeer. As an acute-phase marker, SAA acts similarly in humans and mice, making murine models accessible. This has not been the case with the classical inflammatory marker, C-reactive protein (CRP) which is inapplicable in mice. Urothelial infections have been especially difficult to treat since latent intracellular pools of bacteria remain in urothelial cells, also after treatment with antibiotics, and cause episodes of recurrent cystitis without evident re-infections. When SAA is present and/or under the appropriate conditions/conformation/interaction, it might downregulate and protect from development of recurrent infections. The aim of this study was to understand the role of SAA in murine infections of the urothelium with E.coli. Our experiments revealed that SAA is highly expressed in urothelial cells of E. coli infected mice versus non-infected mice. Especially, there is a high increase in cytoplasmic SAA during infection as compared to the nuclear SAA in urothelial cells. We also have evidence that SAA can be colocalized with cytoskeletal elements which are supposedly the attachment sites for the clusters of E. coli. We propose that SAA can serve as a marker for E.coli urothelial infection where it could also perform bactericidal functions.

Involvement of Peroxisome Proliferator-Activated Receptor (PPAR) gamma in epithelial tolerance against muco-active ribotoxic stress**Hyun Yang and Yuseok Moon**

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Inflammatory bowel diseases (IBD) are aggravated by disrupted epithelial barrier integrity. Some muco-active ribotoxic stresses are known to cause IBD-like symptom including mucosal intolerance by triggering the production of pro-inflammatory mediators by enhancing mRNA stability. Elav-like RNA binding protein 1 (ELAVL1) positively regulates mRNA stability of AU-rich elements (ARE)-containing transcripts such as pro-inflammatory mediators. We investigated the effects of ribotoxins on ELAVL1 translocation and its involvement in the regulation of the pro-inflammatory interleukin-8 (IL-8) mRNA stability. Exposure to the muco-active ribotoxins induced nuclear export of both endogenous and exogenous ELAVL1 protein in human intestinal epithelial cells. Moreover, ELAVL1 was shown to be involved in ribotoxin-induced IL-8 mRNA stabilization. It was also demonstrated that ELAVL1 was regulated by peroxisome proliferator-activated receptor gamma (PPAR gamma). PPAR gamma retarded DON-triggered cytoplasmic translocation of ELAVL1, which led to suppression of IL-8 production. In this study, ELAVL1 was the positive regulator for IL-8 production, and regulated by PPAR γ in anti-directional coupled manner. ELAVL1 and PPAR gamma were critical mechanistic links between ribotoxic stress and the pro-inflammatory cytokine production, and may have a broader functional significance with regard to gastrointestinal insults by muco-active ribotoxic stresses. (This work was supported by the Korea Research Foundation (KRF) grant funded by the Korea government (MEST) (No.2009-0065479)).

Session III: Anti-inflammatory Compounds

Antiinflammatory effect of a leguminous lectin isolated from *Canavalia maritima* seeds**Ana Maria S Assreuy, Nilson V Pinto, Natália VFC Rodrigues, Benildo S Cavada****Laboratório de Fisio-Farmacologia da Inflamação–LAFFIN, Universidade Estadual do Ceará, 60740-000 Fortaleza-CE, Brazil. E-mail: anassreuy@gmail.com**

Lectins are proteins used in the study of inflammation due to their capability to recognize carbohydrate in inflammatory cell membranes. The leguminous lectin isolated from *Canavalia maritima* (ConM) presents vasodilator activity *in vivo* and *in vitro*, but little is known about its anti-inflammatory effect. Here, it was investigated the ConM anti-inflammatory activity and its possible systemic toxicity. Protocols were approved by our Institutional Ethical Committee (Nº. 0559924-4). Edema was measured by hydroplethysmometry before (zero time) s.c. injection of carragenan (2 mg/paw) or dextran (300 µg/paw) into the hind paw of Wistar rats (150-250g) and thereafter (1-4h). Results were expressed as area under the time-course curve-AUC. ConM was injected i.v. (0.01; 0.1 and 1 mg/kg), 30 min before flogistic agents. Participation of the lectin domain was evaluated by incubation (30 min, 37°) of ConM with its binding sugar maltose (0.1M). The formalin test was performed by s.c. injection of formalin (2.5%, 20ml) into the hind paws of Swiss mice (25-30g) and the time that animals spent licking the paws was recorded from 0-5 min (neurogenic phase) and from 15-30 min (inflammatory phase). ConM (0.1-10 mg/Kg; i.v.) was injected 30 min before formalin. The toxicity was assessed after 7 days of rats treatment with ConM (1mg/Kg) analyzing renal and hepatic function (wet weight, creatinine and urea dosage, kinetic of serum transaminase activity, heart and spleen wet weight, body mass, leukogram and osmotic equilibrium (total protein and albumin/globulin)). Results were expressed as Mean ± SEM and analyzed by ANOVA followed by Duncan's test ($p<0.05$ to indicate differences). ConM (1mg/Kg) inhibited the rat paw edema induced by carragenan (200.9 ± 12.7) and dextran (240.7 ± 16.0) about 43% (113.9 ± 10.5) and 27% (176.3 ± 12.4), respectively. ConM inhibited the late phase of the nociception induced by formalin (210.6 ± 17.6 s) at 1 (86.0 ± 11.3 s, 59%) and 10 mg/Kg (96.7 ± 7.0 s, 54%). ConM presented antinociceptive and anti-edematogenic actions without apparent toxicity. The anti-edematogenic effect of ConM seems not to involve the lectin domain. **Acknowledgments:** CNPq, FUNCAP and Gabriela FO Marques-Domingos for technical assistance.

Improved blood lipid and antioxidant status in healthy subjects consuming soy- fortified rich lycopene tomato juice

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Background: Both tomato and soy foods are hypothesized to be beneficial for preventing cardiovascular disease and cancer. However, no studies have investigated the effects of simultaneously consuming soy and tomato. **Objective:** To investigate changes in blood lipids, oxidative stress and inflammatory markers, and metabolism of isoflavones/lycopene in humans consuming a novel functional food, soy-germ-fortified tomato juice, rich in isoflavones/carotenoids. **Design:** Healthy subjects (n=18) consumed 300mL soy-germ-fortified juice/d (containing 22mg isoflavones and 7.3mg lycopene/100mL) during an 8 wk intervention trial after a 1wk washout. Prior and after washout, and after 4 and 8wks of juice consumption, cholesterol, apolipoproteins A-I and B, triacylglycerols, plasma antioxidant capacity, Cu²⁺-mediated LDL+VLDL-C oxidation, C-reactive protein and carotenoids were measured in plasma; 8-isoprostaglandin-2α, isoflavones and metabolites in 24h urine. On day1 after washout, appearance of isoflavones in plasma and urine and lycopene in the triacylglycerol-rich lipoprotein-fraction were monitored 10h postprandial after consumption of 255mL juice. **Results:** Consuming the juice for 8wks significantly improved resistance of LDL+VLDL-C to oxidation (lag-time increase from 138.5±18.2 to 146.3±14.7min, P=0.039), HDL-C (from 47.3±15.8 to 51.7±14.8mg/dL, P<0.001), and the ratio of totalC/HDL-C (from 4.25±1.59 to 3.63±1.16, P<0.001); effects were less pronounced after 4wks. The postprandial trial indicated that 3.1±2.3% of lycopene was absorbed; 49.3±12.1% of isoflavones ingested were recovered within 24h urine pools. **Conclusions:** These studies show that lycopene and isoflavones were readily absorbed from the soy-fortified tomato juice, and that consuming the product for 8wks improved markers of blood lipids and antioxidant status in healthy subjects, making this a promising combination for future clinical studies.

Inhibition of expression of pro-inflammatory genes in cystic fibrosis cells by products from *Citrus Bergamia Risso*

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The inflammatory process of cystic fibrosis (CF) is characterized by production and release of cytokines and chemokines, among which interleukin 8 (IL-8) represent one of the most important. Accordingly, there is a growing interest in developing therapies against CF in order to reduce the excessive inflammatory response in the airways of CF patients. In this respect, extracts from medicinal plants have been reported to exhibit anti-inflammatory activities in several reports. In this study we report that extracts obtained from bergamot (*Citrus bergamia Risso*) epicarps contain two components (citropten and bergapten) displaying a strong inhibitory activity on IL-8 expression. These effects have been confirmed both at the mRNA levels and the protein release in the CF cellular model IB3-1 induced with TNF- α , using RT-PCR and Bio-plex technology respectively. These results indicate that bergapten and citropten could be proposed as potential anti-inflammatory molecules to reduce lung inflammation in CF patients.

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Microarray-based determination of anti-inflammatory genes targeted by fisetin in macrophages**Jihua Chen¹, Makoto Fujii^{1, 2}, De-Xing Hou^{1, 2}**

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3,3',4',7-tetrahydroxyflavone (fisetin) is a common flavonoid that exists in many types of plants including fruits, vegetables and medicinal herbs. To evaluate the anti-inflammatory function and underlying genes targeted by fisetin, gene expression profiling through DNA microarray was performed in mouse macrophages. Among 22,050 oligonucleotides, the expression levels of 406 genes were increased by equal or more than 3-fold in lipopolysaccharide (LPS) - activated RAW264 cells, 223 gene signals of which were attenuated by fisetin (equal or more than 2-fold). Expression levels of 717 genes were decreased by equal or more than 3-fold in LPS-activated cells, of which 417 gene expression were restored by fisetin (equal or more than 2-fold). Utilizing group analysis, 206 genes affected by fisetin were classified into 35 categories relating to biological processes (72), molecular functions (98), and signaling pathways (17) with equal or more than 2-fold change. The genes were further categorized as “defense, inflammatory response, cytokines activities, and receptor activities” and some of them were confirmed by real-time polymerase chain reaction. Ingenuity pathway analysis further revealed that fisetin regulated the relevant networks of chemokines, interleukins and interferons to exert its anti-inflammatory function. Our DNA microarray data, for the first time, revealed the gene expression profiling of fisetin in an inflammatory cell model, RAW264.7. These data provide a basis for understanding the molecular mechanisms of the anti-inflammatory effects of fisetin.

Black rice improves the abilities of memory and cognition through attenuation of inflammation induced by amyloid beta

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Age-related neurodegenerative diseases including Alzheimer's disease are related to inflammation. Therefore, the great effort has been focused on search for anti-inflammatory agents without toxicity. In the present study, the protective effect of black rice against amyloid beta 25-35 ($A\beta_{25-35}$)-induced memory impairment was investigated under *in vivo* Alzheimer's model. The memory and cognition abilities were observed by the tests of T-maze, object recognition and Morris's water maze. Although impairment of recognition and memory was observed by $A\beta$, the oral administration of black rice (50 and 100 mg/kg body weight/day) for 7 days after treatment of $A\beta$ showed improvement of cognition and memory. Furthermore, $A\beta$ treatment significantly elevated nitric oxide (NO) formation and lipid peroxidation, while the black rice significantly decreased NO formation and lipid peroxidation in brain, liver and kidney. In particular, the protective effect against inflammation induced by $A\beta$ was stronger in the brain than other tissue. It suggests that the improvement of memory deficit and cognition ability by black rice is closely related to the attenuation of $A\beta$ -induced inflammation.

Comparing the anti-inflammatory activity of curcumin alone and in a cocktail of plant extracts on cytokine-stimulated cartilage explants

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Curcumin (diferuloylmethane) is used in traditional medicine for treating inflammatory diseases such as arthritis. This study sought to test the hypothesis that curcumin may be more potent when acting synergistically with other plant extracts. The effects of curcumin alone and in combination with other known anti-inflammatory plant extracts, where it represented <20% of the formulation, were evaluated. Curcumin (from *Curcuma longa*) was dissolved in DMSO. A self-formulated cocktail of plant extracts containing <20% curcumin was dissolved in either DMEM or DMSO. Cartilage explants from horses euthanized for purposes other than research were incubated for 5 days with interleukin(IL)-1beta (10ng/ml⁻¹) and either curcumin (25, 50, 75, 100microM) or the cocktail in DMEM or DMSO (0.4, 40, 400micrograms/ml⁻¹). Curcumin significantly reduced IL-1beta-stimulated cartilage glycosaminoglycan (GAG) release at 50microM ($P<0.05$), 75microM ($P<0.01$) and 100μM ($P<0.001$). Prostaglandin E₂ (PGE₂) release was significantly lowered at 25μM curcumin ($P<0.001$). The plant extract cocktail in DMEM significantly increased IL-1beta-stimulated GAG release from 40micrograms/ml and above ($p<0.001$) whereas equivalent concentrations in DMSO reduced GAG release ($p<0.001$). PGE₂ release was significantly decreased by the cocktail in DMSO (40micrograms/ml; $p<0.01$) and in DMEM (400micrograms/ml; $p<0.01$). Micromolar concentrations of curcumin, alone and as part of the cocktail, exerted anti-inflammatory effects on the explants. However, as expected, one of the water-soluble cocktail components, bromelain, increased GAG release in DMEM due to its established proteolytic activity. Thus, the mode of action of key functional ingredients in plant extracts must be carefully considered before interpreting the results from *in vitro* models of cartilage inflammation.

Tumor targeted delivery of pravastatin by liposomes inhibits B16F10 melanoma growth in mice

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Statins have activities that extend beyond their cholesterol-lowering capability. These pleiotropic effects result from the altered post-translational modification of GTP-binding proteins, which regulate many intracellular pathways. Pre-clinical studies have suggested that these altered post-translational modifications could make statins effective anticancer agents. However, required doses are higher than those needed to lower cholesterol levels. Furthermore, in view of their wide-ranging effects on cellular metabolism, target site-specific delivery is preferred. In this study, we investigated tumor-specific delivery of pravastatin using long-circulating liposomes. 5 mg/kg liposome-encapsulated pravastatin inhibited murine B16F10-melanoma growth over 70% as compared to free pravastatin, which was ineffective. At 24 h after injection of the liposomes, 3 microgram of pravastatin could be recovered in the tumor, whereas drug levels were below detection limit (i.e. < 20 ng) after administration in the free form. Neither treatment affected cholesterol metabolism within the time frame of the study. *In vitro* studies on the effects of (liposomal) pravastatin on viability and proliferation of tumor cells, endothelial cells, and macrophages revealed that macrophages were the most sensitive cell type towards (liposomal) pravastatin treatment. Measurement of tumor levels of a panel of 24 proteins involved in inflammation and angiogenesis showed that liposomal pravastatin treatment effectively inhibited production of pro-inflammatory/pro-angiogenic mediators as compared to free drug. Taken together, targeted delivery of statins can improve their tumor growth inhibiting activity. This activity seems to result primarily from a local inhibition of tumor inflammation and angiogenesis.

Curcumin mediated suppression of NF-κB promotes chondrogenic differentiation of mesenchymal stem cells in a high-density co-culture microenvironment**Csaki C¹, U. Matis², A. Mobasher³, M. Shakibaei¹**

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Osteoarthritis (OA) and rheumatoid arthritis (RA) are characterised by joint inflammation and cartilage degradation. Although mesenchymal stem cell (MSC) like progenitors are resident in the superficial zone of articular cartilage, damaged tissue does not possess the capacity for regeneration. The high levels of pro-inflammatory cytokines present in OA/RA joints may impede the chondrogenic differentiation of these progenitors. IL-1 β and TNF- α activate the transcription factor nuclear factor- κ B (NF- κ B), which in turn activates proteins involved in matrix degradation, inflammation and apoptosis. Curcumin is a phytochemical capable of inhibiting the IL-1 β -induced activation of NF- κ B and the expression of apoptotic and pro-inflammatory genes in chondrocytes. Here we present evidence to show that although curcumin alone does not have chondrogenic effects on MSCs, it inhibits IL-1 β induced activation of NF- κ B, activation of caspase-3 and Cox-2 in MSCs as it does in chondrocytes in a time and concentration dependent manner. In contrast, in our high-density co-culture model of OA where primary chondrocytes and MSC are stimulated with IL-1 β , a 4h pre-treatment with curcumin antagonized the adverse effects of IL-1 β on the co-culture, thus promoting chondrogenesis. In IL-1 β stimulated co-cultures, pre-treatment with curcumin significantly enhanced the production of collagen type II, cartilage specific proteoglycans (CSPGs) and β 1-integrin and activation of the MAPKinase pathway. Furthermore, activation of caspase-3 and Cox-2 were suppressed. In conclusion, curcumin treatment may help establish a microenvironment in which the effects of pro-inflammatory cytokines are antagonized, thus facilitating chondrogenesis of MSC-like progenitor cells *in vivo*. This strategy may support the regeneration of articular cartilage.

Quercetin protects HepG2 cells against inflammation induced by TNF- α .**A.B. Granado-Serrano, M. A. Martín, L. Bravo, L. Goya and S. Ramos.****Departamento de Metabolismo y Nutrición. Instituto del Frío (CSIC), José Antonio Novais,
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Quercetin is one of the most common flavonoids found in the diet. It possesses antioxidant, antiinflammatory and anticarcinogenic properties, and it may modulate key proteins involved in signaling transduction pathways connected to the apoptotic and cell survival/proliferation processes (Granado-Serrano et al., *J Nutr* **2006**, 136, 2715-21; Granado-Serrano et al., *J Agric Food Chem* **2007**, 55, 2020-7; Granado-Serrano et al., **2009**, *Nutr Cancer*, in press). These features confer quercetin a great interest in pathological situations associated to oxidative stress, suggesting its potential as a chemopreventive agent. The aim of this study was to evaluate the effect of quercetin as anti-inflammatory agent in a model of inflammation induced by TNF- α in human hepatoma HepG2 cells. In order to establish a model of inflammation in cultured cells, the response of the main proteins involved in the modulation of the NF- κ B pathway (p-I κ B, I κ B, IKK and NF- κ B) to TNF- α , as well as the potential regulation of the NF- κ B pathway to quercetin were studied. Analyses were carried out pre-incubating cells with different quercetin concentrations (0-10 μ M) for 4 h and later stimulating cells with TNF- α . Cell viability and reactive oxygen species (ROS) generation were assayed by crystal violet test and by the dichlorofluorescein assay, respectively, and the inflammatory process was evaluated by analyzing p-I κ B, I κ B, IKK and NF- κ B levels. Treatment of HepG2 cells with all tested concentrations of quercetin did not result in any cell damage or change in the levels of the NF- κ B pathway proteins evaluated. TNF- α , which did not cause any cell damage, induced the NF- κ B pathway, showing the maximum stimulation after 30 min of incubation. Pretreatment for 4 h with 0.5-10 μ M quercetin reduced ROS generation when compared to control, TNF- α and 0.1 μ M quercetin treated cells, which showed similar ROS levels among them. Quercetin (5-10 μ M) greatly prevented the increase of p-I κ B, IKK and NF- κ B levels and the decrease of I κ B values evoked by TNF- α in HepG2 cells. In summary, treatment of HepG2 cells in culture with the natural dietary antioxidant quercetin strongly protects the cells against a pro-inflammatory insult.

The synergistic anti-inflammatory effect of Lycopene, Lutein and Carnosic acid combination**Nurit Hadad and Rachel Levy**

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Macrophages play an important role in host defense by producing a variety of pro-inflammatory cytokines such as TNF α as well as other inflammatory mediators including PGE₂ and nitric oxide (NO), which are synthesized by cyclooxygenase and inducible nitric oxide synthase, respectively. These inflammatory mediators are involved in the pathogenesis of many inflammation-associated human diseases, thus their attenuation may contribute to efficient therapy. The aim of the present research was to study the potency of combinations of Lycopene, carnosic acid, lutein and β -carotene, at concentrations that can be achieved in blood, to inhibit the release of the inflammatory mediators from macrophages exposed to LPS. Preincubation of mouse peritoneal macrophages with Lycopene (1 μ M), Carnosic acid (2 μ M) and Lutein (1 μ M) or β -carotene (2 μ M) before addition of LPS caused an efficient and synergistic inhibition of about 60% of NO and PGE₂ production and 40% of TNF α secretion. This inhibition was much higher than the additive inhibitory effect of each of the phyto-nutrients alone, that had a very low, if any, inhibitory effect. Combinations of the three carotenoids, excluding the polyphenol, carnosic acid, were less effective, suggesting that the synergistic effect was achieved by combinations of polyphenol and carotenoids that probably act by different mechanisms. Omitting Lycopene from combination of carotenoids and Carnosic acid resulted in lower inhibition compared with its presence, indicating the importance of for the process. The effect of these phyto-nutrient combinations is probably due to their anti-oxidant effect, since they caused a synergistic inhibition of around 80% in superoxide production by macrophages exposed to LPS. Taken together, all of the results obtained suggest that combinations of low concentrations of Lycopene, Carnosic acid Lutein and β -Carotene have a very potent anti-inflammatory effect.

Synthesis and Study the Analgesic and Anti-inflammatory Effects of Rigid 6-methoxy Benzopyran 3, 4 Dihydroxy Chalcon (DHC) in Mice

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Although, anti-inflammatory and antinociceptive drugs have some useful applications, but they have some adverse effects. Therefore researchers are trying to find new anti-inflammatory compounds with more ideal applications and less adverse effects. In this research anti-inflammatory and antinociceptive effects of rigid derivative of 6-methoxy benzopyran 3,4- Dihydroxy chalcone (DHC) was evaluated by Formalin, Hot plate and Carageenan tests. At first different doses of DHC 25, 50 and 75 mg/kg were injected to mice and the analgesic and anti-inflammation effects of it was evaluated by three mentioned tests. The result showed that dose of 50mg/kg of DHC induced more significant anti-inflammation and antinociception in formalin test. In addition the effect of DHC was higher in the chronic phase, therefore it seems that DHC has better anti- inflammatory effect rather than analgesic effect. Doses of 50 and 75 induced some lethargy in the mice. DHC induced analgesia and anti-inflammation in hotplate and carageenan test too. The result showed that DHC has a significant anti-inflammatory and antinociceptive effect and more effective compounds can be synthesized by modification of structure of this derivative.

Key words: Methoxy Benzopyran 3, 4 Dihydroxy Chalcon, Anti inflammation, Anti nociception, Mice.

Antinociceptive and anti-inflammatory effects of new rigid 6 Methoxy Benzofurane 3,4- Dihydroxychalcone (M-DHC) in mice

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Introduction: Study of anti- inflammation and antinociception effects of rigid drivetives from 3,4- Dihydroxy chalcone is the revise of this research. In this study the analgesic effect of different doses of rigid drivative 3,4- Dihydroxy chalcone, was injected intraperitoneally to mice and the analgesic and anti- inflammation effects was evaluated by formalin, Hotplate and Carageenan tests. **Methods:** first Different concentration of 3,4- DHC prepared and injected to mice Doses of 12.5, 25 and 50 mg/kg of 3,4- DHC evaluated by formalin, Hotplate and carageenan tests. Effective dose compared by morphine and Ibuprofen. **Results:** The result showed that, 3,4- DHC with dose of 25mg/kg induced Significant antinociception and anti- inflammation compared with control group. In formation test , in additon the effect of 3,4-DHC was higher in the chronic phose of formalin test, therefore it seems that 3,4- DHC has better anti- inflammatory effect rather than analgesic effect. The results showed that, dose of 25 mg/kg of 3,4 DHC, induces significant analgesia in hot plate test in 45 and 60 ominutes the doses of 25 and 50 mg/kg, induced lethargy in mice. The analgesic effect of the most effective dose of 3,4 – Dihydroxy chalcone was lower than morphine (2.5 mg/kg) in all time in formalin, Hotplate tests. The analgesic effect of DHC was higher than Ibuprofen (200mg/kg) in 0-5 minute in formalin test and in 45 and 60 minutes in Hot plate test but in chronic phase of formalin test and one hour after injection carageenan in carageenan test was nearly equal 3,4-DHC. **Conclusion:** The result showed that with madification of stractare of the DHC, this drivative has a significant analgesic effect and it could be used for more studies to access aclinical use of 3,4- DHC as a drug.

Key words: 3,4-Dihydroxy chalcone, Anti-inflammation effect, Anti nociception effect, formalin test, Hot plate test, carageenan test, Mice.

Antinociceptive and anti-inflammatory effect, of new rigid, propoxy benzopyrane-3, 4 Dihydroxychalcone (DHC-P) by Hot-plate, Formaline and Plethysmography tests, in mice

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Introduction: There are many reports indicating the analgesic and anti inflammatory effects of 3,4 dihydroxy chalcones. In this study antinociceptive and anti-inflammatory effects of rigid derivative 3-(3,4-dihydroxybenzylidene)-7-propoxy benzopyran-4-one, (DHC-P) were evaluated by Formalin, Hot plate and Carageenan tests. **Methods:** Experimental doses of 50, 75 and 100 mg/kg of DHC-P were injected to mice and the analgesic and anti inflammatory effects evaluated by Formalin, Hotplate and Carageenan tests. Effective dose compared with Morphine and Ibuprofen. **Results:** The result showed that, DHC-P with dose of 75mg/kg induced significant anti nociception and anti inflammation in Formalin and Carageenan tests. The results showed that the dose of 75 mg/kg of DHC-P induces significant analgesia in 45 and 60 minutes in hot plate test .The analgesic effect of the most effective dose of DHC-P 75mg/kg was lower than morphine (2.5 mg/kg) in all time in Formalin and Hot plate tests. The analgesic effect of DHC-P was higher than Ibuprofen (200mg/kg) in 0-5 minute in Formalin test and in 45 and 60 minutes in Hot plate test, but in chronic phase of Formalin test was nearly equal to Ibuprofen. In Carageenan test, the anti inflammatory effect of DHC-P was higher than Ibuprofen (200mg/kg) and morphine (2.5 mg/kg) in the first and third hours. Therefore it seems that DHGP has better anti-inflammatory effect rather than analgesic effect. The doses of 75 and 100 mg/kg, induced lethargy in mice. **Conclusion:** The results showed that the modification of this structure of DHC-P, may lead to more effective derivative with significant analgesic effect and it could be used for more studies to access a clinical use of 3,4 DHC as a drug.

Key words: 3-(3,4-dihydroxybenzylidene)-7-propoxy benzopyran-4-one, Anti-inflammation, Anti nociception, Mice.

Comparison of the Gastric Ulcerogenicity of Analgesic Doses of Percolated Extract of *A. Occidentale* with Indomethacin in Rats

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In traditional Iranian medicine, the core of the fruit of *Anacardium occidentale* (cashew nut) was used in the treatment of pain. In this research comparison of ulcerogenic effect of percolated extract of *A. occidentale* with indomethacin were investigated. There are five rats with (200-250 g) weight in each group. The extract or indomethacin was administrated orally with dose of 200, 300, 400 and 800 mg/kg. In control group normal saline was administrated with volume of 5 mg/kg. The animals were killed 4h after receiving extract or indomethacin or normal saline. The stomachs were removed and 10ml formalin 2% injected in to the stomach to fix the inner layer of the gastric wall. The stomachs were incised along the greater curvature and lesion in the glandular portion were evaluated, 20 min after formalin exposure. The ulcer index was calculated, using the J- Score. The results showed that the oral dose of 200mg/kg of the extract did not induce any ulcerogenic effect in the rat' stomach. The dose of 300, 400 and 800 mg/kg of extract induced lower ulcerogenic effect than the same dose of indomethacin ($p<0.01$). Therefore according to previous study indicating the analgesic effect of *A. occidentale*, it seems that this plant is suitable for more investigation as an analgesic compound with low gastro intestinal unwanted effect in clinical use.

Key words: *Anacardium occidentale*, Gastric ulcerogenicity, Cashew nut, J-Score

Investigation of *Hypericum Perforatum* Extract on Convulsion Induced by Picrotoxin in Mice

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Therapeutic effect of *Hypericum perforatum* L. has been known for many years. In this investigation, we studied about effects of methanolic extract of this plant against seizure induced by picrotoxin in mice. In first phase, the study was performed on four groups of animals pretreated with different doses of percolated extract of this plant via Intraperitoneal injection. After 20 minutes each animal received picrotoxin for induction of seizure. Latency of seizure, Duration of seizure, Death latency and Mortality percent were determined. The results showed that the latency of seizure was increased in group that pretreated with dose of 50 mg/kg ($p<0.01$). The results showed that the dose of 50 mg/kg of *Hypericum perforatum* L. maybe have some beneficial effect in seizure induced by picrotoxin. More studies are needed in this field.

Keywords: *Hypericum perforatum* L. , Picrotoxin, Seisure, mice.

Evaluation of the Effects of *Annacardium Occidentale* Extract on Histopathology and Hepato - Renal Function Tests in Rats

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Introduction: In Iranian traditional medicine, the core of the fruit of *Anacardium occidentale* (A.O) was used for relief of pain. In this research hepatotoxicity and renal toxicity of percolated extract of A.O were investigated.

Methods: Five groups of six rats in each group were used in this experiments. The extract was administered orally with doses of 200-400 and 800mg/kg every 24 hours for 7 days. In control group, normal saline was administered 5ml/kg. In the end of 7th day, urine, blood and tissue specimen were collected for analysis. In second of the experiment, the blood enzymes including; Alanin transpherase (ALT), Aspartate transpherase (AST), Alkaline phosphatase (ALP), and the serum creatinin (Cr), and the Blood Urea Nitrogen (BUN) and Activity of urinary enzymes including; gama- glutamil transpherase (GGT), and lactate dehydrogenase (LDH), changes in the kidney weights were determined. In third phase of the experiment, histopathological study in kidney and liver were evaluated.

Results: The results showed, the oral doses of 200mg/kg (therapeutic analgesic dose) and 400mg/kg of the extract, did not induce any hepatotoxicity or renal toxicity effects in the rats, but the dose of 800mg/kg induced hepatotoxicity and renal toxicity.

Conclusion; It seems that the A.O is a suitable plant for further investigation for introducing as analgesic drug for clinical use.

Key Words: *Annacardium Occidentale*, Hepatotoxicity, Renal toxicity, Rat, Histopathology

Protective effect of Isoorientin against oxidative stress, inflammation, and apoptosis of high glucose exposed human umbilical vein endothelial cells**Ji Young Hwang¹, Ji Sook Han¹****¹Department of Food Science and Nutrition, Pusan National University, 30 Jangjeon-dong, Busan, 609-735, Korea****hjy770802@nate.com, hanjs@pusan.ac.kr**

Sasa borealis bamboo has been used for making tea in the Far East, and it is known for traditional medicine. Antioxidant activity-guided fractionation of butanol fraction from *Sasa borealis* led to the isolation of Isoorientin which has strong antioxidant activity. The present study examined the protective effect of Isoorientin against high glucose induced oxidative stress, inflammation, and apoptosis in human umbilical vein endothelial cells (HUVEC). To assess the efficacy of Isoorientin, several key markers and activities were measured, including cell viability, lipid peroxidation, intracellular reactive oxygen species (ROS) and nitric oxide (NO), as well as expressions of inflammatory proteins, inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and nuclear factor-kappa B (NF- κ B), and levels of Bcl2/Bax protein. Results showed that high glucose treatment induced HUVEC cell death, but Isoorientin significantly increased cell viability and effectively suppressed lipid peroxidation, ROS generation and NO level from high glucose induced glucotoxicity. In addition, Isoorientin significantly reduced iNOS, COX-2, NF- κ B and pro-apoptotic Bax protein levels, whereas it increased anti-apoptotic Bcl2 protein level. These results suggest that Isoorientin has a protective efficacy against several deleterious effects caused by high glucose exposure in HUVEC.

[6]-Gingerol Rescues BV2 Microglial Cells from β -Amyloid-induced Pro-inflammatory Damages and Cell Death**Jung-Hee Jang****College of Oriental Medicine, Daegu Haany University, Daegu 706-828, Korea****E-mail : pamy@dhu.ac.kr**

β -Amyloid (A β) peptide is responsible for the formation of senile plaques, the neuropathological hallmarks of Alzheimer's Disease (AD) and has been reported to induce apoptotic cell death in neurons and glia via oxidative and pro-inflammatory pathways. Recently, considerable attention has been focused on dietary manipulation of oxidative and inflammatory cell death in neurodegenerative disorders including AD. Therefore, in this study, we have investigated the neuroprotective effect of [6]-gingerol, a pungent ingredient of ginger against A β -induced inflammatory cell death. BV2 microglial cells treated with A β underwent apoptotic cell death as determined by positive in situ terminal end-labeling (TUNEL staining), decreased mitochondrial transmembrane potential, and increased ratio of proapoptotic Bax to antiapoptotic Bcl-2. [6]-Gingerol pretreatment attenuated A β -induced apoptosis in BV2 cells. Moreover, [6]-gingerol effectively inhibited A β -induced cell death by suppressing expression of inducible nitric oxide (iNOS) and subsequent production of nitric oxide (NO) and peroxynitrite. A β treatment caused activation of NF- κ B as an upstream regulator for iNOS expression, which seemed to be mediated by activation of extracellular signal-regulated kinase (ERK) and p38 mitogen-activated protein kinase (p38 MAPK). [6]-Gingerol pretreatment effectively down-regulated DNA binding of NF- κ B, phosphorylation of p65, the functionally active subunit of NF- κ B, and degradation of I κ B α , the cytoplasmic inhibitor of NF- κ B by suppressing upstream kinases. Thses results suggest that [6]-gingerol may have preventive and/or therapeutic potential in the management of neuroinflammation and cell death in AD.

Effect of a novel COX-2 inhibitor on IL-8 expression in *Helicobacter pylori*-infected gastric epithelial AGS cells**Sung Hee Jang and Hyeyoung Kim****Department of Food and Nutrition, Research Institute of Food and Nutritional Sciences,
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Helicobacter pylori (*H. pylori*) infection induces many pathogenic factors including reactive oxygen species (ROS) in gastric epithelial cells. ROS is considered as an important regulator in pathogenesis of *H. pylori* associated inflammation in *H. pylori*-associated gastric diseases. Previously we showed that ROS induced the expression of COX-2 and IL-8 by activating redox-sensitive transcription factors NF- κ B and AP-1 in gastric epithelial AGS cells. In the present study, we determined whether a novel synthetic COX-2 inhibitor suppresses the expression of IL-8 by inhibiting NF- κ B and AP-1 in AGS cells. *H. pylori* strain of HP99 (Korean isolates) was used to stimulate gastric epithelial AGS cells with or without treatment of a new COX-2 inhibitor. A novel COX-2 inhibitor was kindly provided by Prof. E. S. Lee, Yeungnam University, Korea. HP99 induced the expression of IL-8 with the activations of NF- κ B and AP-1 and degradation of I κ B α . A new COX-2 inhibitor (2 or 5 μ M) inhibited the expression of IL-8 dose-dependently and suppressed DNA binding activities of NF- κ B and AP-1 as well as degradation of I κ B α . In conclusion, a novel synthetic COX-2 inhibitor may have anti-inflammatory activity by inhibiting the expression of target genes of NF- κ B and AP-1 in *H. pylori*-stimulated gastric epithelial cells.

Effect of *Rubus coreanus* Miquel added to *Bacillus* sp.-fermented soymilk on high glucose-induced vascular inflammation in human umbilical vein endothelial cells**Hong Eun Ju¹ and Ji Sook Han¹**

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Vascular inflammation is an important factor which can promote diabetic complications. We investigated whether 5% *R. coreanus* Miquel added to *Bacillus* sp.-fermented soymilk (RFS), which had strong antioxidant activity in previous study, suppresses vascular inflammatory process induced by high glucose in human umbilical vein endothelial cells (HUVEC). Western blot analysis revealed that incubation of HUVEC with high glucose increased cell adhesion molecules (CAMs) expression levels. However, high glucose-induced increase of CAMs expression was attenuated by pretreatment with RFS extract (RFSE). The cell adhesion between HL-60 monocyte and HUVEC induced by high glucose was also reduced by pretreatment with RFSE. Pretreatment with RFSE inhibited high glucose-induced intracellular reactive oxygen species (ROS) formation. In addition, RFSE suppressed the transcriptional activity of NF- κ B increased in high glucose condition. The present data suggested that RFSE could suppress high glucose-induced vascular inflammatory process, which may be closely related with the inhibition of ROS and NF- κ B activation in HUVEC.

Acknowledgment: This research was financially supported by the Ministry of Education, Science Technology (MEST) and Korea Institute for Advancement of Technology (KIAT) through the Human Resource Training Project for Regional Innovation.

Anti-aging effect and anti-inflammatory mechanisms of kimchi during fermentation under stress-induced premature senescence cellular system**Boh Kyung Kim¹, Eun Ju Cho¹, Hyun Young Kim², Kun Young Park¹**

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Aging is known to associate with inflammation that accelerates aging process. The anti-aging activity and the related inflammatory mechanisms of kimchi during fermentation was evaluated using stress-induced premature senescence (SIPS) of WI-38 fibroblasts caused by hydrogen peroxide (H_2O_2), a well-established cellular aging model. The methanol extracts of fresh kimchi (pH 5.8), optimally ripened kimchi (Opt-R kimchi, pH 4.1) and over ripened kimchi (pH 3.7) fermented at 5°C were prepared. H_2O_2 -treated WI-38 cells showed the loss of cell viability, the increase of lipid and shortening of the cell lifespan, indicating the induction of SIPS. However, treatment with kimchi, especially Opt-R kimchi, attenuated cellular oxidative stress through increase in cell viability and inhibition of lipid peroxidation. In addition, the lifespan of WI-38 cell was extended, suggesting promising role of kimchi as an anti-aging agent. Furthermore, H_2O_2 -treated WI-38 cells significantly increased the age-related inflammatory gene expression such as nuclear factor kappa B (NF- κ B), cyclooxygenase-2, inducible NO synthase and Bax. However, the treatment of kimchi exerted anti-aging effect through NF- κ B-related gene regulation. These results suggest that kimchi, especially Opt-R kimchi, may delay the aging process by regulation of inflammatory process.

Pine bark extract enzogenol blunted pro-inflammatory tumor necrosis factor-alpha-induced endothelial cell adhesion and transmigration of human monocytic THP-1 cells**Dong Shoo Kim, Min-Soo Kim, Young-Hee Kang****Department of Food science and Nutrition, Hallym University, Chuncheon, Korea****E-mail:** yhkang@hallym.ac.kr

The leukocyte recruitment and transmigration across the endothelial barrier into the vessel wall are crucial steps in promoting atherosclerosis. Cell adhesion molecules mediate the attachment of THP-1 monocytes and facilitate their migration into the subendothelial space, thus contributing to accumulation of mononuclear cells in the vascular wall that is one of the initial steps in the development of atherosclerosis. Vascular endothelial cells are an important target of pro-inflammatory cytokines modulating many genes involved in cell adhesion, thrombosis and inflammatory responses. This study examined whether pine bark extract enzogenol blunts transendothelial migration of THP-1 monocytes through TNF-alpha-activated human umbilical vein endothelial cell (HUVEC). The pro-inflammatory TNF-alpha markedly induced protein expression of intracellular cell adhesion molecule, vascular cell adhesion molecule, and E-selectin with increasing mRNA levels in HUVEC. Nontoxic enzogenol at 5-25 microgram/mL attenuated the expression of all three adhesion molecules in a dose-dependent fashion. Consistently, enzogenol suppressed the enhanced THP-1 monocytes adhesion onto TNF-alpha-activated HUVEC. In TNF-alpha-activated HUVEC was observed IkB dissociation and NF-kappaB nuclear translocation, which was ameliorated by enzogenol. Furthermore, enzogenol hampered the transendothelial migration of THP-1 monocytes by increasing matrix metalloproteinase-9 activity. These results demonstrate that blunting induction of cell adhesion molecules by enzogenol was mediated by their interference with the NF-kappaB-dependent transcription pathways. Thus, enzogenol may have therapeutic potential targeting inflammatory response-associated atherosclerosis. This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MEST) (The Regional Research Universities Program/Medical & Bio-Materials Research Center)

Anti-inflammatory effect of soybean germ extract by regulation of NF-κB in human colorectal cancer cells.

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Inflammation is a complex event modulated by various mediators. Nitric oxide (NO) and prostaglandins (PGs) are known to be an important mediator of acute inflammation. They are synthesized by nitric oxide synthase (NOS) and cyclooxygenases (COX), respectively. In this study, we investigated anti-inflammatory effect of soybean germ extract (SGE) in lipopolysaccharide (LPS)-stimulated HT-29 human colorectal cancer cells. Soybean germ extract inhibited in a dose-dependent manner the protein expression levels of inflammatory mediators such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in LPS-stimulated HT-29 cells. Also, our results showed that the inhibition of iNOS and COX-2 takes place via a down regulation of NF- κB. In conclusion, our study indicates that soybean germ extract (SCE) may be utilized for the development of an anti-inflammatory functional food and further studies can be conducted to estimate its effect *in vivo* animal model and clinical study.

4-[(butylsulfinyl)methyl]-1,2-benzenediol (SMBD) inhibits early atherosclerosis in high cholesterol fed rabbits

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Hypercholesterolemia plays key roles in the production of oxidative stress in vascular cells and stimulates the early stage of the atherosclerosis process. In this study, 4-[(butylsulfinyl)methyl]-1,2-benzenediol (SMBD), sulfur-containing phenolic antioxidants in sea weed, was synthesized and demonstrated to inhibit the Cu²⁺-induced low-density lipoprotein oxidation. Rabbits were fed an atherogenic diet containing 0.5% (w/w) cholesterol and 10% (w/w) coconut oil for 4 weeks, while SMBD or simvastatin was given as an intravenous injection (0.33 mg/kg/day) simultaneously. The SMBD significantly lowered the plasma cholesterol, lipid peroxidation and aortic lipid levels compared to the control group. Furthermore, when compared with the control group, both the simvastatin and SMBD group significantly inhibited the proliferation of aortic intima, resulted in reducing the thickness by 38% and 36% through retarding the aortic reactive oxygen species (ROS) generation. The cyclooxygenase-2 protein levels of these groups were also decreased. In conclusion, SMBD seems to have benefits in the early stage of atherosclerosis through its antioxidant activities, especially with retardation of ROS generation.

Protective effect of purple sweet potato added to *Bacillus* sp.-fermented soymilk from amyloid beta-induced memory impairment through attenuation of inflammation

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Inflammation has been known to play an important role in the progression of Alzheimer's disease (AD). The present study was focused on the protective effect of 5% purple sweet potato added to *Bacillus* sp.-fermented soymilk (PFSM), which had strong antioxidative activity in previous study, from cognition impairment under AD model of ICR mouse induced by amyloid beta (A β). To observe the abilities of memory and cognition, T-maze, object cognition and Morris water-maze tests were carried out. A β injected groups showed the impairments of cognition and memory. However, the oral administration of PFSM extract (100 and 200 mg/kg body weight/day for 7 days) improved cognition and memory. Furthermore, nitric oxide (NO) formation and lipid peroxidation were significantly elevated by A β , whereas the PFSM treatment significantly decreased NO formation and lipid peroxidation in brain, liver and kidney. The present study suggests that PFSM significantly improves memory deficit and cognition ability induced by A β . The protective effect of PFSM against A β -induced impairments of memory and cognition ability is closely related to the attenuation of inflammation induced by oxidative stress.

Acknowledgment: This research was financially supported by the Ministry of Education Science Technology (MEST) and Korea Institute for Advancement of Technology (KIAT) through the Human Resource Training Project for Regional Innovation.

Suppression of inflammatory responses by celastrol, a quinone methide triterpenoid isolated from *Celastrus regelii*

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Background Celastrol, a quinone methide triterpenoid isolated from the *Celastraceae* family, exhibits various biological properties, including chemopreventive, antioxidant, and neuroprotective effects. In this study, we showed that celastrol inhibits inflammatory reactions in macrophages and protects mice from skin inflammation.

Materials and methods Anti-inflammatory effects of celastrol (0-1 μ M) were examined in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. In order to investigate the effects of celastrol (0-50 μ g/mice) *in vivo*, activation of myeloperoxidase (MPO), and histological assessment were examined in the 12-O-tetradecanoyl-phorbol-13-acetate (TPA)-induced mouse ear edema model.

Results Our *in vitro* experiments showed that celastrol suppressed not only LPS-stimulated generation of nitric oxide and prostaglandin E₂, but also expression of inducible nitric oxide synthase and cyclooxygenase-2 in RAW264.7 cells. Similarly, celastrol inhibited LPS-induced production of inflammatory cytokines, including tumor necrosis factor- α and interleukin-6. In an animal model, celastrol protected mice from TPA-induced ear edema, possibly by inhibiting MPO activity and production of inflammatory cytokines.

Conclusions Our data suggest that celastrol inhibits the production of inflammatory mediators and is a potential target for the treatment of various inflammatory diseases.

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Inhibitory effects of synthesized product of XH-14 derivative on expression of pro-inflammatory factors, visfatin and resistin in 3T3-L1 adipocyte

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XH-14 is known as a potent ingredient isolated from Danshen, the dried root of *Salvia miltiorrhiza* Bunge (Lamiaceae), is one of the most commonly used traditional Chinese medicines for cardiovascular indications and structurally identified as a benzo[b]furan lignan. Many biologically active benzo[b]furan compounds are found in nature. Herein, 2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-6-methoxybenzofuran (5-methoxy-XH-14, HC-59) was used in this study. This study tested whether HC-59 can inhibit differentiation of adipocytes and expression of pro-inflammatory visfatin and resistin, visceral-fat-specific adipokines. The 3T3-L1 preadipocytes were differentiated in DMEM-10% FBS for up to 6 days in the absence and presence of nontoxic HC-59 between 1 and 25 microM. Oil Red O staining and Western blotting analyses revealed that HC-59 at more than 5 microM attenuated lipid accumulation and expression of PPAR gamma and C/EBP alpha, indicating that submicromolar HC-59 suppressed adipocyte differentiation. In addition, HC-59 dampened expression of visfatin and resistin proteins highly elevated in differentiated adipocytes. The results demonstrate that compound HC-59 may be a promising agent to disturb adipocyte differentiation and obesity-associated inflammation. This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MEST)" (The Regional Research Universities Program/Medical & Bio-Materials Research Center)

Free radical scavenging activity of fermented soymilk with *Bacillus subtilis***Mi Jin Kim^{a†}, Sun Hee Hong^a, Yeong Ok Song^a**

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Free radical scavenging activities of natural products are important to reduce the oxidative stress which subsequently will retard the inflammatory responses in the body. Health promoting effects of soymilk (SM) products are well known to people and these beneficial effects of SM will increase by fermentation. In this study, free radical scavenging activities of SM fermented with *Lactobacillus acidophilus* or/and *Bacillus subtilis* were examined. Fermentation elevated the radical scavenging activity of SM against DPPH radical, hydroxyl radical, nitric oxide radical, superoxide radical, and peroxinitrite radical. But the radical scavenging activities of various FSMs were differed. FSM by *Bacillus subtilis* showed the greatest radical scavenging activity against DPPH, hydroxyl and peroxy nitrite radicals among FSMs. But nitrite and superoxide scavenging activities of FSMs were not differed. IC₅₀ for DPPH scavenging activity of FSM with *Bacillus subtilis* and its total antioxidant activity expressed as TEAC were 769.23 ug/mL and 0.55mM, respectively, which were 140 and 275% higher than un-fermented SM (UFSM). LDL susceptibility to oxidation was retarded by 60%, compared with UFSM. These results indicate that FSM with *Bacillus subtilis*, beverage type *natto*, would reduce oxidative stress via scavenging free radicals and increasing antioxidant status in the body when we consume it. Further study for FSM against inflammation is needed. (This research was financially supported by MEST and KIAT through the Human Resource Training Project for Regional Innovation.)

Transdermal application of ultradeformable cationic liposome for the treatment of inflammatory skin disease

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Ultradeformable cationic liposome (UCL) has been recently developed to improve transdermal delivery of therapeutically active agents. To alleviate disease conditions associated with inflammatory skin disease, we formulated UCL containing IL-13 antisense oligodeoxynucleotide (ASO) and investigated their physicochemical properties *in vitro*. When applied *in vivo* model of inflammatory skin disease, IL-13 ASO-UCL complexes dramatically suppressed IL-13 production as well as other T helper 2 lymphocyte-secreted cytokines, IL-4, IL-5. Therefore, IL-13 ASO-UDL presents the ability to control inflammatory conditions and further demonstrated the feasibility as an effective therapeutics for inflammatory skin disease.

Curcumin down-regulates HIF-1 α through the regulation of AMPK in hypoxia-induced HT-29 colon cancer cells

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Hypoxia inducible factor 1 α (HIF-1 α), the primary transcriptional regulator has been reported to play an important role in modulating tumor growth and angiogenesis in response to oxygen deprivation. In this study, we investigated the possible cross-talk between HIF-1 α and AMPK (AMP-activated protein kinase), a major signaling molecule for the regulation of energy metabolism, often induced by cellular stress such as glucose deprivation and hypoxia utilizing HT-29 colon cancer cells treated with curcumin. The induction of HIF-1 α and AMPK was observed in cancer cells under CoCl₂ induced hypoxic state. However, curcumin reduced the expression of HIF-1 α , which was increased in cells of hypoxic state with CoCl₂. The effective AMPK activator, curcumin abolished the ability of CoCl₂ to induce HIF-1 α expression. These observations suggest that phytochemical such as curcumin may exert the inhibitory effects on HIF-1 α in hypoxic tumor cells, and thus control tumor cell growth and angiogenesis. [This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MEST) (NO. R01-2008-000-20131-0)]

ATP-gated P2X₁ ion channels negatively regulate neutrophil activation: a novel anti-inflammatory mechanism

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Extracellular nucleotides, secreted by activated blood platelets and leukocytes or by epithelial and endothelial cells submitted to mechanic stress or hypoxia, act as signalling molecules through their interaction with P2Y and P2X receptors. Upon tissue damage or infection, a sudden increase of extracellular ATP concentration occurs, that is thought to contribute to the inflammatory response. We previously showed that ATP-gated P2X₁ ion channels are expressed by neutrophils and promote their chemotaxis through Rho Kinase activation. In this study, we investigated the role of these channels in neutrophil activation both *in vitro* and *in vivo* in murine models of sepsis. *In vitro* studies revealed that neutrophils isolated from the bone marrow of P2X₁^{-/-} mice had enhanced respiratory burst activity. Reactive oxygen species (ROS) production induced by formylated peptide, PMA or serum-opsonized zymosan particles was significantly augmented as compared to that of wild-type neutrophils. A hallmark of neutrophil activation is the shedding of L-Selectin (CD62L). P2X₁^{-/-} neutrophils displayed significantly lower CD62L expression than wild-type neutrophils, indicative of a basal pre-activated state. This observation was confirmed in a model of endotoxemia induced by intraperitoneal injection of a sub-lethal dose of lipopolysaccharide (LPS). We showed that plasma myeloperoxidase (MPO) concentration, an indicator of neutrophil systemic activation, was increased after LPS treatment in wild type mice but reached significantly higher levels in the P2X₁^{-/-} mice. In addition, peripheral P2X₁^{-/-} neutrophils expressed higher levels of CD11b in response to LPS injection, again reflecting their higher activation state. Concomitantly, we observed that the LPS-induced drop in platelet and lymphocyte counts were both worsened in the P2X₁^{-/-} mice as compared to their wild type littermates. Furthermore, immunohistochemistry and MPO activity assay revealed exaggerated neutrophil relocalization into the lungs of P2X₁^{-/-} mice, where these cells formed large aggregates in the capillary lumen. Finally, in a model of septic shock induced by intraperitoneal injection of a lethal dose of LPS, the P2X₁^{-/-} mice exhibited shorter survival time than wild type mice, most likely as a consequence of enhanced neutrophil-mediated organ failure. Taken together, these results suggest that P2X₁ ion channels negatively regulate neutrophil activation, contributing to limit inflammation in response to bacterial challenge.

***Elaeagnus multiflora* extracts exerts anti-inflammatory effects by inhibiting COX-2 and Akt signals in HT-29 colon cancer cells**

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Cancer cells generally show hyper-activated inflammatory molecules, which are related to cancer survival and malignancy. In this study, we have examined the potential of *Elaeagnus multiflora* as a cancer preventive agent through regulating inflammatory signals including COX-2 and Akt. *Elaeagnus multiflora* has been traditionally used in china, and recently, it has reported to exert anti-oxidative effect, anti-inflammatory and anti-cancer effects. We obtained extracts from seed and flesh of *Elaeagnus multiflora* extracted with 95% ethanol and analyzed the effects of COX-2 and Akt activities in HT-29 colon cancer cells. Our results showed that the treatment of seed extracts (200 – 800 µg/ml) for 48 h effectively reduced COX-2 and p-Akt expression, and their inhibitory effect was stronger than that of flesh extracts. In addition, these anti-inflammatory effects seemed to be related to cancer cell death. Both of seed and flesh extracts inhibited cell growth and induced apoptosis in HT-29 cells. These results suggest that *Elaeagnus multiflora* may contribute to suppressing cancer growth through its anti-inflammatory and anti-proliferative effects, and natural products used as oriental medicine have the possibility to control tumor cell growth.

Quercetin exerts apoptotic effects through regulation of AMPK-HIF-1 α pathway in MCF-7 breast cancer cells**Yun-Kyoung Lee¹, Song Yi Park², Young Min Kim² and Ock Jin Park¹**

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AMP-activated protein kinase (AMPK) is a major signaling molecule for the regulation of energy metabolism. Recent studies indicate that AMPK activation regulates several cellular processes including cell proliferation, apoptosis and inflammatory responses. In this study, we have examined the effects of quercetin on hypoxia condition-stimulated cancer cell growth and regulatory mechanism of AMPK on HIF-1 α , the transcriptional regulator of response to oxygen depletion, in MCF-7 breast cancer cells. Our results showed that the induction of HIF-1 α and AMPK was observed in cancer cells under CoCl₂-induced hypoxic state. Quercetin reduced the expression of HIF-1 α , which was elevated in hypoxic state with CoCl₂. However, this inhibitory effect of quercetin on HIF-1 α did not seem to be dependent on AMPK activity. Since the inactivation of AMPK by applying AMPK inhibitor, Compound C did not affect the ability of quercetin to inhibit HIF-1 α . These observations suggest that quercetin exerts its anti-cancer effects through regulation HIF-1 α and AMPK against hypoxic state tumors and may contribute to controlling tumor angiogenesis and metastasis. [This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MEST) (NO. R01-2008-000-20131-0)]

Effects of Celecoxib and Compound C on AMPK/Akt signaling in MCF-7 breast cancer cells

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Most breast cancers have characteristics of over-activated Akt, a major stimulator of cell growth and tumor progression. Thus, suppressing Akt activity can provide an effective way for control tumor. In this study, we have focused on the regulatory effects of AMPK and cyclooxygenase-2 (COX-2) on cell survival signal in MCF-7 breast cancer cells using their specific inhibitors, Compound C and Celecoxib for AMPK and COX-2, respectively. Our results showed that the inhibition of AMPK by Compound C increased both Akt and COX-2 levels. Blocking COX-2 expression by Celecoxib increased AMPK, and surprisingly it increased Akt also in MCF-7 cells. To determine the relationship between Akt and COX-2 signals, we used the cox-2 negative and positive cells. In *cox-2* positive cells, Celecoxib decreased COX-2 level as well as Akt activity, but activated AMPK. In addition, treatment of Celecoxib and LY294002, Akt inhibitor reduced cell growth of MCF-7 cells. Therefore, these results suggest that there might exist mutual regulatory interactions between COX-2 and Akt signals in only cancerous applicable cells. Moreover, AMPK can act as a negative regulator of Akt and COX-2 signals and targeting AMPK to control tumor growth can be an effective way due to its inhibitory effects of Akt against breast cancers. [This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MEST) (NO. R01-2008-000-20131-0)]

CRX-526 - a new TLR4 antagonist - reduces LPS-induced impairment of the intestinal microcirculation in experimental endotoxemia

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Objectives: Toll like receptor 4 (TLR4) represents an important mediator of endotoxin-related signal transduction. Aim of our study was to evaluate whether TLR4 inhibition after onset of experimental endotoxemia is able to improve the intestinal microcirculation, which is crucial in the pathogenesis of septic multiple organ failure.

Materials and Methods: We studied four groups of animals (Lewis rats, n=10 per group): healthy controls (CON group), endotoxemic animals (15 mg/kg lipopolysaccharide, LPS group), endotoxemic animals treated with TLR4 antagonist (1 mg/kg CRX-526, LPS+CRX group), and CRX-526 treated controls (CRX group). Intravital microscopy of the intestinal microcirculation was performed following 2 hours of observation in all animals. Blood samples were taken for cytokine measurements at the end of the experiments.

Results: Following two hours of endotoxemia we observed a significant increase of leukocyte adhesion in the intestinal submucosal venules (e.g., V1 venules: CON 20.4 ± 6.5 n/mm², LPS 237.5 ± 36.2 n/mm², p<.05). Capillary perfusion of the muscular and mucosal layers of the intestinal wall was significantly reduced (e.g., longitudinal muscular layer: CON 112.5 ± 5.9 cm/cm², LPS 71.3 ± 11.0 cm/cm²). TLR4 inhibition reduced leukocyte activation (V1 venules: 104.3 ± 7.8 n/mm²) and improved capillary perfusion (longitudinal muscular layer: 111.0 ± 12.3 cm/cm²) significantly. Cytokine release was not affected.

Conclusions: Administration of the TLR4 antagonist CRX-526 improved intestinal microcirculation in a post-treatment model of experimental endotoxemia. The TLR4 pathway may be a target in clinical Gram-negative sepsis.

EFFECTIVE ANTI-FIBROSIS RESPONSES ON MICE MODEL OF LUNG FIBROSIS BY INTRATRACHEALLY ADMINISTERED ANTIFLAMMIN-1

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Antiflaminin-1 (AF-1, MQMKKVLDS) is a nonapeptide which has a powerful anti-inflammatory effect . It acts as its original full length protein-Clara Cell Secretory Protein (CCSP) and may display potent clinical application value. Our previous studies indicated that Clara Cells on the terminal bronchioles significantly inhibited pulmonary fibrosis induced by bleomycin. In present study, we observed firstly the protective effect of AF-1 against pulmonary fibrosis. Mice were subjected to intratracheal administration of bleomycin (5mg/kg) to establish the fibrotic model and received AF-1(15mg/kg) by intratracheal administration 3 days later. The mRNA expression level of procollagen III and procollagen I in lung tissue were quantified by RT-PCR at 14th day and 28th day respectively. The bleomycin-induced increase of procollagen III and procollagen I mRNA expression were significantly down-regulated by AF-1. The collagen deposition detected by Masson staining and the hydroxyproline content was decreased by AF-1. All together, our results showed that AF-1 could reduce the degree of lung damage and fibrosis. In conclusion, AF-1 has a protective effect on bleomycin-induced lung fibrosis and it might prove useful as an add-on therapy for the treatment of fibrotic disorders of lung such as idiopathic pulmonary fibrosis, a disease that still represents a major challenge to medical treatment.

Lipopolysaccharide promote the glutamate release from human bronchial epithelial cell line (HBEc)

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The glutamate (Glu) is the major excitatory neurotransmitter in the mammalian central nervous system. And the basal levels of extracellular glutamate are maintained primarily by the exchange of extracellular cystine for intracellular Glu via cystine/glutamate transporter (Xc^-) exchange in the nucleus accumbens(NA) in rats. However, the overactivation of NMDA receptor-induced by the accumulation of extracellular Glu can cause excitotoxicity. The expression of xCT mRNA, the light chain of Xc^- , has been confirmed in the bronchiolar epithelium of the lung in lipopolysaccharide (LPS)- induced endotoxemia mice. Our previous study has firstly found that NMDA receptor antagonist MK801 can attenuated LPS-induced acute lung injury (ALI) in mice. It suggests that endogenous Glu take part in LPS-induced ALI mediated by NMDA receptor. However, whether the release of endogenous Glu-induced by LPS through Xc^- is not clear. In present study, we observed firstly that the effect of LPS on Glu release from human bronchial epithelial cell line (HBEc). The results showed that the expression of Xc^- mRNA was positive in HBEc. And LPS promotes HBEc to release Glu which can be inhibited by Xc^- inhibitor homocysteic acid (HCA). In conclusions, LPS can promote the glutamate release from HBEc by Xc^- . This cell may be the sources of endogenous Glu in the pathogenesis of ALI.

Anthocyanins inhibited NF-κB Expressions of HeLa Human Uterine Cervical Cancer Cells at least in part through the inhibition of IκBα phosphorylation.

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Vitis coignetiae Pulliat Named Meoru in Korea has been used widely as a remedy for inflammatory diseases and various cancers. We previously suggested that anthocyanins from *Vitis coignetiae* Pulliat (AIMs) should have apoptotic effects through suppression of Bcl-2 and anti-invasive activity through suppression of MMP-2 and MMP-9. We investigated their effects on NF-κB-regulated gene products and cellular responses in HeLa human uterine cervical cancer cells. AIMs inhibited the proliferation of Hela cells in a dose dependent manner. AIMs inhibited the motility of HeLa cells in a wound healing test and gelatin migration test. AIMs also inhibited the invasion of HeLa cells in a similar dose-dependent manner as determined through a Matrigel-coated chamber assay. They also inhibited expression of MMP-2 and MMP-9 which are key molecules for cancer invasion. AIMs inhibited the NF-κB activity and the IκBα phosphorylation induced by TNF-α. AIMs also suppressed the NF-κB-regulated proteins which are related to metastasis. Taken together, this study indicates that AIMs have anti-cancer effects on HeLa cells through the inhibition of NF-κB activation and downstream proteins. This study suggested that AIMs might have anti-cancer effects on human uterine cervical cancer by inhibiting the NF-κB activity through the inhibition of IκBα phosphorylation. [This work was supported by a grant of the Korea Healthcare technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (A080164).]

Glycerophosphoinositols inhibit pro-inflammatory and pro-thrombotic functions of monocytes**Mariggò S.^{1,2}, Zizza P.^{1,3}, Di Santo A.², Dell'Elba G.², Amore C.², Corda D.³, Evangelista V.²**

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Inflammatory cytokines and pro-coagulants in monocytes/macrophages have a key role in physiological and pathological inflammatory responses. However, the mediators regulating the expression of inflammatory genes committed to restore the normal homeostasis after acute inflammation are poorly defined [1]. Recent investigations have revealed the active involvement of the phosphoinositide derivatives produced by phospholipase A₂ activity: the glycerophosphoinositols (GPIs). Immune cells have a potent and regulated phospholipase A₂ that provides fine modulation of intracellular GPI levels consequent to cell development, differentiation and hormone stimulation (i.e. exposure to lipopolysaccharides [LPS], cytokines, and other pro-inflammatory agents) [2,3]. In this study, we delineate a role of the GPIs as endogenous metabolites that are part of a negative feed-back loop that limits pro-inflammatory and pro-thrombotic responses in human monocytes stimulated with LPS. The pro-coagulant activity of LPS-stimulated monocytes is mainly ascribed to tissue factor expression; in addition, LPS induces increase in the mRNA levels of tissue factor, cyclooxygenase-2, interleukin-1beta, and tumour necrosis factor-alpha. Pre-treatment of monocytes with GPIs before LPS addition resulted in dose-dependent inhibition of tissue factor activity as well as decrease of mRNA levels of all the analysed inflammatory genes. Notably, treatment with the GPIs was consistently associated with decreased LPS-induced nuclear translocation of transcription factors, such as NF-kB. The time course of the effects of different GPIs on NF-kB nuclear levels were consistent with the different timing for the modulation of the mRNA levels of the inflammatory markers. Our study provides new insight into the biology of the GPIs, suggesting that these compounds have roles as endogenous anti-inflammatory mediators for inflammation resolution.

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Antinociceptive and anti-inflammatory activities of the essential oil of *Lippia gracilis*

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Essential oil (EO) of leaves from the tropical plant *L. gracilis* (Verbenaceae) has antimicrobial activities and is traditionally used to externally treat cutaneous diseases, burns, wounds, and ulcers. In this study we evaluated the antinociceptive and anti-inflammatory activities in rats and mice and linked these activities with the chemical composition of the EO. The chemical analysis of the EO was performed using GC/MS and GC/FID. The antinociceptive activity, induced by injection of 1% acetic acid, was investigated using concentrations of 50-200 mg EO (dissolved in NaCl + DMSO)/kg in mice, n=6) and with acetylsalicylic acid as a reference. Anti-inflammatory activity of the EO was evaluated using the paw edema method in rats (n=6) and the peritonitis method in mice (n=6). The paw edema assay was based on diminution by the EO (at 50-200 mg/kg) of local inflammation induced by carrageenan, using dexamethasone (2 mg/kg) as a reference. The paw volume of the rats was measured plethysmographically before and after administering carrageenan for up to 4h. In the peritonitis method, drugs or vehicle were administered orally. Carrageenan was injected intraperitoneally 1h later, and after a further 4 h period the animals were sacrificed and total amounts of leukocytes were estimated using a Coulter counter. In all experiments, 0.9% (w/v) NaCl + DMSO was used as the negative control. The results of the composition analysis of *L. gracilis* EO indicated that the terpenoids thymol (35%), *p*-cymene (14%), methyl thymol (12%) and carvacrol (8%) were the major compounds. At least thymol and carvacrol are known for their antioxidant and anti-inflammatory effects *in vitro*. In the antinociceptive test, the oral treatment with the EO elicited significant inhibitory activity ($P < 0.05$) effect at all concentrations tested. In the anti-inflammatory assay, carrageenan-induced edema formation was reduced by EO at both 100 and 200 mg/kg ($P < 0.001$). Likewise, EO diminished carrageenan-induced leukocyte migration at all concentrations tested ($P < 0.01$). Our results indicate that essential oil of *L. gracilis* leaves shows both antinociceptive and anti-inflammatory activities.

Curcumin and resveratrol counteract the catabolic effects of lipopolysaccharides (LPS) in explant models of canine and porcine cartilage inflammation

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In this study we established an *in vitro* model of joint inflammation mimicking the events that occur in osteoarthritis (OA) and osteochondritis dissecans (OCD) to investigate whether curcumin and resveratrol can counteract lipopolysaccharide (LPS) induced glycosaminoglycan (GAG) release. Cartilage was obtained from canine stifle and porcine hock joints. Explants were incubated in serum-free culture medium and challenged with LPS ($0.25\mu\text{g}/\text{ml}^{-1}$) to mimic joint inflammation. LPS-stimulated samples and controls were co-treated with either curcumin or resveratrol, both at $2.5\mu\text{M}$ for 5 days. Tissue culture media and the papain digested cartilage explants were analysed for GAG content in order to assess the anti-catabolic potential of curcumin and resveratrol using the dimethylmethylen blue (DMMB) assay. There was a significant increase in GAG release from LPS-stimulated canine and porcine samples when compared to controls ($P<0.001$). Co-treatment with curcumin and resveratrol antagonized the degradative effects of LPS and inhibited GAG release from the explants. There was a marked decrease in GAG release from LPS-stimulated canine and porcine samples with $2.5\mu\text{M}$ resveratrol and this difference was found to be statistically significant in the canine explants ($P<0.05$). Differences were also noted between LPS stimulated samples with and without $2.5\mu\text{M}$ curcumin but these did not reach statistical significance. Curcumin and resveratrol antagonized the catabolic and degradative effects of LPS in explant cultures. However, resveratrol had a more significant anti-catabolic effect on canine explants. These plant-derived compounds may have potential as novel anti-inflammatory nutraceuticals.

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A pilot study with a modified filter-type device (Cellsorba EX) for leukocytapheresis using ACD-A as anticoagulant in patients with mild to moderately active ulcerative colitis. LL-37 is generated during apheresis session.

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Background: Leukocytapheresis (LCAP) is a non-pharmacological treatment modality for inflammatory conditions. CellsorbaTM is a medical device for LCAP treatment of ulcerative colitis (UC). Cellsorba EX Global type has been developed from Cellsorba E to minimize the risks of bradykinin generation and for use with ACD-A as anticoagulant. **Aims & Methods:** To evaluate safety and efficacy of the modified CellsorbaTM using ACD-A as anticoagulant in a pilot trial comprising patients with active UC, despite receiving 5-ASA. A total of 10 LCAP treatments were administered. Safety assessment focused on clinical signs and symptoms before, during and after each LCAP session. Hematological variables were assessed before and after all sessions, and levels of bradykinin and IL-6 were measured before and during LCAP. Efficacy was determined using the Mayo clinical / endoscopic scoring index at start, after 8 and 16 weeks as well histological assessment of biopsies taken at 0, 8 and 16 weeks. Additional aim was to evaluate the impact of apheresis system lines and filter on selected regulatory molecules. **Results:** All six subjects completed the trial without any serious adverse events. WBC, platelet counts and levels of bradykinin and IL-6 were not significantly affected. The median Mayo score decreased from 8.0 to 3.5 at week 8 (and to 2 at week 16 for the responders). Two patients were then in remission, and two additional patients were responders. Median histological scores decreased from 3.5 at entry to 2.0 at the end of the study in these four patients. LL-37 increased significantly within the apheresis system lines. **Conclusions:** LCAP using the modified CellsorbaTM EX (Global type) device with ACD-A as anticoagulant for the treatment of active UC was found to be safe and well tolerated. The clinical response experienced in this study was promising as 4/6 patients were responders, out of which 2 were in remission at week 16. Immunoregulatory molecules can be generated within the apheresis apparatus plastic lines.

Anti-inflammatory effects of active principle of Korean cabbage kimchi via suppressing NF-kappa B and increasing NO production in aorta of apoE KO mice**Jeong Sook Noh^a, Hyun Ju Kim^a, Myung Ja Kwon^a, Hongsuk Suh^b, Yeong Ok Song^{a*},**

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In atherosclerotic process, inflammatory response and impaired nitric oxide bioavailability in aorta are associated with pathogenesis. 3-(4'-hydroxyl-3',5'-dimethoxyphenyl)propionic acid (HDMPPA) demonstrated antioxidant activity in kimchi (Korean fermented vegetable) may inhibit expression of pro-inflammatory mediator and adhesion molecules in aorta. Apo E KO mice were fed an atherogenic diet with an intraperitoneal injection (10 mg/kg bw/day HDMPPA) for 8 weeks. HDMPPA treatment increased NO production accompanied by augmenting the expressions of eNOS protein in aortas ($p<0.05$). The concentration of BH4, cofactor of eNOS was elevated by HDMPPA-treatment (control vs HDMPPA; 32.48 vs 40.88 pmole/mg protein, $p<0.01$). The inflammatory protein levels of COX-2 and iNOS was reduced by 45% and 44%, respectively. Significant decreased in the protein level of ICAM-1 and VCAM-1 in plasma of HDMPPA group (40% and 17%) was observed. Furthermore, HDMPPA increased protein level of IkBa ($p<0.01$) while that of NF-kB p65 in nucleus decreased ($p<0.001$). HDMPPA inhibited inflammatory response by down-regulating the pro-inflammatory responses while preserving NO bioavailability in apoE KO mice.

Protective Effect of *Coptis Chinensis* against UVB-induced Pro-inflammatory Responses in Human Keratinocytes and NC/Nga Mice

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Atopic dermatitis (AD) is a complex skin disorder accompanied by severe itching and inflammation with frequently repeated episodes. Genetic as well as environmental factors are involved in the development and pathogenesis of AD. Recently, ultraviolet B (UVB) radiation is regarded as a critical factor aggravating AD-related symptoms. The purpose of this study is to search for naturally occurring medicinal herbs which can protect against UVB-induced pro-inflammatory responses and improve AD-like skin lesions in the *in vitro* cell culture as well as *in vivo* animal models of AD. Human keratinocytes, HaCaT cells were irradiated with UVB in the presence or absence of *Coptis Chinensis*. Rhizome of *Coptis Chinensis* referred as Hwang-ryun has been widely used in traditional Korean medicine to treat a vast array of inflammation-related health problems and medical conditions caused by excessive heat. In HaCaT cells, UVB irradiation led to an increased mRNA and protein expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) and subsequent generation of nitric oxide (NO) and prostaglandin E₂ (PGE₂), which were significantly inhibited by *Coptis Chinensis* pretreatment. In another experiments, topical application of *Coptis Chinensis* to NC/Nga mice suppressed the development of UVB-mediated skin lesions and AD-like dermatitis. Moreover, expression of inflammatory enzymes and production of pro-inflammatory mediators were effectively attenuated by *Coptis Chinensis* treatment. These findings suggest that *Coptis Chinensis* has preventive and therapeutic potential against UVB-induced skin damages in AD.

Luteolin inhibits iNOS expression *via* blockade of NF-kappaB and JNK/AP-1 pathways in TNF-alpha induced HepG2 cells.

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Luteolin (3',4',5,7-tetrahydroxyflavone), an abundant flavonoid in celery, green pepper, parsley, perilla leaf, and dandelion, showed anti-inflammatory activities in previous researches. In this study, the inhibitory mechanism of luteolin on tumor necrosis factor (TNF)-alpha-induced inflammation was investigated in HepG2 cell line. Luteolin significantly suppressed TNF-alpha-stimulated inducible nitric oxide synthase (iNOS) expression in a dose dependent manner without cytotoxicity. Activator protein (AP)-1 and nuclear factor (NF)-kappa B were also measured due to an important role in inflammation and were known to regulate iNOS expression. Luteolin treatment dose-dependently inhibited the phosphorylation and nuclear translocation of p65 and c-jun, one subunit of NF-kappaB and AP-1. In addition, luteolin suppressed TNF-alpha-induced phosphorylation of c-Jun N-terminal kinase (JNK) which is attributable for iNOS expression, and this stimulation was attenuated by the JNK selective inhibitor, SP600125, in HepG2 cells. These results suggest that luteolin attenuates TNF-alpha-stimulated iNOS expression through blockade of NF-kappaB and JNK/AP-1 pathways in HepG2 cells.

Luteolin and chicoric acid synergistically inhibited inflammatory responses *via* inactivation of PI3K-Akt pathway and impairment of NF-kappaB translocation in LPS stimulated murine macrophage cell line

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The synergistic effect of luteolin and chicoric acid, two major constituents of dandelion (*Taraxacum officinale* Weber), was investigated in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells. The optimum mixing ratio of luteolin and chicoric acid was 1:2. The anti-inflammatory activity was compared using mRNA and protein expression levels, which were attributable to the suppression of inflammatory mediators, such as inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), tumor necrosis factor-alpha (TNF-alpha), interleukin-1beta (IL-1beta). Cytoprotective enzyme, heme oxygenase (HO)-1, and its corresponding transcription factor, nuclear factor (NF)-E2-related factor (Nrf)-2, were increased by luteolin and chicoric acid co-treatment. In addition, synergistic effect was further examined whether inflammatory response was related to NF-kappaB activation in the mitogen-activated protein kinase (MAPK) or phosphoinositide 3-kinase (PI3K)-Akt pathway, inflammatory signaling pathways in RAW 264.7 cells. Luteolin and chicoric acid co-treatment inhibited phosphorylation of IkappaB alpha, p65 and Akt, while gave no effect on extracellular signal-regulated kinase (ERK), c-Jun NH₂-terminal kinase (JNK), and p38. These results suggest that two phenolic compounds synergistically attenuate LPS-induced inflammation *via* inactivation of PI3K-Akt pathway and impairment of NF-kappaB translocation in RAW 264.7 cells.

Daidzein suppresses high glucose-induced ICAM-1 and VCAM-1 expression in human umbilical vein endothelial cells**Mi Hwa Park¹, Jae Won Ju¹, Ji Sook Han¹****¹Department of Food Science and Nutrition, Pusan National University, 30 Jangjeon-dong, Busan 609-735, Korea****mf0106@hanmail.net, philo7ju@hanmail.net, hanjs@pusan.ac.kr**

Adhesion molecules are produced by endothelial cells following stimulation with various inflammatory cytokines. The current studies examined the effect of daidzein, an isoflavone and phytoestrogen component of soy, on the expression of adhesion molecules in human umbilical vein endothelial cells (HUVEC) stimulated with high glucose. Adhesion of monocyte to daidzein treated HUVEC was evaluated by co-culture experiments using 2,7-bis(2-carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester (BCECF-AM) labeling of HL-60 cells. The expression levels of vascular cell adhesion molecule-1 (VCAM-1), intracellular cell adhesion molecule-1 (ICAM-1) and nuclear NF- κ B were evaluated by Western blot. Daidzein significantly inhibited the high glucose-induced increase in HL-60 monocyte adhesion to HUVEC as well as downregulated protein expression levels of CAMs and E-selectin on HUVEC. High glucose-induced ROS production was inhibited by treatment of daidzein. Daidzein also reduced nuclear NF- κ B protein expression in HUVEC. These results suggest that daidzein reduced high glucose-induced CAMs activation by inhibiting monocyte adhesion, ROS generation, and NF- κ B in HUVEC.

Modulation of cancer cell proliferation by cell survival signal Akt and tumor suppressive energy sensor AMP-activated kinase in colon cancer cells treated with resveratrol

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It has been well known that resveratrol inhibits the proliferation of various cancer cells. AMP-activated kinase, a sensor of cellular energy status, has emerged as a potent target for cancer prevention and/or treatment. We have found that the activation of AMPK by resveratrol was crucial for the inhibition of growth of HT-29 colon cancer cells. Resveratrol strongly inhibited p-Akt. We have investigated the possibility of AMPK activation with resveratrol was essential to the inhibition of p-Akt. The inhibitory effect of resveratrol on Akt was not observed when AMPK activities were blocked by the treatment with AMPK siRNA at a relatively low concentration of resveratrol. However, at the higher dose of resveratrol, without the activation of AMPK resveratrol inhibited phosphorylation of Akt to a certain degree. Therefore, we have concluded that resveratrol could modulate Akt AMPK-dependently or AMPK-independently. This suggests that resveratrol has molecular targets within the Akt signaling pathways and that the inhibition of Akt along with the activation of AMPK may contribute to the unraveling anticancer mechanism exerted by resveratrol. [This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MEST) (NO. R01-2008-000-20131-0)]

Identification of anti-inflammatory molecules for cystic fibrosis by virtual screening of a furocoumarin database against NF-kB

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Persistent recruitment of neutrophils in the bronchi of CF patients contributes to airway tissue damage, suggesting the importance of intervening on the expression of the neutrophil chemokine IL-8. Since transcription factor NF-kB plays a critical role in IL-8 expression, a virtual screening of database with 1740 furocoumarin (consisting of a furan ring fused with coumarin) compounds has been utilized to select molecules for their ability to inhibit the binding of NF-kB with the consensus sequence identified in the promoter of IL-8. Compounds available in a structure searchable database were retrieved using a substructure searching (2D) method. VS approach was followed against both NF-kB p50 monomer and homodimer. The selection of interesting compounds were based on the docking XP GlideScore rank for each target. Among the compounds exhibiting the highest docking ranks in the dimeric aggregation of the protein, the compound **7f** (a furo(3,2-c)chromen-4one derivative) inhibited: (a) the formation of a stable NF-kB p50/DNA complex in EMSA studies; (b) the PAO1-dependent transcription of IL-8 in IB3-1 cystic fibrosis cells. In conclusion, compounds identified by a NF-kB virtual screening of a furocoumarin database resulted to be useful to identify novel pharmaceutical molecules able to inhibit the expression of the pro-inflammatory chemokine IL-8, which could be beneficial to control lung inflammation in the lung of CF patients.

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Influence of novel selective cyclooxygenase-2 (COX-2) inhibitors on low density lipoprotein oxidation

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Clinical investigations showed a link between the extended use of rofecoxib, a selective COX-2 inhibitor (coxib), and increased cardiovascular risk. This has led to general concern over the coxibs' cardiovascular safety. Subsequently, rofecoxib has been shown to increase oxidation of low density lipoproteins (LDL) and membrane lipids, whereas other coxibs did not, providing a biochemical rationale for differences in cardiovascular risk among coxibs. We investigated the independent effects of eight COX-2 inhibitors (two cyclopentene-/four indole-based sulfonyl derivatives) and two non-sulfonyl indomethacin derivatives, which all were synthesized as lead compounds for development of novel COX-2 imaging radiotracers, on both human LDL lipid (kinetics of conjugated diene formation was followed photometrically) and protein oxidation (formation of γ -glutamyl semialdehyde/ α -amino adipyl semialdehyde in LDL apolipoprotein B-100 was followed by gas chromatography-mass spectrometry) compared to celecoxib and quercetin as controls. The presence of indole derivatives (50 nM to 10 μ M) concentration-dependently increased the lag-time for Cu²⁺- or hemin(Fe³⁺)-mediated formation of conjugated diene in LDL (>10%, p<0.01) and, furthermore, significantly decreased transition metal-catalyzed protein oxidation (>18%, p<0.01). Addition of indole-based coxibs to human plasma increased the oxygen radical antioxidant capacity (ORAC) by 6-14% (p<0.01). By contrast, other coxibs (cyclopentene and indomethacin derivatives) did not show effects on lipid and protein oxidation and, even at suprapharmacologic concentrations, had no significant effect on plasma ORAC values. A radiotracer-based analysis showed that indole derivatives interact differently with LDL subfractions and other lipoprotein particles, than the cyclopentene and indomethacin derivatives, respectively, suggesting a physico-chemical basis for observed differences in anti-oxidant activity. These findings may provide further insights for evaluation of potential cardiovascular risk of selective COX-2 inhibitors.

Antiinflamatory activity of *Lonchocarpus araripensis* lectin in mouse**Alana de F. Pires, Ana Maria S. Assreuy, Márcia M. Marinho, Benildo S. Cavada****Laboratório de Fisiologia e Farmacologia da Inflamação- LAFFIN, Universidade Estadual do Ceará, 60740-000 Fortaleza-CE, Brazil. E-mail: anassreuy@gmail.com**

Anti-inflammatory activities have been demonstrated for leguminous lectins. Here, it was investigated the anti-inflammatory effect of a leguminous lectin from *Lonchocarpus araripensis* (LaL) in the models of mice paw edema and peritonitis. Systemic effects and participation of nitric oxide and arachidonic acid metabolites were also investigated. Protocols using Swiss mice (25- 30g) were approved by our Institutional Ethical Committee (Nº. 0559924-4). Paw edema was induced s.c. by carrageenan (Cg; 300 µg), sodium nitroprusside (10 µmol/Kg), or phospholipase A₂ (1µg), measured by hydroplethysmometry before (zero time) and thereafter (1-5h) s.c. injection of stimuli and expressed as area under the time-course curve (AUC). Vascular permeability (A600nm-µg of Evans blue/g of tissue) was evaluated after mice had received Evans blue (25mg/kg; i.v.) 1h before sacrifice (2^h of Cg-induced edema). Peritonitis was induced by Cg (500 µg; i.p.) for total and differential leukocyte counts (cells/µL) 4h after induction. LaL was injected i.v. (0.1-10 mg/kg), 30 min before flogistic agents. Toxicity was evaluated after a nine-day treatment with LaL (10mg/Kg) analyzing renal and hepatic function, body mass and leukogram. Results were expressed as Mean ± S.E.M (ANOVA and Bonferroni's test; p<0.05). LaL (0.1, 1, 10mg/kg), dose- dependently, inhibited the time-course of Cg-paw edema (15.69 ± 1.58) by 32% (10.68 ± 1.37), 52% (7.45 ± 0.36) and 69% (4.84 ± 0.41), respectively. LaL at 10mg/Kg also inhibited the increase in vascular permeability elicited by Cg (38.7 ± 1.5) about 26% (26.61 ± 1.3). The edema induced both by sodium nitroprusside (10.76 ± 1.08) and by PLA₂ (12 ± 0.89) was respectively inhibited by LaL (10 mg/Kg) about 83% (1.84 ± 0.58) and 48% (6.21 ± 0.86). LaL, dose dependently, reduced about 68% (1168.75 ± 117.9) the leukocyte migration evoked by carragenin (3704.17 ± 184.2), with maximal effect at 10mg/Kg, mainly due to inhibition (86%) upon neutrophils. *L. araripensis* lectin inhibited vascular and cellular events of inflammation with the apparent involvement of NO and prostaglandins showing no signs of toxicity. **Acknowledgments:** CNPq, CAPES, FUNCAP and Gabriela FO Marques-Domingos for technical assistance.

Curcumin synergizes with resveratrol to stimulate the MAPK signaling pathway in human articular chondrocytes *in vitro*: potential for treating osteoarthritis**Shakibaei M¹, A. Mobasher², C. Csaki¹**

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The mitogen-activated protein kinase (MAPK) pathway is stimulated in differentiated chondrocytes and is an important signaling cascade for chondrocyte differentiation and survival. Pro-inflammatory cytokines such as interleukin 1 β (IL-1 β) play important roles in the pathogenesis of osteoarthritis (OA). In this study we investigated whether curcumin and resveratrol together can act synergistically to inhibit the catabolic effects of IL-1 β , specifically the inhibition of MAPK and subsequent apoptosis in human articular chondrocytes. Chondrocytes were either left untreated or treated with 10 ng/ml IL-1 β or 1 μ M U0126 alone for the indicated time periods or pre-treated with 10 μ M curcumin, 10 μ M resveratrol or 10 μ M resveratrol and 10 μ M curcumin for 4 hours followed by co-treatment with 10 ng/ml IL-1 β or 1 μ M U0126 and 10 μ M resveratrol, 10 μ M curcumin or 10 μ M resveratrol and 10 μ M curcumin for 1, 12, 24 and 48 h. Cultures were evaluated by immunoblotting and electron microscopy. Incubation of chondrocytes with IL-1 β resulted in activation of caspase-3, down regulation of β 1-integrins and the extracellular signal-regulated kinase (Erk) and induction of apoptosis. Interestingly, incubation with U0126, a specific inhibitor of Erk1/2 blocked these changes. Furthermore, co-treatment of the U0126-stimulated cells with curcumin and resveratrol blocked activation of the caspase-3 and apoptosis suggesting, at least in part, that this process is a MAPK-dependent pathway. Thus, these results suggest that the MEK/Erk signal transduction pathway is involved in the maintenance of chondrocyte differentiation and survival and this pathway may be activated by combining these natural compounds.

Replacement enzyme therapy and systemic oxidative stress in patients with exocrine pancreatic dysfunction

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Cystic fibrosis (CF) is inherited disorder caused by mutations in a single gene encoding cystic fibrosis conductance regulator (CFTR) protein, resulting in fatal lung disease and exocrine pancreatic insufficiency. Dysfunctional CFTR modifies calcium homeostasis, impairs transport of glutathione, and induces mitochondrial oxidative stress. The disturbance of the redox balance evokes endogenous activation of NF- κ B and exaggerates proinflammatory signaling in CF cells. In this environment, malabsorption of fatty soluble antioxidants secondary to pancreatic insufficiency can affect inflammatory response in CF patients. The aim of this study was to compare inflammatory markers in CF patients with different enzyme treatment regimens. Nineteen CF patients with pancreatic insufficiency were examined at a time of symptomatic exacerbation of their lung disease. Group A (n=11) were regularly received currently recommended lipase/protease intake. Group B (n=8) were treated with pancreatic enzyme supplements during hospitalization period only. The reasons for irregular pancreatic enzyme treatment were social, including parents' preference as well as infrequent follow-up at the CF centres. During exacerbation group B had lower levels of T-cell proliferation than group A ($p = 0.026$). There were no differences in initial plasma malondialdehyde (MDA) levels between the groups. Adequate enzyme therapy improved lymphocyte functions but increased in systemic oxidative stress in the patients from group B. Indeed, their plasma MDA levels were markedly elevated after the treatment ($p=0.021$). For group B, a significant positive linear association between values of plasma MDA and T lymphocyte proliferation has been observed. For group A, neither the same correlation, nor changes in MDA levels and lymphocyte proliferation have been found. In conclusion, rapid enzyme replacement in malnourished CF patients results in drastic influx of lipid targets for peroxidation that introduces a heavy oxidative challenge. Under the circumstances exhausted antioxidant defenses are unable to handle the oxidative insult.

Flavonoid Dihydroquercetin from *Larix sibirica* (*L.*) suppresses inflammation processes, caused by H1N1 pandemic Influenza Virus Infection in Mice

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Despite success with chemotherapy and vaccine prophylaxis, influenza remains a poorly controlled infection causing epidemics and pandemics. The aim of this study was to evaluate anti-influenza properties of dihydroquercetine (DHQ), the flavonoid from larch (*Larix sibirica L.*), on the model of infection in mice, caused by pandemic H1N1 influenza virus. Animals were infected with the influenza virus A/St.Peterburg/5/09 (H1N1)v and treated intraperitoneally with DHQ on a combined (before and after infecting) schedule. On day 3 p.i. level of virus' replication in lung tissue and mean size of foci of inflammation were evaluated by titration in MDCK cells and lung morphology analysis, respectively. No differences in virus' titer was detected between control and DHQ- treated groups of animals. In both cases the virus replicated up to approx. $10^{5.5}$ EID₅₀/(0,2 mg tissue) suggesting that there is no direct anti- viral activity of DHQ. However, DHQ has been shown to possess protective activity against influenza virus infection leading to a decrease in size of foci of inflammation (from approx. 25% of total lung volume in control group to 10% in experimental group). These data suggest that DHQ can be considered as an affordable and prospective tool to be used in complex prophylaxis and/or treatment of severe influenza.

Effects of COX-2 inhibitors on cell proliferation in human hematopoietic cancer cell lines

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The link between chronic inflammation and cancer involves different cytokines and mediators of inflammatory pathways (*i.e.*, reactive oxygen species, cyclooxygenases) and acts at the different steps of tumorigenesis. The cyclooxygenases (COX) are a family of enzymes, which catalyze the rate-limiting step in prostaglandin biosynthesis. COX-2 is the inducible isoform of COX, upregulated during inflammation and overexpressed in various cancers. There is evidence about the role of COX-2 in proliferation of colorectal, breast and prostate cancers, whereas this role remains to be elucidated in hematopoietic malignancies. In our study, we analyzed the role of COX-2 in cell proliferation of a panel of leukemic and lymphoblastic cell lines, including cells expressing COX-2 (U937, Jurkat, HeLa and Raji) and a cell line (K562) expressing low level COX-2. We used two different COX-2 inhibitors: nimesulide and NS-398. We found that both inhibitors were able to inhibit cell proliferation in all COX-2-positive cell lines tested whereas they were completely ineffective in low level COX-2 K562 cells. Focusing our investigations on U937 and K562, respectively as a COX-2 positive and a low level COX-2 cell model, we show that nimesulide and NS-398 led to an accumulation of the cells in the G0/G1 phase of the cell cycle in U937 cells, paralleled by an up-regulation of p27, a cyclin-dependent kinase inhibitor involved in the inhibition of G1/S transition. No upregulation of p27 was observed on K562. The fact that COX-2 inhibitors affect cell proliferation only on COX-2 positive cell lines suggests that this phenomenon may be related to the specific COX-2 inhibition. Possible modulation of autophagy, as a possible explanation to the effects exerted by COX-2 inhibitors on cell proliferation, was also investigated and discussed.

Chemopreventive effect of curcumin on the Wingless signaling pathway in prostate cancer cells**Teiten M-H¹, Gaascht F¹, Henry E¹,Eifes S¹, Cronauer M², Dicato M¹ and Diederich M¹**

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Prostate cancer is the third cause of death from cancer in men. Lifestyle, aspects of the diet and environmental factors as well as genetic factors (e.g. disregulations of the Wingless (Wnt) signaling pathway) promote the malignant transformation of healthy prostatic epithelium. Due to the high prevalence and the slow progressive development of prostate cancer, primary prevention using non-toxic natural or synthetic compounds appeared as an attractive strategy to eradicate prostate cancer. Curcumin (diferuloylmethane), a major component of turmeric plant (*Curcuma Longa*), is mainly characterized for its anti-inflammatory, anti-carcinogenic, anti-proliferative, anti-angiogenic and anti-oxidant properties. Based on the fact that curcumin exhibits an inhibitory effect on the Wnt signaling pathway in colon cancer and that this pathway is highly implicated in prostate cancer progression, we evaluate the potential impact of curcumin on the Wnt pathway in prostate cancer cells. We pointed out that whenever curcumin has the same intracytoplasmic localization in all of the prostate cancer cells tested, the androgen sensitive cells (22rv1, LNCaP) appear more sensitive to curcumin treatment than the androgen independent one (DU145, PC-3). We also reported that curcumin has an impact on the proliferation of the androgen 22rv1 sensitive cells as it induces a cell cycle arrest in G2/M phase confirmed by a decrease of the mRNA and protein level of expression of PCNA (proliferating cell nuclear antigen) and cyclin B1. Curcumin was also shown to decrease the Wnt (β -catenin/Tcf-4) transcriptional activity in 22rv1 cells that could be explained by a decrease of the level of expression of the nuclear Tcf4 transcription factor after curcumin treatment. This leads to a decrease of the mRNA and protein expression level of both c-myc and cyclin D1, two well-known target genes of the Wnt signaling pathway. All of these findings suggested that curcumin could be considered as a potential chemopreventive agent for early but not for late, metastatic stages of prostate cancer.

The Diabetic antigen Glutamic Acid Decarboxylase (GAD 65) in the human peripheral blood**G.P. Tilz^a, J. Dausset^a, M. Wiltgen^b**

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Background: Glutamic Acid Decarboxylase (GAD 65) is a diabetes associated antigen which is generally considered to be strictly intracellular. In order to better understand autoimmunity, this study demonstrates the appearance of GAD 65 in the peripheral human blood and presents implications for diagnosis and therapy of some autoimmune diseases. **Method:** In an experimental set up with fluorescence correlation spectroscopy (FCS), the GAD 65 molecules are detected by their interaction with corresponding monoclonal antibodies, labelled with dyes. These interactions result in changes of the Brownian motion, measured as fluorescence fluctuations. Sera from 153 patients, with Diabetes Mellitus Type 1 and controls, were investigated. To enable an imagination of the molecule as model for further discussions we present structural visualizations of its hydrophobic properties, leading to possible interactions with the cell membrane lipids, and epitope locations. **Result:** The GAD 65 antigen could be measured with the sensitivity of 2.65 microgram/ml in "clean systems", resulting from spiking experiments and human sera. In 8 cases of patient sera the GAD 65 antigen could be identified: 4 children with Diabetes Mellitus Type 1 and 4 adults, which were initially used as controls, who retrospectively showed signs of autoimmunity. **Conclusion:** We conclude that these findings are of significance for the concept of autoimmunity, whereby in an initial step the immune system is primed by the accessibility to GAD 65. Our experimental results may also be important for the therapy of Diabetes Mellitus Type 1 and other autoimmune diseases by the passive administration of GAD 65-antibodies.

Phosphatidylcholine inhibits TNF- α -induced pro-inflammatory signalling and interferes with the compartmentation of TNF-R1 and TNF-R2 to sphingolipid/cholesterol enriched microdomains (lipid rafts).

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Background: Phosphatidylcholine (PC) is a major lipid of the gastrointestinal mucus layer that is reduced in patients suffering from ulcerative colitis [1, 2]. Clinical studies reveal that the therapeutic addition of PC to the colonic mucus using slow release preparations is beneficial [3, 4]. The positive role of PC in this disease is still elusive, however, we have recently shown that PC has an intrinsic anti-inflammatory property [5]. Exogenous PC inhibited membrane-dependent actin assembly and TNF- α -induced nuclear NF- κ B activation. **Methods:** We investigated the hypothesis that exogenous application of PC has anti-inflammatory properties. PC species with different fatty acid side chains were applied to polarized and non-polarized Caco-2 cells treated with TNF- α . We analysed NF- κ B-activation via the transient expression of a NF- κ B-luciferase reporter system. Pro-inflammatory gene transcription was detected with the help of a quantitative (RT)-PCR. We assessed the binding of TNF- α to its receptor by FACS and analysed lipid rafts by isolating detergent resistant membranes (DRMs). Activation of MAP kinases ERK and p38 were analysed by blotting. **Results:** All PC species tested significantly inhibited TNF- α -induced pro-inflammatory signalling. The expression levels of IL-8, ICAM-1, IP-10, MCP-1, TNF- α and MMP-1 were reduced after PC pre-treatment for at least two hours. The effect was not due to a reduced binding of TNF- α to its receptor or a decreased surface expression of TNF- α receptors. PC was also effective when applied to the apical side of polarised Caco-2 cultures if cells were stimulated from the basolateral side. Phosphorylation of ERK and p38 was inhibited and PC changed the compartmentation of the TNF- α -receptors 1 and 2 to DRMs. **Conclusions:** PC induces a prolonged inhibition of TNF- α -induced pro-inflammatory signalling. This inhibition may be caused by a shift of the TNF- α receptors at the surface to lipid rafts. Our results provide a molecular foundation for the clinical studies showing a beneficial effect of PC therapy in ulcerative colitis.

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***Leishmania* infection control and Ehrlich Tumor inhibition by a neutrophil-mediated inflammatory reaction modulated by *Chenopodium ambrosioides* treatment.**

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The leaves of *Chenopodium ambrosioides* L. (Chenopodiaceae) have been indicated for the treatment of tumor diseases. Recently, we showed that the treatment with hydroalcoholic crude extract (HCE) from the leaves of *C. ambrosioides* has a potent anti-tumor and anti-leishmanial effects, which was evident even when the HCE was given after the tumor implantation or after the infection progression. The aim of this study was to investigate the effect of the daily treatment with HCE by different routes on the inflammatory reaction induced by Ehrlich Tumor cells implantation or by *Leishmania* promastigotes inoculation. Tumor bearing or *Leishmania*-infected C3H/HePas mice received HCE (5mg/kg) by intraperitoneal route. The treatment initiated soon after the inoculation of 5×10^5 tumor cells or 4 weeks after the *Leishmania* inoculation and it was maintained by 2 weeks. To determine the solid tumor growth and the infection progression, the footpad was measured with a digital caliper each 2 days or each week in the case of infected mice until the sacrifice of mice, when the paw were cutted, weighted and fixed to histopathological analyses. The results showed that the treatments increased the thickness of the footpad, but this was not related to an increase in the tumor cell number or in the parasite burden, but it was related to an intense inflammatory influx to the footpad, since both, the tumor cells and the parasite burden was significantly decreased in both cases. In fact, the HCE induced an intense infiltration of inflammatory cells, specially neutrophils, suggesting a pro-inflammatory effect of this product, what is fundamental to the controlling of tumor developing and *Leishmania* infection progression. In conclusion, the treatment with *Chenopodium ambrosioides* reduced both the presence of tumor cells and the parasite burden in the site of inoculation, likely because the induction of an intense neutrophilic infiltration. Those results can, at least in part, justify the popular use of this specie to the treatment of tumor diseases and ulcers caused by *Leishmania*.

Keywords: *Chenopodium ambrosioides*, Chenopodiaceae, *Leishmania*, Ehrlich tumor, Inflammation.

A NOVEL ANTI-INFLAMMATORY ROLE FOR ANDROGRAPHOLIDE AND ITS DERIVATIVES IN ASTHMA VIA INHIBITION OF THE NUCLEAR FACTOR-κB PATHWAY**W.S. Fred Wong****Department of Pharmacology and Immunology Program, Yong Loo Lin School of Medicine, National University of Singapore, Singapore**

Persistent activation of nuclear factor (NF)-κB has been associated with the development of asthma. Andrographolide, an active component of a medicinal plant *Andrographis paniculata*, has been shown to inhibit NF-κB activity. We hypothesized that andrographolide may attenuate allergic asthma via inhibition of the NF-κB signaling pathway. BALB/c mice sensitized and challenged with ovalbumin (OVA) developed airway inflammation and airway hyperresponsiveness. Andrographolide dose-dependently inhibited OVA-induced increases in total cell count, eosinophil count, and IL-4, IL-5 and IL-13 levels in BAL fluid, and reduced serum level of OVA-specific IgE. It attenuated OVA-induced lung tissue eosinophilia and airway mucus production, mRNA expression of E-selectin, chitinases, Muc5ac and iNOS in lung tissues, and airway hyperresponsiveness to methacholine. In normal human bronchial epithelial cells, andrographolide blocked TNF- α -induced phosphorylation of inhibitory κB (IkB) kinase-β (IKKβ), and downstream IkBα degradation, p65 subunit of NF-κB phosphorylation, and p65 nuclear translocation and DNA-binding activity. Similarly, andrographolide blocked p65 nuclear translocation and DNA-binding activity in the nuclear extracts from lung tissues of OVA-challenged mice. 14-Deoxy-11,12-dihydroandrographolide is another major component of *A. paniculata*, and has shown similar anti-inflammatory actions in allergic asthma. Our findings implicate a potential therapeutic value of andrographolide and its derivatives for asthma and it may act by inhibiting NF-κB pathway at the level of IKKβ activation. (This work was supported by a BioMedical Research Council grant BMRC06/1/21/19/443 and Academic Research Grant R-184-000-130-112)

The link between multiple sclerosis, T regulatory cells and Japanese rice

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The purpose of the present study was to develop a peptide for treatment of multiple sclerosis (MS). We have tested the effect of a novel antiinflammatory peptide (KGHYAERVG, termed IIIM1) on experimental autoimmune encephalitis (EAE), an animal model of MS. Our findings demonstrate significant reduction in neurological score following oral administration of IIIM1, as compared to the control groups received the vehicle (saline). Structural studies revealed that the entire peptide is required for activity. The peptide caused significant reduction in IL17, interferon gamma and IL12 production by isolated splenocytes and concomitant elevation of antiinflammatory cytokines. IIIM1 elevated T regulatory cells (Tregs, CD4⁺CD25⁺FoxP3⁺) in brain and spleen of EAE mice. Similar proliferative effect was observed in isolated human and mouse Tregs *in vitro*. Stimulation of Tregs by IIIM1 caused production of a new peptide termed RA1 present in Oryza Sativa Japonica group. This Japanese rice peptide ameliorated neurological symptoms in the EAE model. Similar beneficial effect was observed upon oral administration of an extract of Japanese rice. In conclusion, oral treatment with IIIM1 ameliorates EAE symptoms via stimulation of Tregs to proliferate and produce RA1 which reduces EAE symptoms. These findings may explain the relatively low prevalence of MS in Japan and other Japanese rice-eating populations. (This work was supported in part by The Israel Science Foundation grant 747/05).

Novel peptides as potential treatment of systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by a loss of immunologic tolerance, production of auto-antibodies and inflammatory damage in multiple organs. We have tested the effect of anti-inflammatory peptide, a H2A histone fragment, termed IIIM1 on MRL/lpr mice, an animal model of SLE. Oral administration of IIIM1 at early stage of disease caused reduction in proteinuria and serum anti-dsDNA antibodies. Starting the treatment at advanced stage of disease resulted in prolonged animal survival, decreased lymphadenosis and reduced levels of pathogenic or abnormal double negative CD4⁻CD8⁻ cells and B220⁺ cells in lymph nodes and spleen. We discovered that IIIM1 induces the production of an additional peptide, a fragment of alpha-1-antitrypsin, termed UBE. Relatively low dose (1µg/kg) of UBE reduced proteinuria and hematuria in MRL/lpr mice. The beneficial effect of the peptide was corroborated by histological examination. Furthermore, a significant reduction in serum IL17, IL12 and anti dsDNA antibodies was observed in the UBE-treated mice. Isolated CD4 cells incubated with the peptide showed similar cytokine profile. Decreased levels of double negative CD4CD8 and B220⁺ cells were determined in lymph organs of UBE-treated animals. The beneficial effects of both UBE and IIIM1 suggest these peptides as potential drugs for SLE.

Anti-inflammatory effect of fisetin on ovalbumin-induced asthma

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Asthma is a chronic inflammatory disease with the characteristics of airway inflammation, airway hyperresponsiveness, and mucus production. Activation of NF- κ B is highly associated with the pathogenesis of asthma. Fisetin, a natural compound commonly found in fruits and vegetables, has been shown to inhibit the NF- κ B activation in macrophages and epithelial cells. In this study, we investigated whether fisetin can attenuate asthma via inhibition of NF- κ B activity using an ovalbumin (OVA)-induced murine asthma model. Our data showed that fisetin decreased the total inflammatory cell counts, eosinophil count and lymphocyte count in broncholalveolar lavage (BAL) of OVA-challenged mice. Furthermore, fisetin attenuated OVA-induced lung inflammation and goblet cell hyperplasia. The expression of eotaxin mRNA and Th2-associated cytokines (IL-4 and IL-5) in the lungs of OVA-challenged mice was also reduced after fisetin treatment. However, the serum OVA-specific IgE was not altered by fisetin. Notably, expression of Th2-predominant transcription factor GATA-3 and cytokines (IL-4, IL-5) in splenocytes under OVA stimulation were both suppressed by fisetin. Whether fisetin can attenuate airway hyperresponsiveness and NF- κ B activation in OVA-induced asthma mice are currently under investigation.

Anti-inflammatory Activity of Sappan lignum Extract in a Cell Model of Osteoarthritis

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In osteoarthritis (OA), chondrocytes and infiltrating inflammatory cells express proinflammatory mediators such as IL-1 β , TNF- α , nitric oxide (NO) and prostaglandins that contribute to the production of catabolic enzymes (MMPs and ADAMTS). The aim of the present study was to evaluate the anti-inflammatory activity of an extract from *Caesalpinia sappan* (CSE) as well as its main constituent brazilin in a cell model of joint inflammation comprising human chondrocytes and macrophages.

Dose-response experiments indicated that concentrations up to 10 μ g/ml and 20 μ g/ml CSE did not impair viability of THP-1 macrophages and chondrocytes, respectively. CSE and brazilin treatments suppressed the up-regulation of IL-1 β , TNF- α , COX-2 and MMP-3 mRNA induced by IL-1 β in chondrocytes and macrophages. An inhibition of NO production and inducible nitric oxide synthase (iNOS) mRNA expression was observed in IL-1 β stimulated chondrocytes treated with CSE. Transient transfection was carried out in SW1353 chondrosarcoma cells and T/C28a2 chondrocytes using LipofectAMINE PLUS. Cotransfections of the COX-2 promoter luciferase reporter plasmid together with p50 and p65 expression vectors enhanced the COX-2 promoter activity 3.4-fold and 3.5-fold in SW1353 and T/C28a2 cells, respectively. This p50/p65-mediated transactivation of the COX-2 promoter was dose-dependently down-regulated to basal levels by CSE.

These results revealed potent anti-inflammatory activities of CSE and brazilin in joint inflammation. Potential molecular mechanisms might involve the inhibition of NO production via iNOS downregulation as well as the inhibition of p50/p65-mediated COX-2 promoter activation.

Effects of Anthocyanins Isolated from *Vitis coignetiae Pulliat* on NF-κB and NF-κB-regulated Gene Expressions in HT-29 Human Colon Cancer Cells *in vitro* and *in vivo*

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Vitis coignetiae Pulliat has been used as a folk remedy for inflammatory diseases and cancers. We previously suggested that anthocyanins from *Vitis coignetiae Pulliat* (AIMs) should have anti-cancer activities by inhibiting NF-κB activation. Here, we investigated their effects on NF-κB-regulated gene products and cellular responses in HT-29 human colon cancer cells, *in vitro* and *in vivo*. AIMs inhibited the proliferation of HT-29 cells in a dose dependent manner. AIMs inhibited the motility of HT-29 cells in a wound healing test and gelatin migration test. AIMs also inhibited the invasion of HT-29 cells in a dose-dependent manner as determined through a Matrigel-coated chamber assay. AIMs suppressed activation of NF-κB and NF-κB-regulated proteins (MMP-2, MMP-9, COX-2, XIAP, ICAM, and VEGF), which are related to metastasis. AIMs inhibited tumorigenicity of HT-29 cells in xenograft mouse model. AIMs inhibited the activation NF-κB and suppressed the NF-κB -regulated protein levels such as XIAP, COX-2 and ICAM in the HT-29 cells grown in athymic nude mice. Taken together, this study indicates that AIMs should have anti-cancer effects on HT-29 cells through the inhibition of NF-κB activation and downstream proteins. This study provides evidence that AIMs might have anti-cancer effects on human colon cancer. [This study was supported by a grant from the National R&D Program for Cancer Control, Ministry for Health, Welfare & Family Affairs, Republic of Korea. (0820050).]

Session IV: Virus Infections and Innate Immunity

Using immunomodulatory agents to limit a dysregulated host response during severe influenza**Lisa M. Alleva**

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The recent H1N1 influenza pandemic reminded us that the generation of a pandemic vaccine takes some months, leaving antivirals as the only available mainstream treatments. While a report noted oseltamivir to be ineffective in treating children with influenza, and there is growing resistance to oseltamivir in the field, alternative treatments that target the host response rather than the virus are required, particularly in cases of influenza requiring hospitalization and intensive care. In response to literature suggesting a role for statins in treating pandemic influenza, our research program has focussed on using mouse models for influenza to find the best candidate treatments for severe murine influenza, from a range of pharmaceutical and natural sources. Reports indicate that the Chinese herbs Angelica sinensis and Salvia miltiorrhiza decrease serum levels of the pro-inflammatory molecule HMGB1 in a mouse model for lethal endotoxaemia, suggesting a potential utility in the treatment of severe influenza. Glycyrrhizin, from *Glycyrrhiza* spp., would be useful as an influenza treatment, both as an antiviral and as an immunomodulatory treatment. One of the active flavonoids from *Trifolium pratense*, biochanin A, could be used as a “natural” PPAR gamma agonist, as recent evidence gathered in our laboratory and others suggests that activation of PPAR gamma limits the severity of experimental influenza.

The immunogenicity of HIV-1 derived lentiviral vectors is mediated by TLR3 and TLR7

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Lentiviral vectors are promising vaccine vector candidates, which have been extensively tested in pre-clinical models of infectious disease and cancer immunotherapy. They are further used in gene therapy clinical trials, both for the *ex vivo* modification of cells and for direct *in vivo* injection. It is therefore critical to understand the mechanism(s) by which such vectors might stimulate the immune system. We evaluated the effect of lentiviral vectors on myeloid dendritic cells (DC), the main target of lentiviral transduction following subcutaneous immunisation. Activation of DC cultures was independent of the lentiviral pseudotype, but dependent on cell entry and reverse transcription. *In vivo* transduced DC also displayed a mature phenotype, produced TNF-alfa, and stimulated naive CD8 positive T cells. Lentiviral activation of DC was TLR-dependent as it was inhibited in TRIF/MyD88 double knock out (KO) DC. TLR3 KO or TLR7 KO DC were less activated, and reverse transcription was important for activation of TLR7 KO DC. Moreover, lentivirally transduced DC lacking TLR3 or TLR7 had an impaired capacity to induce antigen-specific CD8 positive T cell responses. In conclusion, we demonstrated TLR-dependent DC activation by lentiviral vectors, explaining their immunogenicity. These data allow the rational development of strategies to manipulate the host’s immune response to the transgene.

VIP reduces TLR3 signalling pathways in rheumatoid arthritis and osteoarthritis fibroblast synoviocytes

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Vasoactive intestinal peptide (VIP) is a broadly distributed peptide first isolated in intestine but described later as a mediator produced in nervous endocrine and immune systems. Its anti-inflammatory properties have been proved both “*in vivo*” in several animal models and “*in vitro*” in human tissues and cells. TLRs are a family of receptors that activates the innate immunity in response to bacterial and viral compounds, inducing signals linking innate and adaptive immunity. TLR3 is present on intracellular compartments and it is activated by viral dsRNA and by its synthetic analogue Poly I:C. After TLR3 engagement, it couples to the adaptor TRIF starting a signalling pathway that culminates in activation of different transcription factors, NFκB and AP-1 which induce the production of inflammatory cytokines and IRF3 activation that leads to production of type I IFN. Fibroblast like synoviocytes (FLS) of rheumatoid arthritis (RA) and osteoarthritis (OA) express different TLRs. In the present study we show that RA and OA FLS express TLR3. In RA FLS, the mRNA expression of TLR3 is higher than in OA FLS. Both FLS were stimulated with Poly I:C in the presence or absence of VIP and the IRF3 phosphorylation was measured in the nuclear extracts by TransAM. VIP treatment reduces IRF3 phosphorylation induced by Poly I:C. Moreover, VIP acts as a negative modulator of the IRF3-signalling by overturning the IFNβ expression at mRNA level and its production. These results confirm the role of VIP as a negative regulator of TLR-signalling and corroborate its potential as therapeutic agent.

Relation between Hypertonic saline and Macrophage migration inhibitory factor

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Immunological suppression is a well-recognized consequence of trauma and hemorrhagic shock and contributes to infectious complications, ultimately leading to sepsis and multi-system organ failure. T cell dysfunction after traumatic stress is characterized by a decrease in T cell proliferation. The addition of prostaglandin E₂ (PGE₂), which depressed immune function after hemorrhage and trauma, to T cells decreases T cell proliferation and Hypertonic saline (HTS) restores PGE₂-induced T cell suppression. Recently, it has become apparent that Macrophage migration inhibitory factor (MIF) plays a central role in several immune responses including the modulation of several cytokines and T cell activation. By controlling immune and inflammatory responses, MIF is thought to play an important role in the pathophysiology of septic shock and chronic inflammatory diseases. The role of MIF mediating HTS restoration of T cell dysfunction is unknown. Therefore Jurkat cells were cultured in RPMI media, final concentration of 2.5×10^6 cell/ml. Cell proliferation was suppressed using PGE₂ and treated HTS at 20 and 40 mM above isotonicity. MIF concentrations of the supernatant will be determined by enzyme-linked immunosorbent assay (ELISA) and the cell lysates will be used for Western Blots to determine the expression of MIF. PGE₂ caused a 15.0% inhibition of Jurkat cell proliferation compared to control. HTS reversed PGE₂ suppressed Jurkat cell proliferation to normal. MIF concentrations was increased in PGE₂-suppressed cells compared to controls. Cells treated with HTS had lower MIF concentrations and MIF expression than PGE₂-suppressed cells and controls. The addition of HTS restored PGE₂-induced cell suppression similar to control. The role of HTS in restoring Jurkat cells proliferation would be mediated through a MIF pathway.

B cells regulate innate immune responses via promoting activation of Natural Killer Cells**Kwanghee Kim, Tae-Jin Kim, Sung Tae Kim and Kyung-Mi Lee*****Global Research Laboratory, Department of Biochemistry and Division of Brain Korea, College of Medicine, Korea University, Seoul, Republic of Korea****E-mail: Kwanghee Kim (kimkh0727@korea.ac.kr), Tae-Jin Kim (anaros@korea.ac.kr), Sung Tae Kim (sungtae@korea.ac.kr), and Kyung-Mi Lee (kyunglee@korea.ac.kr)**

Belonging to innate immunity, Natural killer (NK) cells constitute of circulating lymphocytes which provide a body's first line of defense against infections and cancers. In a secondary lymphoid organ, e.g., spleen or lymph nodes, NK cells co-exist with cells in adaptive immunity, T and B cells. Though NK cells are likely to interact with B cells, the functional consequence of this interaction remains uncertain. To address this question, we co-cultured primary NK cells with freshly isolated B cells from the spleen and investigated the influence of B cells on NK cells. Here we show that co-presence of B cells in cultures facilitated NK cell expansion, as measured by ³H-thymidine incorporation and CFSE dilution assays, while augmented NK cytotoxicity against RMA/S tumor targets. NK cells cultured with B cells exhibited higher expression of CD69, B220, CD25 activation markers, and produced more Granzyme B, suggesting that B cells drastically facilitated NK cell activation. Given the fact that NK cells can exert regulatory role in the pro-inflammatory diseases, such as atopic dermatitis and psoriasis, co-presence of B cells can potentiate the role of NK cells via promoting their proliferation and cytotoxicity.

IKK α is required for maintaining skin homeostasis and preventing skin tumor development

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IKK α is required for the formation of the embryonic epidermis. Loss of IKK α prevents keratinocytes from terminal differentiation and promotes keratinocyte proliferation *in vivo* and *in vitro*. Furthermore, downregulation, altered localization, mutations, and/or loss of heterozygosity (LOH) of *Ikk α* have been reported in squamous cell carcinoma (SCC) of the skin, lungs, esophagus, and head and neck in humans, highlighting the importance of IKK α in human cancers. It has long been known that excessive mitotic activity due to H-Ras can block keratinocyte differentiation and cause skin cancer. It is not clear whether there are any innate surveillants that are able to ensure that keratinocytes undergo terminal differentiation, preventing the disease. IKK α induces keratinocyte terminal differentiation and its reduction promotes skin tumor development. However, its intrinsic function in skin cancer is unknown. Thus, our lab generated *Ikk α* conditional knockout mice, deleted IKK α in keratinocytes in mice by using keratinocyte specific Cre mice, and then examined the effect of IKK α loss on skin development and maintenance, and skin tumorigenesis. We found that mice with IKK α deletion in keratinocytes developed a thickened epidermis and spontaneous squamous cell-like carcinomas. Inactivation of epidermal growth factor receptor (EGFR) or reintroduction of IKK α inhibited excessive mitosis, induced terminal differentiation, and prevented skin cancer through repressing an EGFR-driven autocrine loop. We also identified that IKK α repressed expression of several EGFR ligands at their transcription level, thereby inhibiting the pathway of EGFR and Ras. Thus, IKK α serves as an innate surveillant. These findings shed light on therapeutic targets for preventing IKK α -related skin cancer development. We are currently investigating how inflammation effects IKK α defect-mediated skin tumor development.

Autophagy and ATP-induced anti-apoptosis in antigen presenting cells follows the cytokine storm in patients after major trauma**E. M. Schneider¹, Lorenz MR¹, Flacke, S¹, Walther P², Nass, M¹, Huber-Lang M³, Weiss ME¹**

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Introduction. Severe trauma and the systemic inflammatory response syndrome (SIRS) are associated with a so-called cytokine storm which is in part due to ATP released from damaged tissue. This pathology may lead to highly increased numbers of immature phagocytic cells sharing properties of either dendritic cells or macrophages. Clinically, the occurrence of this cell type was related to the reactivation of herpes virus infections and impaired natural killer (NK-) cell function during sepsis. The aim of this study was the comparative analysis of the ultrastructural and functional characteristics of such immature antigen presenting cells (APC). **Methods.** We used high pressure freeze substitution transmission electron microscopy (TEM) to study morphological criteria and caspase measurements to study ATP related effects and to investigate the susceptibility for cell lysis by NK effectors. Herpes virus reactivation was tested by a novel multiplex PCR identifying EBV genes characterizing EBV latency states I, II and III. **Results.** Ultrastructural studies identified impressive macroautophagy, impaired phago-lysosome fusion, mitochondrial degradation and multivesicular body formation in immature APC cells isolated from blood of patients with sepsis following a major SIRS. Caspase 3/7 activation was increased when immature APC were coincubated with NK-92 killer effector cells, but caspase 3/7 activation was significantly downregulated in the presence of exogenous ATP. We found evidence for EBV latency state II infection in these immature APC cells as well as in whole blood of these patients which was most frequently associated with the detection of EBV-specific LMP-1 and EBNA 2 genes. **Conclusions.** The present study illustrates novel aspects of cytokine storm-associated APC maturation deficiency to explain immune dysfunction against virus reactivation in sepsis patients after major trauma. The induction of autophagy is a novel morphological criterion associated with ATP specific anti-apoptosis, and EBV infection.

Bacterial antigens and cytokines influence Toll-like receptor (TLR)2 expression on mast cells**Maciej Wierzbicki, Ewa Brzezińska-Błaszczyk and Łukasz Konopka****Department of Experimental Immunology, Medical University of Łódź, Łódź, Poland, e-mail: mwierzb@csk.umed.lodz.pl**

Nowadays it is well established that Toll-like receptors (TLRs) play a critical role in development of innate immunity and are involved in adaptive immunity processes. TLRs are expressed on both lymphoid and non-lymphoid cells, including mast cells. Taking into account the role of mast cells in antibacterial defense, immunological reactions as well as in inflammatory process in the present study we examined the influence of some TLR ligands and proinflammatory cytokines on mast cell TLR2 expression. Experiments were done *in vitro* on mature tissue rat peritoneal mast cells. We used lipopolysaccharide (LPS) from *Pseudomonas aeruginosa*, lipoarabinomannan (LAM) from saprophytic *Mycobacterium smegmatis*, peptidoglycan (PGN) from *Staphylococcus aureus*, tumor necrosis factor (TNF), interleukin (IL)-6 and chemokine RANTES. Mast cells were incubated with bacterial antigens or cytokines for 1, 3, 6, 12 and 24 hours and flow cytometry was employed to determine membrane expression of TLR2. We found that TNF and IL-6 did not influence TLR2 expression on mast cells. LPS and LAM also failed to alter mast cell membrane TLR2 expression. At the same time, PGN at concentration of 1 mikrog/mL significantly upregulated mast cell TLR2 expression after 1- and 3-hour incubation ($p < 0.01$). RANTES at concentrations of 1 pg/mL and 100 pg/mL induced statistically significant downregulation of TLR2 expression after 24-hour incubation ($p < 0.05$ and $p < 0.01$, respectively). Our results indicated that bacterial cell wall component PGN as well as chemokine RANTES, modulate mast cell TLR2 expression and thereby might influence TLR2-dependent mast cell activation. This work was supported by grants from the Medical University of Łódź (grant 502-12-606 and 502-12-759) and financial resources for research project (N N401 010236).

Session V: Chronic Inflammatory Pathologies

Low level neuroinflammation in severe psychiatric disorders**Karl Bechter**

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Immune and inflammatory mechanisms are increasingly detected in subgroups of patients with severe psychiatric disorders. We analysed treatment resistant hospitalised affective and schizophrenic spectrum disorder patients regarding albumin, IgG, IgA, IgM, oligoclonal IgG and specific antibodies in paired cerebrospinal fluid (CSF) and serum samples .Numerical and graphical interpretation of CSF protein data was performed by Reibergrams with a new CSF statistics tool for nonlinear group analysis with reference to a large control group (n=4100). In 41% of the psychiatric patients (n=63) we observed CSF pathologies: 14% displayed intrathecal humoral immune responses, 10% slightly increased CSF cell counts (5-8/ μ L) and 29% had moderate blood-CSF barrier dysfunctions, in 24% as the only pathological sign with normal IgG, IgA and IgM concentrations in CSF ($p=0.9$ testing the null hypothesis for intrathecal synthesis with reference to Qmean of the reference group). In the group of affective (n=24) spectrum disorders 20% displayed a systemic immune reaction as detected by oligoclonal IgG. CSF analysis and interdisciplinary clinical approach revealed 6% of psychiatric patients likely to represent a virus-specific, bacterial or autoimmune associated disorder with CNS involvement. Elevated CSF neopterin concentration in 34% of the patients was interpreted as an increased release from astrocytes or from other glia cells. The low level immune response and barrier dysfunctions are discussed on the base of a mild encephalitis pathomechanism in subgroups of psychiatric patients. CSF analysis is known a useful diagnostic tool for differential diagnosis in neurological disorders, and may become increasingly important in psychiatric diseases. Therefore low level neuroinflammation may represent an underlying cause as proposed by the Mild Encephalitis Hypothesis in subgroups of severe psychiatric disorders. The broad diagnostic spectrum involved is not surprising as the validity of psychiatric diagnoses appears limited presently, to be enriched by biological markers close to the neurobiological mechanisms. Overlap between present psychiatric diagnostic categories and findings has for example also been found regarding genes. Such psychoneuroimmunological scenario would be similar to that found in autoimmune disorders in general, that is interaction between environment (infectious agents), genes, and immune system. Antiinflammatory, antiinfectious and/or immunemodulatory treatments of therapyresistant psychiatric disorders have begun.

NANOTECHNOLOGY IN LIGATION-INDUCED PERIODONTITIS: PROTECTIVE EFFECT OF A DOXYCYCLINE GEL WITH NANOPARTICULES.**Marco Botelho****Nanotechnology, Evidence Phamaceuticals, 1905, Padre Valdevino, ST, Fortaleza, Brazil**

Objectives: We aimed to investigate the effect of a nanostructured doxycycline gel 8.5% in the experimental periodontal disease (EPD) model in rats, using the (AFM) tap mode technique. **Material and Methods:** EPD was induced in twenty-four Wistar rats. Animals were treated with a nanospheres doxycycline gel (NDG) topically, immediately after EPD induction and 3 times a day for 11days. Controls did not receive any treatment naïve and non-treated group (NT) and another group received only a vehicle gel (V). In order to investigate topographical changes in histological sections, we used a new simple method for sample preparation, etching with xylol the sections from paraffin-embedded specimens. **Results:** Comparing the AFM images of periodontal surface, several grooves was present in the alveolar bone and other periodontal structures surface of untreated maxillae (NT) and vehicle gel group (V), the depths of the grooves was significantly deeper when compared with the DOX group ($p<0.05$). **Conclusions:** The doxycycline gel was able to provide periodontal surface preservation, with flatter grooves. Periodontal structures were brought into high relief confirming to be a simple and cost-effective method for AFM imaging with ultrastructural resolution.

Key words: Nanotechnology. Doxycycline. Periodontitis. Atomic force microscopy. Nanospheres.

**EXPERIMENTAL ASTHMA IN MICE SELECTED FOR MAXIMUM (AIRmax)
ACUTE INFLAMMATORY RESPONSE**

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Introduction and Aim: Asthma is a chronic inflammatory disease characterized by airway inflammation and hyperresponsiveness (AHR) to methacholine. In addition, asthmatic lung usually respond to allergen by two distinct phases: an early-phase response (EPR) and a more prolonged late-phase response (LPR). The early or late phase responses are not easily reproduced in mouse models of asthma. Because AIRmax and AIRmin mice differ in mobilization of inflammatory cells we used these mouse strains to study bronchial responses and airway allergic inflammation. **Methods and Results:** AIRmax and AIRmin mice were sensitized twice with OVA/Alum on days 0 and 7 and challenged with aerosolized OVA (2% in PBS) on days 14 and 21. Immediately after the last OVA challenge mice were placed in whole-body plethysmograph (Buxco apparatus) for LPR determinations. Twenty-four hours later, AHR to increases doses of methacholine and airway inflammation were also determined. AIRmax but not AIRmin mice developed a clear LPR from 3-6 hours after allergen challenge ($\text{Penh}_{180\text{min}}=1,75\pm0,63$ and $\text{Penh}_{360\text{min}}=2,02\pm0,76$). Twenty-four hours after OVA challenge, the bronchial response to methacholine in allergic AIRmax mice was 130% higher when compared to control mice whereas in AIRmin was only 30% higher than control animals. As expected, airway inflammation and type 2 cytokines were more robust in AIRMax than in AIRmin mice (AIRmax $108,3\times10^5\pm7,3$ vs AIRmin $6,7\times10^5\pm1,2$), eosinophils (AIRmax $88,6\times10^5\pm7,5$ vs AIRmin $1,8\times10^5\pm0,5$), and IL-5 cytokine (AIRmax $267,3\pm64,1$ vs AIRmin $114\pm8,6$ pg/mL). **Conclusion:** We conclude that AIRmax animals are of value as a model of asthma because these animals develop allergen-induced late phase reaction, airway eosinophilia campaigned AHR to metacholine. **Financial support:** Supported by CNPq and FAPESP.

Effects of PTEN deficiency on wound-healing during experimental colitis

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Inflammatory bowel diseases, represented mainly by ulcerative colitis and Crohn's disease are thought to result from inappropriate activation of the mucosal immune system driven by penetration of normal luminal flora through the intestinal epithelial barrier. This barrier is primarily regulated by the apical junctional complex consisting of tight junctions and adherens junctions. After resolution of inflammation, mucosal repair involved epithelial cell proliferation and migration. Our recent works have demonstrated the critical role of the lipid phosphatase activity of the PTEN tumor suppressor in stabilizing adherens junctions, and in reverting EMT and invasiveness. PTEN is a multifunctional protein that dephosphorylates the phosphoinositides generated by PI3K. Interestingly, PTEN is inactivated by reactive oxygen species produced by immune cells, and accumulated under the phosphorylated/inactive form in ulcerative colitis. In this context, our study aimed to investigate the implication of PTEN in tissue repair during intestinal inflammation. Experimental colitis were induced in Villin-Cre PTEN^{flox/flox} and wild-type mice using sodium dextran sulfate (DSS). DSS is directly toxic to gut epithelial cells and affects the integrity of the mucosal barrier. The intestinal invalidation of PTEN was associated with a lower sensitivity to DSS-induced acute inflammation as evidenced by the lower incidence of anal bleeding and by the decreased weight loss in Villin-Cre PTEN^{flox/flox} mice as compared to wild-type mice. At histological level, we evidenced an improved intestinal repair in villinCre PTEN^{flox/fox} mice as compared to wild-type mice, after DSS treatment. These results might be explained by the enhanced proliferative activity of intestinal epithelium of PTEN defective mice, and/or an increased in cell motility related to EMT. In this context, preliminary results suggest an increased accumulation of snail transcripts in the colon of villinCrePTEN^{flox/fox} mice. Our results suggest that PTEN might be a valid target directed at restoring epithelial barrier function after intestinal injury.

Thrombomodulin mutations predispose to atypical hemolytic-uremic syndrome via impaired complement regulation

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Atypical hemolytic uremic syndrome (aHUS) is characterized by microangiopathic hemolytic anemia, thrombocytopenia, and renal failure. Disease-associated polymorphisms in complement regulatory genes account for half the patients, but the etiology of the rest remains unknown. Thrombomodulin (TM) is a ubiquitously expressed endothelial membrane protein with anticoagulant and anti-inflammatory properties. To determine if aHUS is associated with TM mutations, we sequenced the TM gene (THBD) of 152 patients with aHUS and 380 controls. Using purified proteins and cell expression systems we investigated whether TM modulates complement and evaluated the mechanisms. Effects of aHUS-associated TM missense mutations on complement activation were assessed by expressing the mutant forms of TM in cultured cells. Results: Six different heterozygous missense THBD gene mutations were identified in seven unrelated aHUS patients. In vitro, TM binds to factor H (CFH) and C3b, and interferes with complement activation by facilitating factor I (CFI)-mediated inactivation of C3b to iC3b in the presence of cofactors, C4b binding protein (C4bBP) or CFH. By enhancing generation of the plasma carboxypeptidase B, TAFIa, TM also promotes inactivation of anaphylatoxins C3a and C5a. Cultured cells expressing aHUS-associated TM mutants, were less effective at promoting the inactivation of C3b and in activating TAFI, and were therefore less protected from complement hyperactivation. Conclusions: TM is a clinically important negative regulator of complement on the endothelium, and mutations that impair its function, predispose to aHUS and possibly to other thrombotic microangiopathies. The findings establish a molecular link between complement and coagulation, and provide insights for novel diagnostic and therapeutic strategies.

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White adipose tissue (WAT) exhibits a higher cellular heterogeneity than previously thought. We recently identified fibrosis areas in human obese WAT where preadipocytes are suggested to be a major fibrotic cell. The non-adipocyte fraction contains, many immune cell types such as macrophages. Mast cells (MC) were identified as another potentially important cell type, since a MC-deficient mouse model ($Kit^{W-sh/W-sh}$) was resistant to diet-induced obesity (collaboration with G.P. Shi team, Boston). Importantly, reconstitution with bone marrow MC restored the wild-type phenotype. Thus, MC have been positioned as an actor in the development of obesity. We first determined the presence of MC in human obese WAT by immunohistochemistry with tryptase and chymase. These two proteases specifically contained in MC granules were detected also in explants and MC secreted media from obese WAT. Second, as observed in other fibrotic pathologies, MC tryptase staining was predominantly found in the fibrotic regions and particularly close to vessels. In subcutaneous WAT sections from obese subjects, a strong positive correlation was found between the number of MC in fibrotic area and the amount of collagen quantified by picrosirius staining ($R=0.85$ $p<10^{-4}$ $n=29$). To determine whether the WAT microenvironment could modify MC activation we compared IL-6 secretion, a cytokine previously detected in WAT MC media, in two different types of 3D matrices. When cultured in a collagen I matrix supplemented with fibronectin, which reproduces fibrosis, MC secreted more IL-6 than in matrigel (a matrix largely composed of basement membrane proteins). Finally adipose tissue endothelial cells cultured with MC conditioned media exhibited higher secretion of IL-6 and IL-8. Altogether, our data suggest a potential major role for MC in fibrosis, by activating endothelial cells and maintaining the fibrosis inflammatory state.

Human endothelial cells of aortic and microvascular origin differ in Toll-like receptor signaling and strength of chemokine production

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Toll-like receptors (TLRs) are pattern recognition receptors of the innate immune system and binding of microbial ligands or endogenous danger signals leads to a pro-inflammatory immune response. We found that human endothelial cells can - except TLR8 in aortic endothelial cells - express the whole repertoire of the currently known 10 receptors. We now were interested in differences between endothelial cells of aortic and microvascular origin regarding the functional TLR expression as a possible explanation for the involvement of large vessels in pro-inflammatory processes of atherosclerosis, while small vessels remain unaffected. In a first step, we checked TLR gene expression pattern of resting and pro-inflammatory pre-activated primary human aortic endothelial cells (HAoEC) against primary human dermal endothelial cells of microvascular origin (HDMEC). We found only small differences comparing both types of endothelial cells that do not explain the selective involvement in pathology. Interestingly, challenging resting as well as pre-activated aortic and microvascular endothelial cells with a set of specific TLR-ligands and measuring IL-8 release, we observed a narrow functional receptor repertoire for both endothelial cell types but measured a much higher IL-8 release in HAoEC - up to a factor of 10 in resting and up to a factor of 20 in pre-activated cells. Thus, chemotactic gradient-directed homing and leukocyte infiltration to large vessels is much more likely than to microvasculature and might explain why large vessels are predetermined for atherosclerotic lesion formation.

Cellular and molecular mechanisms involved in TNF α -mediated inhibition of erythropoiesis**Grigorakaki C., Morceau F., Dicato M., Diederich M.****Laboratoire de Biologie Moléculaire et Cellulaire du Cancer, Luxembourg****Fondation de Recherche Cancer et Sang, Luxembourg**

Objective: Cancer-related anemia appears to be mediated by pro-inflammatory cytokines including tumor necrosis factor (TNF) α . This pathology is treated by human recombinant erythropoietin (hrEpo), which was recently incriminated to stimulate tumor growth. In order to assess the corresponding molecular mechanisms, we investigated the effect of TNF α on erythroid progenitor cell maturation mechanisms induced by Epo. **Methods:** CD34+ cells, purified from umbilical cord blood by magnetic cell sorting, were cultured in serum-free medium complemented by IL3, SCF and Epo, for cell expansion and differentiation before TNF α treatment. Hemoglobinization was assessed by benzidine staining and cellular morphology by Giemsa staining. **Results:** TNF α reduced the ability of EPO to differentiate CD34+ cells as shown by a decrease of 36% of hemoglobin-positive cells without any detectable erythroid cell maturation. Our results show that TNF α treatment induced a decrease of erythrospecific gene expression, such as γ -globin, and Epo receptor (EPOR) whose mRNA and protein decreased both by 1,8-fold. Moreover, major erythroid-related transcription factor GATA1 and its Friend of GATA (FOG)-1 cofactor mRNA were increased (1,7-fold) after TNF α treatment. Furthermore, our results show that PU-1, a GATA-1 inhibitor, as well as GATA2 protein were also increased (ex GATA2 1,9-fold). **Conclusion:** Despite the induced expression of GATA1, our results show that TNF α blocks erythrospecific gene expression under the control of GATA1. Results are correlated with the overexpression of PU-1, an inhibitor of GATA-1 transcriptional activity. GATA-2 overexpression is in agreement with an inhibition of GATA1 activity. This study provides insight into the molecular mechanisms involved in the inhibition of erythropoiesis triggered by increased TNF α expression and will thus contribute to the improvement of the cancer anemia therapies.

Protective effect of VIP on autoimmune diabetes in NOD mice: balance between different T cell subsets.

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Type 1 diabetes is an autoimmune T-cell mediated disease associated with overexpression of inflammatory mediators and the disturbance of different T cell subsets. Vasoactive Intestinal Peptide is a pleiotropic peptide, produced in nervous, endocrine and immune systems. It has immunoregulatory actions both in innate and acquired immunity, with potent anti-inflammatory and regulatory effects on activated T cells. Since the equilibrium among different T cell subsets participate in the final outcome leading to tolerance or autoimmunity, we studied the evolution of markers for these T cells in VIP-treated and non-treated NOD mice. The study of different transcription factors, cytokines or cytokine receptors in pancreas and spleen, shows that VIP treatment in NOD mice switches the balance between Tregs/Th17 cells subsets, leading to tolerance. In addition, NOD mice show declining values of Th17/Th1 balance with the progression of the disease that could be due to an increase of Th1 cell subsets or a decrease of Th17 cell subsets. Treatment with VIP reverts this balance, increasing Th17 cells relative to the Th1 cell subset. Similarly, in the pancreas of NOD mice, Th1 cell subset predominates over Th2 during the development of the autoimmune pathology, however the treatment with VIP reverts this balance. The change in the balance between different T cell subset induced by VIP during the development of diabetes in NOD mice has a reflex upon inflammation and destruction of pancreatic islet cells. In cell transfer experiments the transfer of splenocytes from VIP-treated mice delayed the onset of diabetes adoptively transferred to NOD-SCID mice. Taken together, these results demonstrate that VIP provides significant protection against spontaneous diabetes modulating T cells subsets and counterbalancing tolerance and immunity.

The immunoregulatory peptide VIP is produced by fibroblast-like synoviocytes from osteoarthritis and rheumatoid arthritis patients and is up-regulated after TLR4 stimulation

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Vasoactive intestinal peptide (VIP) has shown potent anti-inflammatory effects in murine arthritis model and *ex vivo* in human rheumatoid arthritis (RA) fibroblast-like synoviocytes (FLS) and peripheral blood lymphocytes (PBL). TLRs are a family of receptors that activates both the innate and acquired immunities in response to bacterial and viral compounds, leading to the production of proinflammatory and antiviral mediators. FLS from osteoarthritis (OA) and RA patients expressed Toll-Like receptor-4 (TLR4) which is the receptor of the exogenous ligand LPS. The aim of this study was to investigate the potential participation of the endogenous VIP in the RA pathogenesis in FLS, and the significance of TLR4 stimulation in this system. Therefore, we have analyzed the expression and regulation of VIP and its functional receptors at basal and LPS-stimulated condition in FLS from OA and RA patients. VIP expression was detected in FLS at mRNA and protein levels, and it was significantly decreased in RA compared to OA-FLS. VPAC₁ receptor was the main AC coupled receptor in OA-FLS, in contrast to RA-FLS where VPAC₂ was the dominant receptor. LPS stimulation produces an up-regulation in the expression of VIP, VPAC₁ and VPAC₂ receptors in both OA and RA-FLS. On the other hand, our previous data have demonstrated that VIP downregulates TLR4 expression and its signal transduction pathway in FLS from OA and RA patients, resulting in the decrease of proinflammatory mediators. Thus, although LPS-induced TLR4 stimulation increases the endogenous production of VIP and its receptors, it is not enough to generate the reduction of proinflammatory mediators. In summary, only the exogenous addition of VIP produced the final reduction of proinflammatory mediators that ameliorates the pathogenesis of RA.

DNA-protein interaction in discoid lupus erythematosus inflammatory disease**E. Keyhani⁽¹⁾, M. Ahadi⁽²⁾, J. Keyhani⁽¹⁾, Z. Naraghi⁽²⁾ and S. Shahmohammadi⁽²⁾**

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Discoid lupus erythematosus (DLE) is a form of local inflammatory autoimmune disease limited to the skin and involving essentially the face, scalp and ear; ultraviolet irradiation is believed to be a major factor in the onset of DLE. Lesions consist of erythematous raised patches with adherent keratotic scaling and follicular plugging; they slowly expand, with active inflammation at the periphery, leaving depressed scars, telangiectasia, and depigmentation. Diagnosis is usually made by clinical examination and histopathology; additional laboratory tests occasionally performed include anti-nuclear antibodies titers and presence of circulating anti-dsDNA antibodies. DLE patients have about a 10% chance of eventually developing systemic lupus erythematosus (SLE), a systemic autoimmune disease that results in inflammation and damage to a range of organ systems.

In this study, we used an electrophoretic mobility shift assay (EMSA) in parallel with an enzyme-linked immunosorbent assay (ELISA) for the detection of anti-dsDNA in DLE patients. IgG from the sera of 24 DLE patients and 24 healthy individuals randomly collected were isolated by affinity chromatography and tested for the presence of anti-dsDNA. Routine laboratory tests used for the detection of anti-dsDNA antibodies include immunofluorescence, radioimmunoassay (RIA) and ELISA; they reveal the presence of anti-dsDNA antibodies in 20 to 55% of DLE patients. We previously developed an EMSA which showed a much higher sensitivity than the routine tests for the detection of anti-dsDNA in patients with the systemic disease, SLE; we used the assay again in this study. The EMSA gave positive results for all DLE patients while the ELISA was positive for only 36% of them. Both were negative for all healthy individuals. The results presented here suggest that the EMSA is much more sensitive than the routine tests used for the detection of anti-dsDNA in DLE. They also relate to a number of emerging models that illustrate the activation of autoreactive T and B lymphocytes and stress the role of antibodies with specificity for nucleic acids in autoimmune inflammatory diseases such as SLE and DLE.

Plasma membrane DNA as a tool in the detection of systemic lupus erythematosus**Jacqueline Keyhani⁽¹⁾, Ezzatollah Keyhani⁽¹⁾, Genevieve Servais⁽²⁾ and Jean Duchateau⁽²⁾**

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Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease in which the immune system attacks the patient's tissues, resulting in inflammation and damage.

In this report, we show that DNA associated with the plasma membrane of Wil2 lymphocytes is a choice antigen for the detection of anti-dsDNA antibodies in the sera of SLE patients. Wil2-NS ("Wil2"), a non-immunoglobulin secreting human B lymphocyte line, was cultured and used for the detection of immunoglobulins directed against its plasma membrane DNA (pmDNA) in the sera of 12 SLE patients as well as 12 healthy individuals. Freely cultured cells were incubated with the sera and then with fluorescein-conjugated anti-IgG for the detection of anti-pmDNA IgG at the cell surface by immunofluorescence microscopy. Furthermore, pmDNA was isolated from Wil2 plasma membrane, purified, and incubated with the IgG fraction of the sera. Formation of an IgG-pmDNA complex was assessed by an electrophoretic mobility shift assay (EMSA). By immunofluorescence microscopy, all SLE patients tested positive for the presence of IgG directed against pmDNA, while healthy individuals tested negative. In addition, for patients responsive to a treatment with cyclophosphamide, the sera became negative for the presence of anti-pmDNA IgG; on relapse, the sera were positive again for anti-pmDNA IgG. In comparison, routine anti-DNA determination done by ELISA on calf thymus DNA did not give positive results for all SLE patients before treatment; furthermore, although the assay would give negative results after treatment, these results remained negative even when the patient was in relapse. Thus the test for anti-pmDNA as reported here was more sensitive and gave results that paralleled the patient's conditions more reliably than the ELISA. EMSA results showed the formation of a complex between pmDNA and IgG from SLE patients but not from healthy individuals.

Data emphasized the potential role of pmDNA in anti-dsDNA production in SLE and the ability of some IgG present in SLE patients' sera to bind directly to pmDNA.

Nrf2 reduces IL-1beta and IL-6 expression in foam cells upon lipopolysaccharide-stimulation**Anne-Marie Kuhn¹, Andreas von Knethen¹ and Bernhard Brüne¹****¹Institute of Biochemistry I, Faculty of Medicine, Goethe-University Frankfurt,
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Atherosclerosis is a chronic inflammatory cardiovascular disease, causing high mortality and morbidity in industrialized countries. Upon uptake of oxidized low density lipoprotein (oxLDL), macrophages differentiate into foam cells in atherosclerotic lesions. To elucidate their contribution to chronic inflammation, we mimicked the *in vivo* situation *in vitro* by pretreating RAW264.7 cells or peritoneal macrophages for 15 h with 40 µg/ml oxLDL. These foam cells display a desensitized macrophage phenotype, since they produce significant lower amounts of the pro-inflammatory cytokines IL-1beta and IL-6 in response to lipopolysaccharide (LPS)-stimulation compared to control macrophages. Considering that the uptake of oxLDL activates the anti-inflammatory and anti-oxidative nuclear factor erythroid 2-related factor (Nrf2), we were interested to define its contribution towards foam cells deactivation. Therefore we used foam cells generated from macrophages of Nrf2 knockout mice. As expected Nrf2^{-/-} foam cells showed restored expression of pro-inflammatory mediators after LPS-treatment compared to wildtype foam cells. Taking into consideration that heme oxygenase 1 (HO-1), catalyzing the degradation of heme to biliverdin, iron and carbon monoxide (CO), is Nrf2-dependently induced we explored its implication. Corroborating this assumption the CO-releasing compound (CORM2) decreased the expression of IL-1beta and IL-6 after LPS-treatment. Moreover we noticed transrepression of C/EBPbeta and reduced production of reactive oxygen species (ROS) upon LPS-stimulation in foam cells. Therefore we suggest that Nrf2 intervenes with LPS signaling by inducing HO-1 and attenuating ROS production, thus reducing C/EBPbeta transactivation in foam cells and consequently inhibiting the expression of pro-inflammatory mediators.

Rapid Detection of *Pseudomonas aeruginosa* in Clinical Samples of Burned Patients by Fluorescent in Situ Hybridization (FISH)**Mojtaba Moosavian, Freshteh. Badie****Dept. of Microbiology, School of Medicine, Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Iran.**

Aims of the study : The burn wounds have suitable conditions for colonization of bacteria, especially *P. aeruginosa* as an opportunistic pathogen which could be the cause of septicemia. The aim of this study was using of fluorescent in situ hybridization (FISH) as a molecular technique that has been developed for detection of pathogenic agents.

Methods: One hundred of specimens (42 blood positive cultures and 58 wound samples) collected from the hospitalized burn patients, were examined. We could identify *P.aeruginosa* by microscopic examination, culturing of samples and standard biochemical tests on isolated colonies. Also, a part of each specimen used for FISH technique, after preparation and fixation of it.

Results and discussion: This study detected 39 *Pseudomonas* isolates (*aeruginosa* and *nonaeruginosa*) in surveyed specimens by both culture and FISH methods, but the results were difference only in 3 blood culture and wound specimens. Sensitivity of FISH for detection of *P.aeruginosa* in blood and wound samples were 94.7% and 100%, respectively. Also, specificity of FISH for identification of this bacterium in the same samples was 100 and 93.3%, respectively.

This results showed that FISH is a rapid , sensitive and specific test for detection of *P.aeruginosa* and needs to 3 hours of time for doing of all steps of this test. Identification of bacterial agents in a short time, is important for treatment and prevention of patient death.

Key words: FISH, *Pseudomonas aeruginosa*, burn

Transcriptomic profiling and pathway modeling of retinoic acid, Wnt and Hedgehog signaling pathways in experimental chronic renal allograft damage**Christine von Toerne¹, Hermann-Josef Gröne², Thomas Werner³, and Peter J. Nelson¹**

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Chronic renal allograft damage (CAD) is seen in virtually all transplanted kidneys. CAD is manifested by a smoldering inflammation leading to diffuse interstitial fibrosis, glomerular changes, tubular atrophy and loss of tubular structures. There are limited therapeutic options for the treatment of CAD. Retinoic acid (RA), an important regulator of differentiation during vertebrate embryogenesis, can moderate the damage observed in experimental models of CAD. Using recently developed methods for enhanced microarray analysis linked to a systems biology approach, we show here that Wnt and Hedgehog pathway signaling is recapitulated during CAD in a Fischer 344 (RT1l^{vl}) to Lewis (RT1l) rat renal allograft model. Subsets of HH/Wnt modules were identified within this experimental system that specifically reiterates the emergence of that phenotype in patients and linked to the pathophysiology of progressive fibrosis and chronic dysfunction. Treatment with 13cis-retinoic acid selectively moderated the dysregulation of gene and protein expression seen in the HH and canonical Wnt pathways during CAD. The interplay between these three developmental pathways may help explain the therapeutic effects of retinoic acid treatment in CAD and may suggest future directions for moderating chronic renal damage.

Role of macrophage metalloelastase in inflammatory bowel disease

Sylvia L.F. Pender, Rebecca Walsh, EV Vorobeva, MG Buckley, CK Li , A Di-Sabatino, TT MacDonald , SD Shapiro

Matrix metalloproteinases (MMPs) play a major role in many physiological and pathological processes such as angiogenesis, wound healing, and inflammation, including that found in inflammatory bowel disease (IBD). In our previous studies, we have shown the important role of stromelysin-1 (MMP-3) in Inflammatory bowel disease (IBD). In this study, we provide evidence of a potentially role for human macrophage metalloelastase (MMP-12) in IBD. MMP-12 is a macrophage specific enzyme, which could play an even more important role in the causation of IBD than MMP-3. Using gene arrays analysis, we have shown that MMP-12 stands out among the 23 MMPs we screened (including MMP-3) as being the most markedly upregulated in a T cell-mediated model of gut injury and in IBD. MMP-12 expression was 11.9 and 7.3 fold higher than in controls in the PWM-stimulated explant cultures respectively. In IBD tissues we have shown MMP-12 is increased 7.3 fold in UC and 3.6 fold in Crohn's disease compared to controls. Western blotting has demonstrated that MMP-12 is significantly increased in IBD compared with the control subjects, and that UC has the highest expression. Western blotting was also performed on the culture supernatants of explant cultures and it detected high concentrations of both latent and active forms of MMP-12 in four independent experiments, suggesting that the production of MMP-12 is directly related to T cell or macrophage hypersensitivity as seen in IBD. When purified MMP-12 (3ug/ml) was used to treat tissue explants, we found that in four independent experiments, the epithelium of all the explants was completely destroyed after four days of culture. At a tenth of that dose (0.3ug/ml), the epithelium of the explant was partially abolished. Charge staining revealed that glycoaminoglycans were lost from the mucosa in a dose response manner. Immunostaining confirmed the disappearance of the epithelium after MMP-12 treatment. The basement membrane and smooth muscle actin of the explant were still intact at low doses of MMP-12 treatment; however, they were completely destroyed by high doses. When TNBS was administrated to the colons of MMP-12 knockout mice (n=14) for 7 days, no mucosal damage was seen. However, severe colitis was observed in wild type control mice (C57BL/6, n=29). Colons of wild type mice but not knockout mice were significantly thickened after TNBS treatment. In another gut inflammation model in which sodium dextran sulphate was fed to mice for 7 days, severe wasting and bloody diarrhea were observed in wild-type mice. MMP-3 was upregulated in DSS-treated wild type mice, but not in MMP-12 knockout mice is again protected from the treatment. Conclusions: These data indicate that local immune responses can increase MMP-12 expression in resident lamina propria macrophages, without the need for a blood-borne component, and that MMP-12 could play a significant role in tissue inflammation in IBD. The inhibition of this enzyme could be a future therapeutic application in IBD.

Biphasic activation of heat shock system in monocytic cells exposed to live *Helicobacter pylori***Piotr Pierzchalski¹, Marek Cieszkowski¹, Jolanta Jaworek¹, Małgorzata Pierzchalska²**

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Helicobacter pylori(*Hp*), curved gram-negative bacterium, can colonize mucous layer of human stomach and persists there for many years. This unique *Hp* feature is possible due to the presence of a special enzyme – urease catalyzing the hydrolysis of urea to ammonia and formation the alkaline microenvironment. This adaptation have been found to damage the gastric mucosa and to cause chronic gastritis, atrophy or even cancer. The immune system takes an active part in the process of gastritis due to the infiltration of gastric mucosa with monocytes, neutrophils and lymphocytes in response to *Hp* originated neutrophil activating proteins or epithelium produced cytokines. For many years fact of the direct contact between *Hp* and immune system phagocytes was being questioned. Break through papers of Zu et al. and El-Zimaity showing the *in vivo* phagocytosis of *Hp* from the lumen of gastrointestinal tract have clarified that problem. The aim of this study was to establish weather *Hp* is responsible for acceleration of monocyte apoptosis. Monomac 6 cell line was used as a model for studying bacteria-monocyte interactions. The presence of the live *Hp* bacteria in Monomac 6 cell line cultures resulted in the increase of heat shock system activity. In those cells biphasic – early and late – reaction of heat shock system was observed. Translocation of all nuclear heat shock proteins to the cytoplasm within the first 6 hours, where its presence could protect cells against toxic products of engulfed bacteria, was a general feature of the early phase. At the same time, the activation of heat shock genes transcription were noticed. The nuclear factor, HSF-1, was phosphorylated on the serine 230 and translocated to the nucleus, what was shown in gel retardation assay. The late phase of the shock response started 24 hours after the exposure of Monomac 6 cells to the bacteria. Experiments employing blocking the protein synthesis with cycloheximide and fluorescence microscopy have confirmed that during that phase, cellular pool of “used/consumed” heat shock proteins was regenerated by *de novo* protein synthesis. Cell exposed to the bacteria for 48 hours did not reveal any signs of apoptotic cell death. Therefore we conclude that the biphasic process of activation of heat shock system led to the prolongation of phagocytes life. Such reaction *in vivo* might be responsible for the extended contact of phagocyte with pathogen and eventually its efficient elimination.

The effects of splenectomy and autologous spleen transplantation on total white blood cell count, differential leukocyte count and cell morphology in pigs

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Subject of this research were nineteen Landras piglets aged 3 months. After induction of anesthesia piglets were randomly divided in to three groups: sham-operation (n=6), total splenectomy (n=6), and splenic autotransplantation (n=7) with spleen chips autotransplanted into the greater omentum. The blood samples were taken just before the surgery, and on 1st, 5th, 12th, 26th and 40th postoperative day. EDTA blood samples were used to determine total white blood cell count and blood smears were made to perform differential leukocyte count and morphologic evaluation of the cells. All groups showed leukocytosis following the operation but this was not regarded as a change specific for splenectomy or autotransplantation, rather than a post-injury inflammatory response due to tissue lesions during operation. In addition, up to 12th postoperative day, increase of the absolute differential number of band neutrophils and sporadic findings of granulocyte precursors and dividing cells were found, suggesting an increased bone marrow activity and release of immature neutrophils and their mobilization from the marginal pool into the microvasculature. Increase of the absolute neutrophil number and initial decrease of the lymphocyte number was found in all groups, followed by the later recovery of the lymphocyte number. But, no unique pattern was determined because these changes had different onset time and intensity in each group. Described variation in number of neutrophils and lymphocytes was typical for the physiological immune response of the peripheral white blood cells to different stress factors, and correlate with the patients' clinical status. Inflammatory/immune response caused by stress factors can be effectively evaluated using a Neutrophil-lymphocyte stress factor (NLSF), and in this research, the highest values were found in the group of splenectomized piglets. Postoperative recovery of lymphocyte number was slowest in this group, which could indicate a possible lymphopoietic function of the autotransplanted splenic chips. Morphological evaluation revealed some reactive, granulated and cytotoxic lymphocytes on peripheral blood smears of all groups on the 1st and 5th day after the surgery. On the 12th day granulated lymphocytes were frequently found in the group with autotransplanted splenic chips. These morphological changes were probably caused by the alarmins release from the destructed and autotransplanted cells, which triggered the reaction of humoral and cellular immunity.

Phagocytosis and digestion of pH-sensitive fluorescent dye-transfected E. coli in whole blood assays from patients with severe sepsis and septic shock**Schreiner, L¹, Wiedenmann, J², Lorenz, MR¹, Huber-Lang, M³, Weiss ME¹ E. M. Schneider¹**

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Introduction. In infectious diseases, hyperinflammation may impair several immune functions. The function of phagocytosis and digestion is crucial to overcome sepsis. Using E. coli transfected with a pH sensitive fluorescent dye, Eos-FP (Eos-Fluorescent Protein*), the successful fusion of phagosomes with lysosomes can be followed by flow cytometry over time. We questioned the influence of sepsis on phagocytic function and the kinetics of phagosome-lysosome fusion in leukocytes from patients before and after granulocyte colony-stimulating factor (G-CSF, Neupogen) administration. **Methods.** Heparinized blood (0.5 mL) was diluted 1+4 and incubated with 10^7 Eos-P transfected E. coli for various time points. Erythrocytes were lysed and leukocytes were fixed before measuring cell-bound green and red fluorescence by either flow cytometry or confocal laser scanning microscopy. **Results.** The phagocytic activity was determined by mean fluorescence intensities (MFI) after 30', 60', 120' and overnight (o/n) incubation. The healthy donors displayed a fairly homogenous Eos-FP-E. coli-phagocytosis, with median MFI values of 526(30'), 540(60'), 563(120'), and 549(o/n), respectively. The septic patients had a lower activity at 30' (median MFI 472), but already after 60', the septic patients had a higher median MFI 564(60'), 698(120'), and 1209(o/n), respectively. The phagosome-lysosome fusion event was determined by a decline of red fluorescent light emission and was quantified by calculating the green/red-ratio of median MFI. The majority of the septic patients had a faster digestion index : The green/red ratios were 2.3(30'), 3.6(60'), 6.1(120'), and 29.6(o/n), whereas the controls and a minority of the sepsis patients ratios were as low as 1.0(30'), 1.4(60'), 2.0(120') and 10.3(o/n), respectively. In the G-CSF-treated patients' group, there was a strong impact of G-CSF substitution to up regulate both, phagocytosis and digestion of the Eos-FP E. coli. **Conclusions.** Inflammation induced anti-apoptosis in neutrophils of patients with sepsis is also associated with an increased capacity to ingest and degrade bacteria as determined by Eos-FP phagocytosis assays established for whole blood analysis. This effect is further stimulated by G-CSF therapy in vivo. The minority of patients not upregulating phagocytosis require more detailed analysis of the neutrophil function.

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Expression of TNF- α , IL-6, resistin and visfatin during inflammation in diabetic piglets

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Recently, chronic, low-grade inflammation is seen as a primary event linking obesity with insulin resistance. In the experimental models, mainly murine, it was found that overproduction of TNF- α in adipose tissue is an important feature of obesity and causes insulin resistance. Moreover, interleukin-6 (IL-6), resistin and visfatin have an immunological activity and are produced in adipocytes of visceral and epicardial adipose tissues. To better understand the mechanism underlying the interaction of endocrine and immune systems we focused our attention on the crosstalk between metabolism and inflammation signals in diabetic piglets. Experiment was carried out on the 8 weeks old piglets treated with streptozotocin (STZ) and glucocorticoids. Plasma levels of IL-6, TNF- α and visfatin were significantly increased in STZ-treated pigs clearly showing the low-grade systemic inflammation parallel with development of diabetes. In contrast, glucocorticoid injections caused significant decrease of the IL-6 and increase of resistin plasma levels. Analysis of the concentration of all tested peptides in the epicardial adipose tissues showed that STZ caused decrease of IL-6 and increase of TNF- α . Quantitative PCR showed the modulation of the expression of resistin and visfatin mRNAs in epicardial adipose tissue. The obtained results clearly showed that TNF- α , IL-6, resistin and visfatin from the epicardial adipose tissue participate in the induction and maintenance of the subacute inflammatory state associated with insulin resistance and diabetes. The results also indicated that piglets are more useful model for the inflammatory and insulin resistance study than rats or mice.

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Session VI: Inflammation and Cancer

Cancer immunoediting from immunosurveillance to tumour escape in microvillus-formed niche. A study of syngeneic orthotopic rat bladder cancer model in comparison with human bladder cancer

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Cancer cells can develop an attenuated immunogenicity and/or create an immunosuppressive tumour microenvironment that prevent tumour eradication by the host immune system; the so-called “cancer immunoediting” hypothesis. We attempted to test this hypothesis by utilising a rat bladder cancer model in comparison with human bladder cancer. An orthotopic bladder cancer model was induced in Fisher rats by inoculating Fisher rat bladder cancer cell line AY-27. The cancer cells, rat and human bladder cancer tissues, and publicly available microarray data from human bladder cancer were analysed by means of bioinformatics and morphology. Results showed that 10 of 23 differentially expressed pathways were concordant in connection to cell cycle and proliferation between rats and humans and that 13 of 23 pathways, including major histocompatibility complex pathways, were related to host immunosurveillance with activations of CD8- and CD4-evoked T cells, and KIR-evoked natural killer cells in rats. The differentiated pathways and morphogenesis of this rat model corresponded more closely with those of human muscle-invasive tumour rather than non-muscle-invasive tumours. A unique ultrastructure displaying microvillus-formed niche that was interconnected with desmosomes between cancer cells and without infiltration of lymphocytes was found in small areas within the tumour in both rats and humans. Expression of E-cadherin, E-, L- and P-selectins, PGP9.5, VEGF, caspase-3 and BCL2L1 was lower in the tumour than in adjacent normal epithelium. In conclusion, a sub-population of the cancer cells elude immunosurveillance within microvillus-formed niches.

ADAM 12 is highly expressed in, but not restricted to, oligodendrogiomas

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ADAM 12 belongs to a ADAM family of proteases. ADAMs have been implicated in several cell physiological processes including cell adhesion, cell fusion, proteolysis and signalling, and are considered to play important roles in the immunopathogenesis of immune-mediated disorders of the nervous system. In addition, many tumors show elevated expression of ADAM 12. In the human CNS ADAM12 is predominantly (but not exclusively) expressed in oligodendrocytes. In the present study, the expression of ADAM 12 was investigated in various human glial neoplasms by immunohistochemistry and real-time PCR. While 9 of 16 astrocytomas of different malignancy exhibited moderate expression levels of ADAM 12, all (11of 11) oligodendrogiomas showed strong ADAM 12 immunopositivity independently of the grading. The percentage of ADAM 12-positive tumor cells was higher in low-grade oligodendrogiomas compared to anaplastic oligodendrogiomas. Additionally, invasion borders of tumors showed increased immunoexpression. Real-time PCR also detected significantly higher amounts of ADAM 12 mRNA in low-grade compared to high-grade astrocytomas or oligodendrogiomas. Our data indicate that ADAM12 expression is not related to the grade of malignancy in oligodendrogiomas.

A mouse model of inflammatory signaling in prostate tumorigenesis

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Prostate cancer is the second leading cause of death among men in industrialized countries. A growing amount of evidence suggests that inflammation and inflammatory signaling are involved in the tumorigenic process, but the exact role remains a controversial issue. In the current project, we aim to elucidate the role of inflammatory signaling in prostate tumorigenesis by developing and analyzing a mouse model of inflammation-induced prostate cancer. To this end, three mouse lines are being crossed, one conferring prostate-specificity to the model by organ-specific expression of the Cre recombinase, one simulating a genetic event by partially deleting a tumor suppressor gene (phosphatase and tensin homologue, PTEN), and one imitating signaling during a chronic inflammatory state by expressing a constitutively active form of the IkappaB kinase 2 (IKK2ca). Analysis of mice sacrificed at different ages indicated that constitutive IKK activity alone is not sufficient to induce changes reminiscent of pre-malignant lesions. However, combined activation of IKK signaling and partial loss of the tumor suppressor PTEN increased tumor mass compared to partial tumor suppressor loss alone. Histologically, these tumors presented as organ-confined intraepithelial lesions forming frequent cribriform structures, showing an enhanced stromal response and loss of smooth muscle cells encircling the epithelial ducts. Acute inflammation by the infiltration of immune cells was observed. Taken together, our results indicate that inflammatory signaling can cooperate with genetic lesions to increase tumor mass but not enhance invasiveness in prostate tumors.

Sox9-associated overexpression of IFIT3 leads to pancreatic cancer progression by activation of “pseudoinflammatory” pathways.

Peter Camaj, Ivan Ischenko, Hendrik Seeliger, Andrea Renner, Karl-Walter Jauch, Christiane J. Bruns

The understanding of invasion, angiogenesis and metastasis is essential for the development of new targeted molecular therapy against cancer. Inflammation plays important role in tumour initiation and progression. Here we report the role of the transcription factor Sox9 for regulation of IFIT3 (interferon-induced protein with tetratricopeptide repeats 3) an inflammation-related and tumour-promoting protein in pancreatic cancer. For in vivo and in vitro experiments we utilized the following human pancreatic cancer cell lines: low metastatic FG, high metastatic L3.6pl, and the stable transfected cell line FG-IFIT3. To demonstrate effects on primary tumor growth and metastases in vivo we orthotopically injected the different cell lines in the pancreas of nude mice. To evaluate the VEGF depending angiogenic capacity of the different cell lines ELISA technology was used. By One STrEP technology we were able to identify IFIT3-binding partners. Chromosomal immunoprecipitation (ChIP) using anti-Sox9 antibody, followed by PCR amplifying the IFIT3-promoter was used to identify the interaction of the IFIT3 promoter with the transcription factor Sox9. To investigate Sox9-depending expression of IFIT3 (protein and RNA) we used stable transfected L3.6pl-Sox9-shRNA cells under control of the Tet-CMV promoter in presence or absence of tetracycline, respectively. Analysis of differential gene expression by gene array technology demonstrated that the IFIT3 gene is up-regulated in L3.6pl cells as compared to FG cells. Results of animal experiment and in vitro experiments clearly demonstrated tumor-promoting, pro-metastatic and pro-angiogenic features of IFIT3. RT-PCR has revealed that both treatment with IFN α as well as NF κ B led to up-regulation of IFIT3-RNA expression. One STrEP experiments identified JNK and STAT1 as binding partners of IFIT3. ChIP has demonstrated binding of the transcription factor Sox9 to the IFIT3 promoter. RT-PCR and immunoblot data demonstrated constitutive up-regulation of Sox9 expression in L3.6pl cells. By Western blotting and RT-PCR we could show that diminishing of Sox9 expression in stable transfected L3.6pl Sox9-shRNA cells leads to a significant down-regulation of IFIT3-expression on the RNA and protein level. The inflammation associated protein IFIT3 is up-regulated in metastatic L3.6pl human pancreatic cancer cells and is in part responsible for the aggressive primary pancreatic tumor growth in vivo. This gene is up-regulated by IFN α and NF κ B. Interestingly Sox9 binds to the IFIT3P and activates its expression. Since in L3.6pl cells Sox9 is constitutively over-expressed, IFIT3 is up-regulated independent on the presence of the cytokine IFN α . Therefore, the pro-inflammatory IFN α -signaling pathway is activated even without actual inflammation in absence pro-inflammatory cytokine. The activation of such a “pseudo-inflammatory pathway” seems to be in part responsible for pancreatic cancer progression.

First detection of OXA-60 β -lactamase in Tunisia in clinical strains of *Proteus mirabilis***CHOUCHANI Chedly^{1,2}, BELHADJ Omrane².**

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Like other countries worldwide, Tunisia has a growing problem with extended-spectrum- β -lactamases especially with OXA- β -lactamase-producing *Enterobacteriaceae* species. Six major clonally related strains have been identified among OXA producers. We firstly detected here the OXA-60 Extended-Spectrum- β -lactamase produced by clinical strain of *Proteus mirabilis* in Tunisia. In order to determine the genetic support of these resistances, plasmids were extracted and transformed into *E. coli* DH5 α conjugative mating was attempted on agar. MICs were determined by agar dilution. β -Lactamases were typed by isoelectric focusing; antibiotic resistance genes were identified by PCR, and sequenced in order to identify these resistance gene. OXA-60, have a pI of 5.1, that was expressed only after β -lactam induction. After amplification and sequencing, this plasmid-encoded oxacillinase shared 19 percent amino acid identity with OXA-22. *bla*_{OXA-60}, carried by a 70 kb plasmid, was detected in one of six strains studied. Conjugative transfer of cefotaxime resistance was only achieved in this strain; plasmids from both strains were transferred by transformation. The plasmid from one strain additionally carried *bla*_{TEM-1} (variably) *bla*_{OXA-60}; the plasmids from other five strains carried *bla*_{TEM-1} consistently and *bla*_{OXA-22} genes. *bla*_{OXA-60} was plasmid-mediated in clinical strain of *P. mirabilis* and was linked to other antibiotic resistance genes. The prevalence of OXA production among ceftazidime-resistant *P. mirabilis* isolates is relatively high in both hospitals. Infection control measures have been challenged and further improvements in such measures are required to prevent dissemination of these isolates among hospitals. This is the first report of OXA-60 ESBL in *P. mirabilis* in Tunisia.

Key words: Resistance, OXA-60, β -lactamase, *Proteus mirabilis*.

Decreased nuclear expression of Smad4 in uterine cervical carcinogenesis

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Squamous cell carcinoma of the uterine cervix is one of the most frequent gynecologic cancers worldwide. Infection with high-risk human papillomavirus types leads to cervical carcinogenesis. Escape from growth inhibition by transforming growth factor β (TGF- β) has been demonstrated in cervical cancer cell lines. However, little is known about changes in the activity of the intracellular Smad downstream of TGF- β signaling during cervical carcinogenesis. We used immunohistochemistry to analyze Smad2, nuclear Smad3, nuclear Smad4, and inhibitory Smad7 in epithelial cells of normal, intraepithelial neoplasia, and invasive cervical cancer to assess the tendency of Smad expression according to the progress of the carcinogenesis. Specimen comprised 20 tissue cores of invasive cervical cancer, 36 cases of cervical intraepithelial neoplasia. Immunoreactivities of Smad2 and Smad7 were observed exclusively in the cytoplasm of cervical tissues, while Smad3 and Smad4 expressions were exclusively localized in the nuclear compartments of cervical cells. Positive Smad4 and Smad7 expression was observed in 40 (71.4%) and 19 (33.9%) cases, respectively. In the current study, the mean levels of Smad4 nuclear expression were statistically different among early CIN group, advanced CIN group and invasive carcinoma group ($P = 0.032$) while those of Smad7 cytoplasmic expression were not ($P = 0.054$). The expressions of Smad4 and Smad7 were inversely correlated with each other ($P = 0.005$) in cases of invasive cervical cancer. These data suggest that decreased nuclear Smad4 expression may be associated with the progression to invasive cervical cancer, and enhanced expression of the TGF- β signaling inhibitor Smad7 may present one of the mechanisms of TGF- β resistance in cervical carcinoma.

Upregulation of monocyte chemoattractant protein-1 and macrophage inflammatory protein-1 α expression in microglia infiltrating into glioma**Kuan-Min Fang¹, Ying-Lan Wang¹, Ming-Chao Huang², Henrich Cheng², Shun-Fen Tzeng¹****¹Department of Life Sciences, National Cheng Kung University, Tainan, Taiwan.****²Neural Regeneration Laboratory, Department of Neurosurgery, Neurological Institute, Taipai Veterans General Hospital, Taipei, Taiwan. email: stzeng@mail.ncku.edu.tw**

Glioma is the common primary brain tumor derived from the abnormal proliferation of the glia. The infiltration of microglia, brain macrophages, is a characteristic feature of glioma. Several studies have addressed that chemotactic factors secreted around glioma may have the regulatory role in the induction of microglial activation, which is to modulate glioma formation. ATP, an important intercellular regulator in the immune and nervous systems, is released primarily from activated glia and glioma cells. In the study, we used an animal brain tumor model by injecting C6 glioma cells into the rat cerebral cortex, and found that Iba1⁺ and ED1⁺ microglia were infiltrated in the tumor center at 1 week after injection (AI). However, Iba1⁺ cells were the major microglial population observed in the tumor center at 2 week AI. The expression of monocyte chemoattractant protein-1(MCP-1) and macrophage inflammatory protein-1 α (MIP-1 α) were also observed in Iba1⁺ or ED1⁺ microglia situated at the periphery of rat brain tumor at 3 day AI. In vitro study showed that the application of ATP increased the release of microglial MCP-1 and MIP-1 α . Treatment of microglia with BzATP, the agonist of P2X₇R, a surface receptor specific for ATP, also improved MCP-1 and MIP-1 α . Together, based on our in vivo and in vitro results, we suggest that injected C6 cells and activated glia around tumor may release a mass amount of ATP molecules to induce P2X₇R-mediated increase in MCP-1 and MIP expression in microglia around glioma tumor.

Antitumor activity of *in vitro* and *in vivo* treatment with *Orbygnia phalerata* Mart.

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Introduction and aim: *Orbignya phalerata* Mart is a palm native from North of Brazil where cover an area of approximately 10 million hectares. It is popularly known as babassu and is very important to the economy of the region. The mesocarp from babassu fruit is largely used in the treatment of various diseases such as inflammations and tumors. Several tests have been made in order to test biological activities. The aim of this study was to evaluate the antitumor effect of the treatment with aqueous extract of babassu mesocarp (BM) *in vitro* and *in vivo*. Methodology: To evaluate the antitumor effect the Ehrlich tumor on the forms of ascites and solid tumor was used. For the *in vitro* tests, the tumor cells were adjusted to 5x10⁶/mL and 100 mL of this suspension were placed in each well of culture plates 96 well flat bottom and cultured in the presence or absence of the extracts at concentrations of 62.5, 125, 250 and 500 ug/mL, for 24 hours when the quantification and analysis of viability was performed. After this procedure, the tumor cells were inoculated in Balb/c and C57Bl/6 mice strains (N= 30 per group), by intraperitoneal or subcutaneous routes (ascitic and solid tumors, respectively). The solid tumor development was followed by the measurement of paw length thickness by 18 days. The ascitic development was evaluated at 10th day by measuring the ascitic volume, the tumor cell number and the survival. Results: The *in vitro* treatment did not induce death in tumor cells. Despite this, the pretreatment *in vitro* before the inoculation of tumor cells induced a significant decrease in the paw's thickness in both mice strains. However, only in the C57Bl/6 strain there was a decrease in the ascite volume, although both strains had an increased lifespan. Conclusion: The results indicate that the inoculation of tumor cells, pre-treated *in vitro* with BM induces an increase in anti-tumor immune response, which can be related to an immunological deviation of tumor associated macrophages to the M1 pattern. However, more investigations are necessary to clarify this mechanism.

Keywords: Babassu mesocarp; *in vitro*; *in vivo*; Ehrlich of tumor.

Tamoxifen enhances the anti-proliferative effect of roscovitine (ROSC), a selective cyclin-dependent kinase inhibitor on human ER-positive human breast cancer cells**David Gritsch, Margarita Maurer and Józefa Węsierska-Gądek****Cell Cycle Regulation Group, Division: Institute of Cancer Research, Department of Medicine I, Medical University of Vienna, Borschkegasse 8a, 1090 Vienna, Austria. E-mail: Jozefa.Gadek-Wesierski@meduniwien.ac.at**

We reported recently that roscovitine (ROSC), a selective cyclin-dependent kinase (CDK) inhibitor, arrested human ER-ve MCF-7 breast cancer cells in G₂ phase of the cell cycle and concomitantly induced apoptosis^{1 2}. The effect of ROSC was diminished in MCF-7 cells maintained in the presence of estrogen-mimicking compounds^{3 4}. Therefore, we decided to prove whether combining ROSC with anti-estrogen therapy would modulate the efficacy of ROSC action. Exposure of MCF-7 cells to tamoxifen (TAM) for 24h decreased the number of living cells by approximately 10%. This was associated with an accumulation of G₁ cell population by approximately 25% and diminution of S-phase cells. Unlike TAM, estrogen had a very low effect on cell cycle progression of MCF-7 cells within 24h. The proliferation-promoting effect of estrogen became evident only after cultivation of cells for 48h or longer. Addition of estrogen to MCF-7 cells 1h prior to TAM administration did not alter the anti-estrogen-induced G₁ arrest. Simultaneous treatment of MCF-7 cells with ROSC and TAM strongly enhanced the anti-proliferative effect of ROSC. This was potentiated after co-treatment with estrogen. These results clearly evidence that the efficacy of the therapy of ER-positive breast cancers by ROSC can be enhanced by combination with anti-estrogens.

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Tumor-associated macrophage is associated with maintenance of glioma cancer stem cell populations

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Recent studies showed a correlation between the numbers of tumor-associated macrophage and poor prognosis for diverse human cancers. Tumor-associated macrophage is also associated with cancer progression including increasing angiogenic potential, invasion, and metastasis. However, the mechanisms underlying acquisition of malignant properties of human cancers associated with macrophage have remained unclear. In this study, we show that activated THP-1 macrophage is involved in maintenance of cancer stem cell populations in human brain cancer cells. Positive populations for CD133, Nestin and Musashi-1 possessing tumor-initiating potential in human glioma cells were expanded after treated with LPS-activated THP-1 macrophage conditioned media. Cells treated with conditioned media have increased anchorage-independent growth on soft agar and tumor formation. LPS-activated THP-1 macrophage conditioned media also induced a marked activation of both PI3K-Akt and PKCdelta signaling pathway. Inhibition of PI3K or PKCdelta with chemical inhibitors or specific siRNA resulted in suppression of tumorigenic potential in cells treated with THP-1 macrophage conditioned media. Moreover, inhibition of PI3K or PKCdelta led to a decrease in expressions of CD133, Nestin and Musashi. The data we elucidated in this study indicate that tumor-associated macrophage is associated with maintenance of cancer stem cell populations in human brain tumor cells through activation of both PI3K and PKCdelta signaling pathways, and may provide pivotal points for therapeutic intervention in brain cancer treatment.

β-Ionone enhances TRAIL-induced apoptosis in hepatocellular carcinoma cells via Sp1-dependent upregulation of DR5 and downregulation of NF-κB activity**Sang-Hycuk Kang,¹ Moon-Soo Heo,¹ Yung Hyung Choi,² and Gi-Young Kim¹**

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β-Ionone (ION), an end-ring analogue of β-carotenoid, has been known to inhibit tumor cell growth and induce apoptosis in various types of cancer cells. Nevertheless, its apoptosis-related molecular mechanisms remain unclear. Here, we first investigated the molecular mechanisms by which ION sensitizes cancer cells to the therapeutic potential of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). Notably, treatment with subtoxic concentrations of ION and TRAIL effectively inhibited cell viability in the hepatocellular carcinoma cell line Hep3B and other cancer cell lines such as colon carcinoma cell line HCT116 and leukemia cell line U937. Combined treatment with ION and TRAIL was also more effective in inducing DR5 expression, caspase activities, and apoptosis than treatment with either agent alone. ION-mediated sensitization to TRAIL was efficiently reduced by treatment with a chimeric blocking antibody or siRNA specific for DR5. EMSA and a chromatin immunoprecipitation assay confirmed that ION treatment upregulates the binding of transcription factor Sp1 to its putative site within the DR5 promoter region, suggesting that Sp1 is an ION-responsive transcription factor. In addition, ION significantly increased hepatocellular carcinoma cell sensitivity to TRAIL by abrogating TRAIL-induced NF-κB activation and decreasing the expression of antiapoptotic proteins such as XIAP and IAP-1/2. Taken together, these data suggest that ION is a useful agent for TRAIL-based cancer treatments.

Role of Junb in mast cell – mediated tumor angiogenesis

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The AP-1 transcription factor subunit Junb was recently identified as a critical regulator of angiogenesis *in vivo*^{1, 2} and of mast cell biology³ *in vitro*. Junb-deficient bone-marrow derived mast cells (BMMC) display a severe degranulation defect due to impaired expression of SWAP-70, SYT-1 and VAMP8 that represent central molecules for Ca²⁺-release (SWAP-70), Ca²⁺-sensing (SYT-1) and SNARE complex function (VAMP8). Moreover, reduced expression of the cytokines IL-3, IL-4, IL-10 and the growth factors TNF-alpha and, most importantly, VEGF-A in BMMC lacking Junb results in a diminished vascular network formation of endothelial cells (EC) on matrigel. Since the role of mast cells in tumor growth and tumor angiogenesis is currently a subject of increasing interest, we aim to study the contribution of Junb to mast cell function in this context *in vivo*. Therefore, we are generating a novel mast-cell specific Junb knockout mouse that will serve as a tool to dissect the putative regulatory effects of Junb for mast cell function *in vivo*. In particular, maturation, migration and activity of Junb-deficient MC will be studied applying the murine models of contact hypersensitivity and of passive systemic anaphylaxis. The contribution of Junb-deficient mast cells to tumor progression will be analyzed by the use of transplantable syngenic tumor models injecting B16 melanoma or Lewis lung carcinoma cells into mice. Our experimental approach will provide insights into the importance of mast cell recruitment to the tumor site with respect to tumor growth or metastasis and reveal whether loss of Junb in mast cells affects the angiogenic response to implanted tumors.

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Induction of apoptosis in multidrug-resistant human multiple myeloma cells by Roscovitine, a small molecule CDK inhibitor: Cooperative action with functional p53 protein improves its therapeutic efficacy**Oxana Komina, Ellen Noßke, Margarita Maurer and Józefa Węsierska-Gądek**

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Recently, a number of new targeted therapies were developed. Unfortunately, cancer cells develop resistance against a wide-range of anti-cancer drugs. Therefore, new therapeutic settings are necessary to overcome the failure of many chemotherapeutic protocols. Currently, targeting of cell cycle regulators raised hope for cancer cure. Small molecule inhibitors of cyclin-dependent kinases (CDKs) show high therapeutic potential against transformed cells. Their high preclinical effectiveness against rapidly dividing cancer cells and malignancies characterized by accumulation of transformed cells due to deregulation of apoptosis such as chronic lymphatic leukemia or multiple myeloma became evident^{1,2}. In the present study we raised the question whether pharmacological CDK inhibitors like roscovitine (ROSC) would be effective against human multiple myeloma cells that acquired drug resistance to doxorubicin. For this purpose we selected an experimental model of human multiple myeloma-sensitive (RPMI 8226_S) and doxorubicin-resistant (RPMI-8226_DOX40) cell lines. RPMI-8226 cells are known to express temperature-sensitive p53 mutant. Exposure of RPMI 8826 cells to ROSC at restrictive conditions (37°C) markedly increased the ratio of hypoploid population representing cells undergoing apoptosis in both sensitive and resistant cell lines. After 12h the number of subG₁ RPMI-8226S increased up to approximately 40% and RPMI-8226_DOX40 cells up to 27%. Unlike ROSC, DOX at high dosage did not elevate the apoptosis rate in RPMI 8226_DOX40 cell line. Remarkably, under permissive conditions (30°C) wt p53 induced spontaneous apoptosis in both tested cell lines. However, treatment of multiple myeloma cells maintained for 24 h at 30 °C with ROSC for another 24 h increased in both cell lines the apoptosis rate by approximately 30% or 15%, respectively. Our results show that ROSC has capacity to induce apoptosis in the RPMI 8226_DOX40 drug-resistant cell line overexpressing P-gp glycoprotein under restrictive and permissive conditions. Moreover, the outcome of ROSC treatment under permissive conditions indicates its cooperative action with wt p53 protein. Considering the fact that ROSC inhibits not only cell cycle-related CDKs but also negatively regulates kinases involved in the regulation of transcription, its administration to quiescent multidrug-resistant cells might be of advantage. Inhibition of transcription of pro-survival genes such as *BCL-2* or *MCL1* as well as destabilization of survivin seems to facilitate therapeutic efficacy.

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Astragalus saponins exhibit anti-angiogenic effect in colon cancer cells via inhibition of mTOR signaling and pro-inflammatory mediators

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Astragalus membranaceus has been demonstrated to induce colonic protection against experimental colitis through modulation of cytokines in our previous study^[1]. Further studies indicate that total *Astragalus* saponins extract (AST) in the herb, exhibit anti-inflammatory and anti-carcinogenic effects using different models^[2-3]. In this study, the correlation between inhibiting pro-inflammatory mediators and the anti-angiogenic effects of AST in HCT 116 human colon cancer cells under hypoxic condition was investigated. The protein and gene expression of angiogenic factors was assessed using Western immunoblotting and RT-PCR, respectively. The involvement of mTOR signaling in the anti-carcinogenic effect of AST was investigated by using its inhibitor rapamycin. Expression of tumor necrosis factor-alpha (TNF- α), heat shock protein 90 (HSP 90), hypoxia-inducible factor (HIF-1 α) and vascular endothelial growth factor (VEGF) under hypoxia conditions were examined using cobalt chloride (CoCl₂) as hypoxia-mimetic agent. AST (80 μ g/ml) significantly inhibited the protein expression of the key angiogenic growth factors VEGF in normoxic condition. Our results showed that the protein expression of PTEN, an upstream regulator of PI3K-Akt signaling, was increased. This was followed by reduced Akt phosphorylation (pAkt) and downregulation of its downstream target mTOR. Rapamycin alone could decrease the protein expression of VEGF, while co-treatment of rapamycin and AST further downregulate these proteins. HIF-1 has been considered to be a pivotal transcription factor linking between the inflammatory and oncogenic pathways. Under CoCl₂ mimicked hypoxia, the induced HIF-1 α and VEGF expression in HCT 116 cells was suppressed by AST or rapamycin alone. Co-treatment of AST and rapamycin could further decrease their protein expression. Preliminary results have shown that CoCl₂ could induce expression of the pro-inflammatory cytokine TNF- α and of the chaperon protein HSP90, which were dropped to control levels after AST treatment. Our data suggest that AST could downregulate the angiogenic factors HIF-1 α and VEGF through inhibition of mTOR signaling and pro-inflammatory mediators in HCT 116 cells under hypoxic condition.

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Expression of cyclooxygenase-2 in uterine sarcomas

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Objectives: Uterine sarcomas are rare gynecologic malignancies characterized by clinical aggressiveness with very limited therapeutic options. Cyclooxygenase-2 (COX-2) is a well known enzyme that promotes tumor growth and metastasis. COX-2 is up-regulated in a number of human epithelial tumors, but data about its significance in mesenchymal tumors are lacking. The purpose of this study was to evaluate COX-2 expression in uterine sarcomas and to identify if a relationship exists between COX-2 expression and clinicopathologic outcomes.

Materials and methods: Immunohistochemical staining for COX-2 was performed on paraffin-embedded tissue blocks of 32 uterine sarcomas (16 leiomyosarcomas, 14 endometrial stromal sarcomas, and 2 malignant mixed müllerian tumors).

Results: Median age was 41 years (28-65). Of 32 uterine sarcomas analyzed, 4 (12.5%) were positive for COX-2 expression (3 leiomyosarcomas and 1 endometrial stromal sarcoma). No significant association between COX-2 expression and age, tumor histology, or stage was observed. Patients with COX-2 expression showed lower survival rate than those without COX-2 expression ($P=0.048$).

Conclusion: Our results indicate that immunohistochemically determined COX-2 expression has a significant impact on the outcome of patients with uterine sarcoma.

Keywords: cyclooxygenase-2; uterine sarcoma.

Cyclooxygenase-2 down-regulates 15-hydroxyprostaglandin dehydrogenase expression: Implications for inflammation-associated colon carcinogenesis

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Colorectal cancer is one of the leading causes of cancer-related deaths in the United States and many other western societies. Abnormal up-regulation of COX-2 expression has been considered as a key oncogenic event in inflammation-associated human colon carcinogenesis. It was reported that 15-PGDH, a physiological antagonist of COX-2 in its catabolism, is ubiquitously lost in human colon cancer. However, the molecular mechanisms by which 15-PGDH exerts tumor-suppressing functions in colon carcinogenesis remain largely unresolved. In the present study, we have observed that 15-PGDH is negatively regulated by COX-2 in colon cancer cell lines. The mRNA levels and protein activity of 15-PGDH were upregulated by inhibition of COX-2 using designed siRNA. In line with the in vitro data, 15-PGDH was constitutively expressed in mouse colonic mucosa, which was abrogated by treatment with 2.5% SDS for 7 days in drinking water. Under the same experimental conditions, expression of COX-2 was elevated in the DSS-treated mice mucosa, which was not detectable in the normal mice colon. To determine whether 15-PGDH is negatively regulated by COX-2, we utilized a selective COX-2 inhibitor celecoxib. Thus, oral administration of celecoxib increased the 15-PGDH expression while the same treatment decreased COX-2 expression in DSS-treated mouse colon. Moreover, 15-PGDH expression in colonic mucosa following treatment with AOM plus DSS was more prominent in COX-2 knockout mice than that observed in COX-2 wild type animals. Likewise, levels of constitutively expressed 15-PGDH were higher in COX-2 knockout mice. In patients with colon tumors, the expression of 15-PGDH was markedly reduced in adenomas and carcinomas, compared with normal surrounding tissues. These finding suggest that expression of 15-PGDH is negatively regulated by COX-2, which may contribute to the inflammation-associated colon carcinogenesis.

Degradation of Sgs1 in response to rapamycin treatment in yeast *Saccharomyces cerevisiae***R. Marrakchi^{1,2}, C. Chouchani^{1,2}, and D. Ramotar³**

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In yeast *Saccharomyces cerevisiae*, rapamycin an immunosuppressant inhibits the TOR (Target of Rapamycin) complex, homologous of mTOP in mammalian cells. It triggers events that mimic the effect of nutrient starvation including inhibition of ribosome biogenesis, protein translation and inducing autophagy and G₀ entry. TOR proteins integrate signals from growth factors nutrients, stress and cellular energy levels to control cell growth. Rrd1 is a cis/trans prolyl isomerase that required signalling responses to the immunosuppressant rapamycin and mutants devoid of rrd1 display striking resistance to the drug. Preliminary data revealed that this effect was blocked by removing the helicase Sgs1. Then our objective was to investigate what is the link between Rrd1 and Sgs1. Sgs1 is a nuclear DNA helicase of the RecQ family involved in genome integrity maintenance. RecQ family is conserved from bacteria to humans; Sgs1 is a homolog of human BLM and WRN proteins implicated in Bloom and Werner syndromes. Deletion of SGS1 leads to diverse phenotypes including sensitivity to genotoxic agents, hyper-recombination, chromosome missegregation, and meiotic defects. Mutations in the SGS1 gene lead to defects similar to those seen in human cells from the RecQ family disorders. Sgs1 mutant is sensitive to rapamycin. Significantly, deletion of Sgs1 in the Rrd1 mutant causes the mutant to no longer show resistance to rapamycin. Reintroduction of Sgs1 will restore to the rrd1 mutant resistance to rapamycin. The sensitivity to Rapamycin remains at the same level even if Sgs1 is over expressed in the wild type strain and Rrd1 mutant. Sgs1 may belongs to the pathway that signals stress caused by rapamycin. Rapamycin treatment induces rapid degradation of Sgs1 and this degradation is dependent on Rrd1 function. It has recently been demonstrated that Rrd1 is required to isomerize the C-terminal domain of RNA polymerase II and caused its release from the chromatin for degradation. Based on this observation, Rrd1 could most probably alter the structure of Sgs1 then it gets degraded in response to rapamycin. Sgs1 accumulates in Rrd1 mutant after rapamycin treatment as detected by immunofluorescent analysis. This finding reveals a lower level after rapamycin treatment in parent strain and accumulation in Rrd1 mutant. These observations support that Rrd1 is necessary to degrade Sgs1 in the cell, but mechanisms of this degradation are not yet known.

Key words: yeast; Sgs1; Rrd1; rapamycin

Triazoloacridone C-1305 abrogates the restriction checkpoint in cells lacking functional p53 and promotes their accumulation in G₂/M phase of the cell cycle**Margarita Maurer, Oxana Komina, Andrzej Składanowski and Józefa Węsierska-Gądek****Cell Cycle Regulation Group, Division: Institute of Cancer Research, Department of Medicine I, Medical University of Vienna, Borschkegasse 8a, 1090 Vienna, Austria. E-mail: Jozefa.Gadek-Wesierski@meduniwien.ac.at**

It has been recognized that conventional anti-cancer drugs exert a variety of adverse side effects and, unfortunately, may lead to the generation of a second malignancy. Therefore, development of new, more selective and less cytotoxic chemotherapeutics is necessary. Recently, triazoloacridone C-1305, a potent topoisomerase II (Topo II) inhibitor, was generated. Although C-1305 shows structural similarity to other acridines like m-amsacrine (m-Amsa), it has some unique properties. C-1305 is a Topo II poison which leads to the formation of cleavable complexes. It was shown to exhibit potent cytostatic activity toward different tumors under *in vitro* and *in vivo* conditions. Interestingly, mouse cells lacking functional poly(ADP-ribose) polymerase-1 were much more sensitive to C-1305 than their normal counterparts¹. These observations indicate that the efficacy of C-1305 may depend on the cellular context. In the present study we addressed the question whether the functional status of p53 in tumor cells might have an impact on the efficiency of C-1305. Therefore, we performed experiments on human HL-60 promyelocytic leukemia cells which are p53-/ and on human MCF-7 breast cancer cells harboring functional p53. Exposure of cancer cells to C-1305 reduced the number of viable cells in a time- and concentration-dependent manner. Remarkably, HL-60 cells were stronger affected than MCF-7 cells. The measurement of DNA concentration in single cells revealed that C-1305 arrested tested cancer cells at the transition between G₂ and M phases. In concordance with the results of cytotoxicity tests, HL-60 cells were much stronger affected than MCF-7 cells. The analysis of the cell cycle and apoptosis regulators revealed that C-1305 strongly elevated phosphorylation of CDK1 at the inhibitory sites (Thr14/Tyr15) in HL-60 cells. Furthermore, C-1305 increased phosphorylation of Rb protein and of CDK2 at Thr 160 in HL-60 cells but not in MCF-7 cells. Unlike malignant cells, normal human diploid fibroblasts as well as human mononuclear cells isolated from peripheral blood of healthy volunteers were only negligibly affected by C-1305. These observations suggest that C-1305 abrogates the restriction checkpoint and promotes G₁/S transition in cells lacking functional p53.

¹ Wesierska-Gadek et al 2004. Increased susceptibility of poly(ADP-ribose) polymerase-1 knockout cells to antitumor triazoloacridone C-1305 is associated with permanent G₂ cell cycle arrest. *Cancer Res.* 64: 4487.

Function of S100A8 and S100A9 protein complex in mouse models of inflammation-associated carcinogenesis

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Cytokines produced by activated innate immune cells are key players in the functional interaction between the inflammatory microenvironment and tumour cells. They stimulate tumour growth and progression by activation of transcription factors like AP-1 and NF-κB. The NF-κB signalling pathway has been recently shown to participate in inflammation-induced cancer progression. In the inflammation-associated liver cancer model of *Mdr2* deficient mice, NF-κB plays an important role in the development of hepatocellular carcinoma (HCC) as the specific inhibition of the NF-κB pathway in hepatocytes due to the expression of an IκB superrepressor (DNIκB_{hep}) leads to a significant reduction of tumour incidence and a later onset of tumour development. In the present study, we performed a functional genomic approach with HCCs derived from *Mdr2*^{-/-} (*KO*) and *IκB-SR Mdr2*^{-/-} mice (*DM*) and identified a NF-κB-dependent gene regulatory network involved in inflammation-associated liver carcinogenesis. These analyses revealed a comprehensive list of known and novel putative NF-κB target genes, including *S100a8* and *S100a9*. We detected increased co-expression of S100A8 and S100A9 proteins in mouse hepatocellular carcinoma cells, in human HCC tissue and in the HCC cell line Hep3B upon ectopic RelA expression. Importantly, we identified *S100a9* as a direct NF-κB target gene in HCC cell lines. Finally, we found a synergistic function for S100A8 and S100A9 in Hep3B cells resulting in a significant induction of reactive oxygen species (ROS) and protection from TNF induced apoptosis. Furthermore, S100A8 and S100A9 coexpression in epithelial tumour cells induce activation of NF-κB-dependent gene transcription. These data imply that S100A8 and S100A9 are not only inducers of NF-κB signaling, but also NF-κB-dependent target genes, supporting the existence of a feed-forward-loop promoting inflammation and neoplastic transformation.

Bergapten antagonizes cell survival and induces p53 gene promoter together with p38 MAPK activation in breast cancer cells

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In the present study, we demonstrated how the psoralen Bergapten in breast cancer cells is able to influence transductional pathways involved in the regulation of cell survival and apoptosis. The biological effects induced by this compound were evaluated in MCF-7 and SKBR-3 cells where Bergapten increases p53 and p21waf, thus activating p53 gene promoter. Site-direct mutagenesis studies showed that the binding sequence of the nuclear factor NF -Y on this promoter is necessary for drug-responsiveness. In addition p38 MAPK signalling is activated by psoralen treatment and this signaling was required for the Bergapten- induced of p53 gene promoter transactivation . The same p38 kinase is required to enhance NF-Y nuclear translocation. All these effects were no longer noticeable in the presence of the p38 MAPK kinase inhibitor (SB203580). The up-regulation of the p53 oncosuppressor protein induced by Bergapten corresponds to the activation of caspase 8 and caspase 9 in the two cell types, even though caspase-9 is also activated at lower concentrations of psoralen in SKBR-3 cells compared to MCF-7 cells. Moreover, in hormone-dependent MCF-7 cells psoralen is also able to antagonize the stimulatory effect of estradiol and IGF-I on phospho-AKT survival signal.

RAGE in inflammation-associated hepatocellular carcinoma.

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The Receptor for Advanced Glycation-End products (RAGE) is a multiligand receptor and member of the immunoglobulin superfamily of surface receptors mainly involved in chronic inflammatory disorders. RAGE engagement by extracellular inflammatory mediators such as S100 proteins sustains and prolongs the inflammatory response mainly via activation of NF- κ B signalling. Activated NF- κ B promotes not only transcription and overexpression of inflammatory mediators but also, in a feed-forward loop, expression of both RAGE and its ligands. Besides chronic inflammatory diseases, RAGE and its potential ligand S100A8/A9 were also shown to be overexpressed in several types of tumor cells, affecting tumor progression and metastasis by still unknown mechanisms. Moreover RAGE-deficient mice are protected in a model of inflammation-derived skin tumorigenesis. In a murine model of inflammation-associated hepatocellular carcinoma (*Mdr2*^{-/-}), NF- κ B activation was shown to be essential for tumor development. The inhibition of NF- κ B activity resulted in a reduced progression and incidence of tumors but did not affect the early transformation phase nor the onset of hepatitis. In this model, S100A8/A9, described as novel NF- κ B targets, were shown to protect cancer cells from apoptosis and induce ROS production. We hypothesize that RAGE may play a fundamental role in inflammation-associated tumor progression, sustaining the onset and progression of cancer through enhancement of the inflammatory state and promotion of chronic NF- κ B activation. To unravel the role and molecular mechanisms exerted by RAGE, we knocked-out *Rage* expression in *Mdr2*^{-/-} mice. *Rage*^{-/-} *Mrd2*^{-/-} mice were analyzed through the different stages of inflammation, promotion of chronic hepatitis and cancer onset to determine how lack of RAGE may affect the mechanism of inflammation-dependent tumor formation.

Bacteriolytic therapy for cancer: A role of immune and inflammatory response

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The therapeutic/oncolytic effects of *Clostridial* bacteria are very convincing so far. Both mildly-pathogenic and non-pathogenic strains have shown their clinical effects in different cancers since early 1890s. However, how clostridia interact with the immune system, a clear understanding of which would be crucial for the success of this therapy in the clinic. In this study, we have evaluated anti-tumor effects and resultant inflammatory cascades of *Clostridium* spore delivery in BALB/c mice bearing synegeic subcutaneous CT26 colorectal cancer. Mice with or without tumors were given 10^7 *C. sordelli* spores intravenously and those showing a complete, partial or no tumor regression were culled on day 3 and 7 respectively. In order to establish clinical efficacy and inflammatory immune response, tumor volumes were monitored and a Bioplex (BioRad) based cytokine assay was conducted using serum harvested from mice. A panel of nine different cytokines was examined based on previous studies and their current roles in cancer inflammatory networks. Our results showed that there was an increase in the levels of some of the cytokines in spore- treated mice without tumor; however, the levels were significantly much higher in spore-treated group with tumors. More specifically, the levels of IL-6, IL-17, IFN-gamma, G-CSF, MIP-2 and KC were up-regulated in spore-treated group with tumor whilst the levels remain unchanged for TNF- alpha and IL-4 in all groups. Interestingly, the levels of IL-1alpha and IL-1beta were detected at lower levels in spore-treated mice (with or without tumor) when compared to mice with no spores (with or without tumor). In conclusion, a substantial antitumor activity was achieved in response to *C. sordelli* spore-mediated bacteriolytic therapy (a clinical response of 64.28%). Further, a potent immune response was generated, which can be correlated to the tumor regression in treated mice, though at an expense of increased systemic toxicity. It is anticipated that the results from this study may help comprehend the balance between favourable (anti-cancer) and un-favourable (against host itself) immune responses to achieve the ultimate goal of complete tumor reduction with less toxic effects.

Antiproliferative effect of natural tetrasulfides in human breast cancer cells is mediated through the inhibition of the cell division cycle 25 phosphatases

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For many centuries, *Allium* vegetables, such as garlic and onion, have been used to prevent a large variety of health disorders. Several epidemiologic studies have supported an inverse correlation between dietary intake of *Allium* vegetables and cancer risk. In addition to these preventive effects, recent studies performed *in vitro* and *in vivo*, revealed that such vegetables are able to suppress the proliferation of various types of cancer cells. These anticarcinogenic properties were attributed to biologically active sulfur compounds such as allyl- and propylpolysulfides found in *Allium* vegetables. This study reports the inhibitory effect of two tetrasulfides, found in garlic and onion, diallyl- (DAS_4) and dipropyl-tetrasulfide (DPS_4), towards the human cell division cycle (Cdc)25 phosphatases. Both compounds have emerged as interesting *in vitro* irreversible inhibitors of the Cdc25 isoforms A and C. These enzymes are important regulators of eukaryotic cell cycle progression and play a crucial role in the activation of cyclin-dependent kinases (Cdk) by dephosphorylating their phospho-Thr14 and phospho-Tyr15 residues. Furthermore, experiments performed on cultured cells have shown that growth of both sensitive (MCF-7) and resistant (Vcr-R) human breast carcinoma cells was significantly decreased by these tetrasulfides. The observed antiproliferative effect appeared to be associated with a G₂-M cell cycle arrest. Cdc25s are attractive targets in cancer therapy because their over-expression was reported in various types of human malignancies, including breast cancer, and this has been correlated with either poor prognosis or tumor aggressiveness. Thus, our results suggest that Cdc25 phosphatases as possible targets of naturally occurring polysulfides and contribute to their anticancer properties.

Inhibition of isoprenylation enhances cisplatin-induced apoptosis in human A549 lung cancer cells**Franziska Weber, Peter Siska, Matthias Kramer and Józefa Węsierska-Gądek**

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Lung cancer arising from a complex series of genetic and epigenetic alterations of proto-oncogenes and tumor suppressor genes is the leading cause of cancer-related deaths. The molecular complexity of lung cancer makes efficient therapy difficult. Currently the first-line therapy is based on platinum-derivatives combined with other agents. Despite great curative efforts the prognosis for lung cancer patients is poor. Therefore, development of new drugs or innovative therapeutic strategies by combining existing agents is needed. In the present study we compared the effect of cisplatin (CP)¹, a strong DNA damaging compound with that of roscovitine (ROSC), a selective inhibitor of cyclin-dependent kinases (CDKs), on wt p53 positive human A549 lung adenocarcinoma cells harboring mutated *K-RAS* gene. Asynchronously growing A549 cells are relatively resistant to CP treatment for 24h. After exposure to CP at higher dose an accumulation of S-arrested cells was observed. However, after post-incubation of CP-treated cells in a drug-free medium for a further 48h the number of living cells was markedly reduced. Combining CP with L-744,832, a small molecule FPTase inhibitor (FTI)², enhanced its anti-proliferative effect. Interestingly, FTI sensitized A549 cells to CP-induced apoptosis. ROSC inhibited A549 cells at the G₂/M boarder resulting in a marked decrease of the number of viable cells within 24h. The continuous treatment with ROSC for 48h abated the frequency of living cells by induction of apoptosis. Unlike CP, ROSC did not benefit from the inhibition of Ras protein processing pathway. Our preliminary results indicate that functional p53 contributes to the outcome of the therapy in human A549 cells by distinct anti-cancer drugs.

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Immune And Inflammatory Responses In Clostridial Oncolytic Therapy: Friend or Foe?**Ming Q. Wei¹, Preetinder Pal Singh¹, Asferd Mangesha¹, Chun Li¹, Jozef Anne²**

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Oncolytic clostridia have shown significant promise as effective anticancer agents. Culture supernatants of oncolytic clostridia reproducibly and effectively kill tumour cells in culture. In experimental tumour models in immune competent animals, there exist complicated clostridia-hosts interactions that influence oncolytic activities, including immune and inflammatory responses. Clearly, immune and inflammatory responses to clostridia, especially the spores, the form of clostridia that we used for delivery, be it innate or acquired, may restrict spore penetration, colonisation, germination, and promote bacterial clearance, thus limiting the antitumor effect of the therapy. On the other hand, immune and inflammatory responses against dead tumour cells (debris) may recognise them as new antigens, thus enhancing tumour destruction, as specific host immunity and inflammatory responses may develop against these newly release tumour antigens. In this study, we design an experiment to investigate whether the immune and inflammatory response is a welcome friend or a meddlesome foe? Or is it both or neither? Two synergetic tumour models, one of subcutaneous colorectal cancer (CT26) and one for lung cancer (D122) were established. Mice with or without tumours were administered with once or twice 10^7 of two *C. sordelli* and two *C. bifermantans* spores intravenously. Serum from these mice was collected 24 hours after spore injection and at the end of the experiments. Mice showing a complete, partial or no tumour regression were culled on day 3 and 7 respectively. A panel of nine different cytokines was examined based on previous studies and their current roles in cancer inflammatory networks. Our results showed there emerge a significant difference in the pattern of cytokine responses. In the group of animals that had experienced complete oncolysis, the levels of proimmune and proinflammatory cytokines increased significantly when compared with the partial oncolytic group, which in turn were much higher than the non-responsive group. Further experiments are underway to establish the exact degree of increases and decreases of each of the nine cytokines. In conclusion, a substantial immune and inflammatory response was generated in response to *Clostridial* spore-mediated bacteriolytic therapy. The mechanisms of these responses will be discussed, and their potential in anti-cancer therapy will be determined.

A rat model of intravesical delivery of small interfering RNA for studying urinary bladder cancer

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The siRNA has been widely used for research in loss-of-function studies *in vitro*, but not successfully for research *in vivo* and clinical application. The aims of the present study was to establish *i*) rat models of *in vivo* delivery of siRNA into bladder cancer, *ii*) potential targets for siRNA, and *iii*) methods to evaluate therapeutic effects. The rat model of human bladder transitional cell carcinoma (TCC) and its derived cell line (AY27) was induced by the tobacco-related chemical carcinogen, either *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (FANFT) or *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine (BBN). A syngeneic orthotopic bladder cancer model was further established by inoculation of AY27 cells. A fluorescence-labeled negative control siRNA with cationic liposomes was tested both *in vitro* (AY-27 cells) and *in vivo*. The siRNA were highly accumulated in the cancer cells for at least 24 hours after a single dose *in vivo*. Numerous lymphocytes, but not the tumor infiltrating lymphocytes, appeared in the tumor area. Bioinformatics analysis revealed a list of concordantly highly expressed genes, possible siRNA targets, in both the animal models as well as in human TCC. Literature search of *in vitro* bladder cancer provided a list of genes used as siRNA targets. The methodology and data presented in the present study provide a number of opportunities for basic research of TCC carcinogenesis and translational research for evaluation of new therapeutic strategies (including siRNA) for bladder cancer in the native organ, where hormonal, neural and immunological processes more closely resemble the clinical situation.

Expression and significance of Tumor-associated macrophages(TAMs)and microvessel density(MVD) in Human Ameloblastomas

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Abstract: This study was to investigate the influence of Tumor-associated macrophages (TAMs) infiltration in the human ameloblastomas (ABs), the correlation between TAMs and angiogenesis in ABs. **Methods:** The expression of TAMs and microvascular density (MVD) in ABs were investigated by immunohistochemical SP method using specific monoclonal antibody to CD68 and CD34. **Results:** The mean macrophage counts in ABs was significantly higher than the counts in normal mucosa ($P<0.05$). The mean macrophage counts in primary ABs, recurring ABs and canceration ABs were different. The mean microvessel counts in ABs were higher than that in normal mucosa ($P<0.05$). The mean microvessel counts in primary ABs, recurring ABs and canceration ABs were not significantly different, but both microvessel counts were strongly correlated with TAMs counts in ABs ($P<0.01$). **Conclusion :** Tumor associated macrophages may play a major role in the regulation of angiogenesis in ABs.

Effect of anti-estrogen combined with roscovitine, a selective CDK inhibitor on human breast cancer cells differing in the expression of ER**Nora Zulehner, Margarita Maurer and Józefa Węsierska-Gądek****Cell Cycle Regulation Group, Division: Institute of Cancer Research, Department of Medicine I, Medical University of Vienna, Borschkegasse 8a, 1090 Vienna, Austria. E-mail: Jozefa.Gadek-Wesierski@meduniwien.ac.at**

Inhibition of cyclin-dependent kinases (CDKs) by small molecule inhibitors is a very effective weapon against malignant cells. Roscovitine (ROSC), a selective CDK inhibitor primarily affecting CDK2, CDK1, and CDK7, reduces the number of human cancer cells in a concentration-dependent manner. At lower doses ROSC arrests cell cycle progression and at higher doses it induces apoptosis. ROSC inhibits efficiently proliferation of human ER+ve MCF-7 breast cancer cells by induction of cell cycle arrest at the G₂/M transition¹ and concomitantly initiates apoptosis by a p53-dependent pathway². However, the effect of ROSC was much weaker in MCF-7 cells maintained in the presence of estrogen-mimicking compounds^{3, 4}. Therefore, we decided to examine the action of ROSC on other breast cancer cell lines differing in the status of p53 and ER and to prove the impact of selective estrogen receptor modulators (SERMs) on the efficacy of a CDK inhibitor. ROSC was effective on all tested breast cancer cell lines. It arrested MCF-7 and BT-20 cells at G₂/M transition and SKBR3 cells in G₁ phase and additionally induced apoptosis. The effect of ROSC on distinct pro-survival and inflammatory factors was studied. Interestingly, tamoxifen (TAM), a SERM of first generation, strongly affected all tested cell lines irrespective of their ER status. Its combination with ROSC had a different impact. It enhanced G₁ or G₂ arrest. Our results evidence that ROSC can be combined with anti-estrogen therapy and show that the mode of ROSC action depends on the cellular context. The strong effect of TAM on ER-negative cancer cells indicates that SERMs crosstalk with other steroid hormone receptors.

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Session VII: Cell Death and Inflammation

Markers of Inflammation and Apoptosis in Chronic Generalized Periodontitis

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In is known that chronic generalized periodontitis is accompanied by more or less pronounced changes in the concentration of cytokines (interleukin-1 β , interleukin-4, tumor necrosis factor- α , interferon- γ) in the saliva. For instance, considerable increase in the concentration of interferon- γ and interleukin-8 in the oral fluid maintains the inflammatory process and promotes destructive changes in the mucosa. For evaluation of the degree of tissue damage we used the method of laser correlation spectroscopy allowing measurement of the percent proportion of various particles in biological fluids. It was demonstrated that the contribution of particles with diameters of 223-300 nm and >988 nm into light scatter in oropharyngeal washout fluid increased in patients with generalized periodontitis (n=31) compared to healthy controls (n=35). These large particles can correspond to either lipoproteins or DNP and RNP particles. Elevation of cytotoxic activity of inflammatory mediators in periodontitis modifies the quality of nuclear material. We counted nuclear abnormalities (karyorrhexis, karyolysis, binuclear cells, pyknosis) in buccal epithelial cells. In each preparation, 1000 Giemsa-stained cells were analyzed at magnification of 10x90. Preliminary analysis revealed increased incidence of cells with karyorrhexis in patients with chronic generalized periodontitis. Thus, parallel processes take place in chronic generalized periodontitis: development of inflammatory reaction leading to necrosis, on the one side, and apoptosis responsible for timely elimination of damaged cells, on the other. Changes in the balance between these two processes open new prospects for improving the efficiency of therapy.

Organism defence systems status integrated method of estimation using cytotoxicity

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Laser correlation spectroscopy (LCS) and analysis of cytotoxicity of patient serum and urine for bovine spermatozoa were first used in allergological clinical practice. Test system consisting of bovine spermatozoa suspension mobile culture was developed for endotoxicity level of biological fluids measurement and, respectively, organism non-specific defense system evaluation. We examined 249 patients ($34,4 \pm 2$ years) with documented allergic disease of medium severity and 145 healthy individuals ($38,1 \pm 1$ years). Significant differences were revealed in the functional state of the defense systems in these groups. It was found that endogenous intoxication develops in the majority of patients; it is characterized by reduced index of blood toxicity and elevated index of urine toxicity (IUT). During therapy we observed a decrease in IUT, which attested to correction of the state of humoral defense system of the organism, what was confirmed clinically. Method of LCS allows determining the dispersion composition of the studied fluid by the relative contribution of particle components into light scattering. Investigation of blood serum of patients with bronchial asthma showed that with the growth of disease severity increases first contribution to light scattering by small immunoglobulins, and then - large immune complexes. In the urine samples of patients predominate, as compared with "normal" spectra, large particle size of 221-1500 nm, which is defined as "allergy similar shifts" according to semiotic classifier. In LC-spectra of blood serum increased contribution to light scattering by particles of small size, indicating that the accelerated degradation of molecules. Shown that changes in immune function induced by the allergen, leads to not only local symptoms, but also to the development of chronic endogenous intoxication, manifested by changes in the molecular composition of biological fluids. The data produced by the above two methods were in good correlation. Our results suggest that new methods are highly informative and safe and should be introduced into allergologist's practices.

Resistance to apoptosis mediated by agonist Fas antibody in peripheral T-lymphocytes from Crohn's disease patients at onset.

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Background: Defective apoptosis has been reported in mononuclear cells from the lamina propria of Crohn's Disease (CD) patients. Few studies have evaluated the apoptotic capacities of peripheral lymphocytes and these studies lack homogeneity. Furthermore, the relationship between the two compartments has not been properly characterized. **Aims:** To study the susceptibility to apoptosis of non-activated peripheral lymphocytes from active-CD patients after activation *in vitro* and after addition of an external apoptotic stimulus (agonist Fas antibody). **Materials & Methods:** Heparinised blood samples were obtained from healthy subjects (n=11) and from patients at onset of CD and yet to begin any specific medication (n=20). Patients were diagnosed according to Leonard-Jones criteria and disease activity was scored based on the Harvey-Bradshaw index. Lymphocytes were isolated by Histopaque gradient centrifugation, followed by negative purification of non-activated T cells (Depletion Dynabeads®). Isolated cells were cultured for 5 days in the presence (to activate) or absence of bounded anti-CD3 and anti-CD28 diluted in X Vivo-15 medium (Lonza). Afterwards, Fas antibody (Fas Ab) was added (1µg/mL) and apoptosis was detected by flow-cytometry (Annexin-V) after incubation for 5h and overnight, ON (24h). **Results:** Activation of T-Lymphocytes by CD3/CD28 antibodies exposure resulted in a significant increase of apoptosis in both controls and active-CD patients (53.8 ± 17.2 vs 8.3 ± 2.7 and 46.4 ± 18.2 vs 7.8 ± 4.9 , respectively, data in % of apoptotic cells). There are no differences in apoptosis induced by activation between patients and controls. The addition of the Fas Ab significantly increased the percentage of apoptotic cells (67.0 ± 10.9 vs 53.8 ± 17.2 in controls and 62.9 ± 17.1 vs 46.4 ± 18.2 in CD patients). Overnight exposure to Fas Ab was necessary for showing a significantly less induced apoptosis in active-CD lymphocytes than in controls (56.4 ± 16.7 vs 69.6 ± 11.9 , respectively). **Conclusions:** Resistance to apoptosis may be a feature of peripheral T-lymphocytes before cells reach the intestinal mucosa. This intrinsic alteration in their apoptotic capacities helps to clarify the relationship between adaptative and innate immune responses in CD and the physiopathology of the disease.

Nanoceria antagonizes apoptosis via ROS scavenging

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Cerium oxide, when in form of nanoparticles (nanoceria), contains oxygen vacancies or defects in the lattice structure, that cause a change in the local electronic and valence arrangement that stabilizes the trivalent oxidation state. Moreover, the mixed Ce³⁺/Ce⁴⁺ valence states on the surface of nanoceria confer anti-oxidant ability allowing scavenging free radicals. These peculiar features allowed proposing nanoceria as a biologically active agent, possibly neutralizing oxygen in O₂-donors enzymes such as those involved in the inflammatory response on the one side, and on the other, by scavenging excess oxidation in biological processes, with the goal of contrasting the many pathologies and disturbances caused by deregulated or excessive oxidation. Since apoptosis is a cellular process implicated in many of the oxidation-dependent pathologies, and some forms of apoptosis do depend on deregulated intracellular oxidative processes, we analyzed the effects of nanoceria on apoptosis induced by the chemotherapeutic agent etoposide on the human leukocytes cell lines U937 and Jurkat. The experiments were performed adding cerium oxide nanoparticles, with average particles sizes of 5 nm in diameter, to the culture medium. We found that nanoceria reduces the extent of etoposide-induced apoptosis by about 30%. Nanoceria also inhibits apoptotic rise of ROS and superoxide, demonstrating in vivo anti-oxidant ability. To understand whether the anti-apoptotic ability of nanoceria was due to its oxidant scavenger ability, we evaluated the effect that nanoceria exerts on each of the two apoptotic subtypes, the oxidation-independent budding and the oxidation-dependent cleavage. Nanoceria only and strongly affects the ROS-dependent "cleavage" pathway, in the same guise as known anti-oxidant do, demonstrating that nanoceria antagonizes apoptosis by its radical scavenging ability.

Pro-inflammatory context mediated by Electroporation as promising approach in DNA vaccination protocols against cancer

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Eradication of cancer cells is imperative for a successful treatment of tumors. In addition to the existent chemotherapy or radiation therapy, other novel immunotherapeutic strategies are currently under investigation. Naked-DNA vaccination has arisen as an approach aimed at inducing effective immune responses in the host against defined tumor antigens. Recent advances in both the immunological and biotechnological research field, made it possible to enhance significantly the DNA vaccine potency and naked-DNA vaccination protocols, combined with electroporation (EP), provide a promising approach for cancer immunotherapy. We demonstrated that EP induces transient morphological changes in the muscle with early production of endogenous cytokines responsible for signaling danger at the local level. Moreover, it causes the recruitment of inflammatory cells, independently of the DNA injection, and the activation of a danger pro-inflammatory pathway, which results in the recruitment and triggering of cells involved in antigen presentation and leads to T-lymphocyte migration. Collectively, our data indicate that a moderate tissue injury and the generation of a pro-inflammatory context with immediate cytokine release induced by the EP treatment, have adjuvant-like properties able to enhance the immune response in vaccination protocols against cancer.

Reaction to DNA damage caused by ionizing radiation in embryonic fibroblasts WI-38

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Aim: The aim of the present study was to determine changes in cell cycle distribution, protein expression and activity of beta-galactosidase in emryonic fibroblasts WI-38 after irradiation.

Methods: Cells were irradiated with the doses of 2, 6 and 20 Gy for the determination of changes in cell cycle distribution by flow-cytometry. For the determination of senescence and protein expression cells were irradiated with the dose 20 Gy. Senescence has been established by analysis of activity of beta-galactosidase and by western blotting detection of proteins. Results: Irradiation with the dose of 2 Gy has no effect on cell cycle distribution, whereas after the doses of 6 as well as 20 Gy 40% of cells are arrested in G2 phase as early as the first day after irradiation. Enhanced activity of beta-galactosidase can be observed first day after the irradiation with the dose of 20 Gy and this activity is increasing in time. Ionizing radiation caused significant increase in expression of p53. Third day after irradiation the level of protein p53 increases and reaches its maximum. 6 day post irradiation the level is decreased and is rapidly decreasing in time. Phosphorylation of p53 at serine 15 occurs already 4 hours after irradiation and its level is decreased compared to control in 6 days. Maximal phosphorylation at serine 392 reaches its maximum 4 hours after irradiation as well and has the same tendency as phosphorylation at serine 15. The negative regulator of the protein p53 mdm2 is activated and follows the tendency of p53. Ionizing radiation also induces the expression of p21 protein. Its level is increased 1 and 3 days after irradiation and than it goes back to the control level. The expression of p16, which is associated with cellular senescence, increases in time since the day 3 post irradiation. Conclusion: Irradiation of embryonic fibroblasts with the doses of 6 and 20 Gy causes decrease in 40 % of cells provokes arrest in G2 phase of cell cycle. Since the first day after the irradiation with the dose of 20 Gy cells enter senesce, which was proved as increased beta-galactosidase activity and elevated expression of protein p16. Acknowledgement: This work was supported by the project MSM 0021620820.

Adult Retinal Pigmental Epithelium (ARPE) 19: a model to study cell death evasion and inflammation.

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Human Adult Retinal Pigmental Epithelium (ARPE) 19 cells, which are located behind the photoreceptors, are constantly exposed to various stress conditions (e.g. visible/UV light-derived ROS). These cells are responsible for photoreceptor homeostasis by degrading and allowing the renewal of outer segments, they participate in the visual cycle and are involved in the outer hemato-retinal barrier. The importance of these cells in retinal function and their position in the most oxidative area of the body make them extremely resistant to various apoptotic and pro-inflammatory stimuli, possibly in correlation with a high autophagy rate. In this regard, we have investigated the effect of HMA [5-(N,N-hexamethylene)amiloride], an inhibitor of Na⁺/H⁺ exchanger that causes intracytosolic acidification, on ARPE19, focusing on the correlation between autophagy and apoptosis evasion. We found that a treatment with HMA for 8 to 24 h induced the apoptotic process as demonstrated by the activation of caspase-3; interestingly, apoptosis was only triggered but never executed, while autophagy occurred. It seems that autophagy can face and block cell death through the elimination of unwanted pro-apoptotic proteins. The analysis of PARP-1 protein status showed that it is initially cleaved and then confined into autophagic vacuoles. This selective elimination of PARP-1 is interesting because PARP-1 is an important enzyme involved in inflammation regulation; indeed, some PARP-1 inhibitors exert an anti-inflammatory response.

PUMA mediates cytokine- and ER stress-induced pancreatic beta cell apoptosis

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Type 1 diabetes (T1D) is an autoimmune disease characterized by chronic inflammation and progressive pancreatic β -cell loss. The pro-inflammatory cytokines IL-1 β and IFN- γ contribute to β -cell apoptosis in T1D, at least in part via induction of endoplasmic reticulum (ER) stress, but the ultimate mechanisms leading to β -cell death remain to be clarified. We presently demonstrate that IL-1 β + IFN- γ induce the expression of the Bcl-2 homology 3 (BH3)-only activator member PUMA (p53 upregulated modulator of apoptosis) in human and rodent β -cells. We observed that cytokine-induced transcriptional activation of PUMA is regulated by nuclear factor- κ B and endoplasmic reticulum stress but is independent of p53. PUMA activation leads to mitochondrial Bax translocation, cytochrome *c* release and caspase-3 cleavage, ultimately resulting in β -cell apoptosis. The anti-apoptotic Bcl-XL protein is localized at the mitochondria of the β -cells and antagonizes PUMA action, but our data indicate that Bcl-XL is inactivated by the BH3-only sensitizer DP5/Hrk, previously shown by our group to contribute to cytokine-induced β -cell death. Interestingly, siRNAs targeting PUMA or DP5/Hrk prevented at least in part cytokine- or ER stress-induced β -cell death, but there was no additive protection by blocking both proteins. The present data support a *hierarchical* activation of the BH3-only members DP5/Hrk and PUMA controlling the intrinsic pathway of β -cell apoptosis downstream of cytokine- and ER stress-induced signaling.

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Curcumin induces apoptosis in cervical cancer cells through the activation of GADD153**Eun-Ji Jung¹, Min-Jeong Kim^{2,3}, Su-Hyeon Kim¹ and Yong-Sang Song^{1,3,4}**

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Curcumin, a major yellow pigment of tumeric derived from *Curcuma longa* plant, is known to have anticancer and antioxidant effects. Recently, many studies have shown that curcumin induces apoptosis by a different mechanism depends on the cell type. Here, we examined the apoptotic effect of curcumin on cervical cancer cells and its mechanism. Cell growth was inhibited by curcumin treatment in a dose and time dependent manner. The decreased cell viability by curcumin was associated with cleavage of caspases-3 and PARP. The event was resulted from the activation of caspase-8 and caspase-9 with Bid cleavage. The anti-apoptotic Bcl-2 expression was decreased in a time dependent manner, whereas the pro-apoptotic Bax expression was not changed. We also showed that GADD153, a growth arrest and DNA damage inducible gene, was increased at mRNA and protein level. Taken together, we demonstrate that curcumin suppresses the growth of cervical cancer cell via apoptosis. In addition, this event is induced by the activation of caspase-8 and -9, which could involve GADD153 activation.

Monensin-induced growth inhibition of PC-3 human prostate cancer cells via cell cycle arrest and apoptosis

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Monensin is a carboxyl polyether ionophore that potently inhibited the growth of various cancer cell lines. But, the mechanism by which monensin acts remains unknown, especially in human prostate cancer cells. Here, we demonstrate that monensin inhibited the proliferation of prostate cancer cells. Particularly, monensin induced a G1 cell cycle arrest in PC-3 cells. Showing that monensin decreased the levels of CDK2, CDK4, CDK6, cyclin A, cyclin D1 and cyclin E proteins. Monensin also induced the apoptosis in PC-3 cells through ROS production and a loss of mitochondrial membrane potential. It suggested that apoptotic process of PC-3 cells was associated with changes of Bax, Bcl-2, caspase-3 and PARP. In conclusion, this is the first report that monensin potently inhibits the proliferation of human prostate cancer PC-3 cells.

Salinomycin induces reactive oxygen species-mediated apoptosis in human prostate cancer PC-3 cells

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Salinomycin is an anionic polyether that has been used extensively as a microbial antibiotic. There is limited information on the cytotoxicity effect of salinomycin on human prostate cancer cells. To decipher the mechanism of this effect, we studied the sequence of signaling events leading to cell growths inhibition in PC-3 human prostate cancer cells. We found the production of reactive oxygen species (ROS) to be a critical mediator in salinomycin-induced inhibititon of cell growth. The event was reversed by *N*-acetyl-L-cysteine (NAC), inhibitor of ROS. Further investigation revealed that salinomycin triggered the mitochondrial apoptotic pathway, as indicated by differential regulation of Bax/Bcl-2 proteins, resulting in a loss of mitochondrial membrane potential, release of cytochrome c, activation of caspase-3 and cleavage of PARP. Our data suggest that salinomycin should be considered as an effective anticancer agent that modulates ROS-induced cell death via a mitochondrial death pathway in PC-3 cells.

Apoptosis of immortalized keratinocyte HaCaT cells

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Camptothecin (CPT) is the first line chemotherapeutic agent for colon cancer because of its potent antiproliferative and apoptotic properties, but it is also used to treat psoriasis, an epidermal hyperproliferation skin disease, in China. A comparative study showed that the human epidermal immortalized HaCaT keratinocytes were more sensitive to the cytotoxicity of CPT than the human colon cancer HT29 cells, with a stronger expression of apoptotic BAX protein and active caspase 3 in HaCaT cells as demonstrated by western blotting and immunofluorescence analysis. In addition, the transcription of coiled-coil alpha helical rod protein 1 (CCHCR1), a marker protein for psoriasis, was also up-regulated in HaCaT cells. When the cells were depleted with CCHCR1 protein by small interfering RNA (siRNA), the CPT-induced expression of active caspase 3 and cell apoptosis were altered, implicating a possible role of this protein with apoptotic changes in keratinocytes.

The caspase-cleaved form of lyn mediates a psoriasis-like inflammatory syndrome in mice

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We showed previously that Lyn is a substrate of caspases, a family of cysteine proteases involved in the regulation of apoptosis and inflammation. Here, we report that expression of the caspase-cleaved form of Lyn (LynDN) in mice mediates a chronic inflammatory syndrome resembling human psoriasis. Genetic ablation of TNF Receptor 1 in a LynDN background rescues a normal phenotype indicating that LynDN mice phenotype is TNF-α-dependent. The predominant role of T cells in the disease developed by LynDN mice was highlighted by the distinct improvement of LynDN mice phenotype in a Rag1 deficient background. We also established by pangenomic profiling that LynDN mice exhibit increased expression of STAT-3 and inhibitory members of the NFkB pathway. Accordingly, LynDN alters NFkB activity underlying a link between inhibition of NFkB and LynDN mice phenotype. Finally, analysis of Lyn expression in human skin biopsies of psoriatic patients detected Lyn cleavage product whose expression correlates with activation of caspase 1. Our data identify a new role for Lyn as a regulator of psoriasis through its cleavage by caspases.

Involvement of NF- κ B activation in tipping the balance in favor of survival or apoptosis in 3'-azido-3'-deoxythymidine (AZT) treated cells

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In this study we investigated the role of NF- κ B activation in molecular mechanisms underlying susceptibility/resistance to apoptosis induced by AZT. To this purpose, we firstly evaluated induction of apoptosis by AZT in a panel of different cell lines, observing a higher susceptibility of the U937 cell line to AZT-induced apoptosis, in comparison with other cell lines. Then, the transcriptional activity of apoptosis-, cell cycle- and DNA-damage-related genes was detected in AZT-treated U937 cells by SuperArray analysis and confirmed, in selected cases, at protein level, by flow cytometry and western blotting analysis. Surprisingly, AZT induced the transcriptional activity of both pro- and anti-apoptotic genes. Interestingly, several genes, whose transcription was up-regulated by AZT were NF- κ B related. In fact, AZT, at a pro-apoptotic concentration, after an initial inhibition of NF- κ B activation with respect to control, induced a transient, but consistent, increase of NF- κ B binding activity. As a consequence, the effects of AZT on apoptosis in cells in which NF- κ B activation was impaired by stable transfection with a dominant-negative I κ B-alpha or by pharmacological treatment, were ascertained, showing that inhibition of NF- κ B activation increased apoptosis in AZT treated cells and impaired the up-regulation of antiapoptotic genes in response to AZT treatment, with respect to control cells. These results indicate that NF- κ B activation by AZT plays an important role in protecting target cells from apoptotic cell death, improving our understanding of the toxicology and the therapeutic usage of this drug. Given basic knowledge concerning the biology of both viral infections and tumours, modulation of NF- κ B activation could be a critical step in determining the adverse or desired effects of AZT.

Histopathologic study of apoptosis and angiogenic factors in large bowel wall in patients with inflammatory bowel disease.

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Current researches strongly suggest, that angiogenesis and apoptosis play important role in pathogenesis of inflammatory bowel disease (IBD). Investigations on animal models show that extent and intensity of inflammation correlate with increased angiogenesis. Several factors increase angiogenesis in the course of IBD, including vascular endothelial growth factor (VEGF) and its receptors (VEGFR). There are also anti-angiogenic factors like thrombospondin. Their interactions seem to promote changes in the microvascular density (MVD) of bowel wall. Apoptosis – a programmed cell death, correlates with inflammation in IBD. It's been shown that decreased apoptosis in the population of lymphocytes may be a key factor in pathogenesis of IBD. Several studies have demonstrated that increased epithelial apoptosis may be responsible for epithelial barrier dysfunction occurring in the course of IBD particularly in Crohn's disease. The purpose of our study was to demonstrate the correlation between angiogenic factors and apoptotic activity of cells in IBD. For our investigation full thickness tissue sections of the large bowel wall were used. Sections were taken from specimens after surgical resection in patients with Crohn's disease (CD) and ulcerative colitis (CU). We performed morphometrical analysis of all tissue samples. Immunohistochemical expression of VEGFR and anti-angiogenic factors was demonstrated with antibodies against Flt-1 [Sigma-Aldrich] and thrombospondin [Sigma-Aldrich]. Epithelial apoptotic activity was assessed with antibodies against caspase-cleaved cytokeratin 18 - M30 CytoDEATH [Roche]. Our research showed correlation between angiogenic and anti-angiogenic activity in IBD, with no significant differences between CU and CD. We also demonstrated increased apoptotic activity in epithelial cells of intestinal mucosa notably in inflammatory polyps (CU) and in proximity of ulcerations (CD).

On the mechanisms of cell death mediated by a lysosomal protease: the cathepsin D.

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It is widely accepted that classical caspase-dependent apoptosis occurs almost in the absence of inflammatory response. However, studies have shown the induction of caspases-independent cell death describing other proteases effectors like calpains or cathepsins. This occurs mostly in differentiated cells and in neurons. The pro-inflammatory effects of these alternative pathways have not been studied. It has been shown that, in a Light Induced Retinal Degeneration model of photoreceptor demise, the LEI/L-DNase II pathway is activated. This is a caspases independent cell death pathway and recent results indicate that it is activated by cathepsin D in this paradigm. As LEI, the precursor of L-DNase II is a cytoplasmic protein, the main fact in its activation is the release of cathepsin D from the lysosome. In this work we study the mechanism and the pro-apoptotic activities of lysosome permeabilization. We mainly focus on the effects of cathepsin D translocation to the cytoplasm and the molecular pathways activated. To do that we use two different cell lines treated with ciprofloxacin an antibiotic which has been previously shown to permeabilize the lysosomes. The kinetics of cathepsin D release and of L-DNase II activation is described. The description of alternative pathways of cell death is essential in the understanding of cellular reactions involved in inflammatory processes.

Fractalkine has anti-apoptotic and proliferative effects on human vascular smooth muscle cells via epidermal growth factor receptor signalling

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Aims: Fractalkine (CX₃CL1) is a membrane-bound chemokine that signals through the G protein-coupled receptor CX₃CR1 that is implicated in the development of atherosclerosis. We have previously reported that CX₃CR1 is expressed by primary human coronary artery smooth muscle cells (CASM), where it mediates chemotaxis towards CX₃CL1. We sought to determine the effect of CX₃CL1 on CASM survival and proliferation and elucidate the signalling mechanisms involved. **Methods and results:** CX₃CL1 significantly reduces staurosporine-induced apoptosis of CASM, as quantified by caspase 3 immunostaining and Annexin-V flow cytometry. Furthermore, CX₃CL1 is a potent mitogen for primary CASM and induces phosphorylation of ERK and Akt, measured by western blotting. Inhibition of either ERK or PI3K signalling abrogates proliferation, while only PI3K signalling is involved in the anti-apoptotic effects of CX₃CL1. We describe a novel and specific small molecule antagonist of CX₃CR1 (AZ12201182) which abrogates the mitogenic and anti-apoptotic effects of CX₃CL1 on CASM. Pharmacological inhibition of the EGFR blocks CASM survival and DNA synthesis, indicating a previously undocumented role for EGFR signalling in response to CX₃CL1 involving release of a soluble EGFR ligand. Specifically, CX₃CL1 induces shedding of epiregulin and increases epiregulin mRNA expression 20-fold within 2 h. Finally, antibody neutralization of epiregulin abrogates the mitogenic effect of CX₃CL1. **Conclusion:** We have demonstrated two novel and important functions of CX₃CL1 on primary human SMCs: anti-apoptosis and proliferation, both mediated via epiregulin-induced EGFR signalling. Our data have important implications in vascular pathologies including atherosclerosis, restenosis, and transplant accelerated arteriosclerosis, where the balance of SMC proliferation and apoptosis critically determines both plaque stability and vessel stenosis.

Evaluation of pro-apoptotic effect of purine analogs combined with alkylating agent on CLL cells after *in vivo* and *ex vivo* treatment

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Chronic lymphocytic leukemia (CLL) is considered as a cancer-involving, mainly deregulated apoptosis. However, apart from a population consisting of long-lived resting malignant lymphocytes, a small subpopulation, representing rapidly dividing cells, also occurs. Despite advances in the medication of CLL this hematological cancer still remains incurable with standard therapies. Thus, elaboration of more effective treatment protocols slowing the disease progression and impeding relapsing is needed. Recently, besides conventional chemotherapy or chemoimmunotherapy a new approach based on agents which broadly inhibit cyclin dependent kinases was developed. In the present study, the *in vivo* response of CLL cells obtained from peripheral blood of patients cured with cladribine or fludarabine, combined with cyclophosphamide (CC or FC), was compared with that under *ex vivo* conditions. Moreover, in *ex vivo* experiments the impact of R-roscovitine, a tri-substituted purine analog inhibiting cyclin-dependent kinases, was additionally determined. R-roscovitine was administered alone as well as in combination with alkylator-mafosfamide. Leukemic cell samples were isolated from blood of untreated CLL patients prior to the onset of therapy (day 0.) and during administration, i.e. on day 1 and 3 of CC and FC regimen as well as 14 days after termination of the first treatment cycle. For control, mononuclear cells isolated from blood of healthy volunteers (PBMNC) were tested. The effect of the examined agent(s) applied alone or in combinations on human cells was evaluated by two independent methods. Reduction of the number of living cells and apoptosis rate was determined by Vybrant apoptosis assay #4 using flow cytometric measurement of stained cells. Cellular levels and functional status of chosen apoptosis-related proteins (Mcl-1, Bax, caspase-9, caspase-3, PARP-1, and lamin B) were examined by immunoblotting. Evaluation of our results revealed that the apoptosis rate of leukemic cells assessed by Vybrant assay and confirmed by DNA fragmentation differed between *in vivo* and *ex vivo* treatment. In leukemic cells obtained from some *in vivo* treated patients a translocation of histone H1.2 from the nucleus into the cytosol was observed. Furthermore, experiments performed under *ex vivo* conditions revealed that R-roscovitine is much more effective than other purine analogs and induces fast apoptosis of CLL cells at high rate. Our results suggest that R-roscovitine emerges as a powerful agent for improvement of CLL therapy outcome.

LATE ABSTRACT

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Astragalus saponins exhibit anti-angiogenic effect in colon cancer cells via inhibition of mTOR signaling and pro-inflammatory mediators

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Astragalus membranaceus has been demonstrated to induce colonic protection against experimental colitis through modulation of cytokines in our previous study [1]. Further studies indicate that total *Astragalus* saponins extract (AST) in the herb, exhibit anti-inflammatory and anti-carcinogenic effects using different models^[2-3]. In this study, the correlation between inhibiting pro-inflammatory mediators and the anti-angiogenic effects of AST in HCT 116 human colon cancer cells under hypoxic condition was investigated. The protein and gene expression of angiogenic factors was assessed using Western immunoblotting and RT-PCR, respectively. The involvement of mTOR signaling in the anti-carcinogenic effect of AST was investigated by using its inhibitor rapamycin. Expression of tumor necrosis factor-alpha (TNF- α), heat shock protein 90 (HSP 90), hypoxia-inducible factor (HIF-1 α) and vascular endothelial growth factor (VEGF) under hypoxia conditions were examined using cobalt chloride (CoCl_2) as hypoxia-mimetic agent. AST (80 $\mu\text{g}/\text{ml}$) significantly inhibited the protein expression of the key angiogenic growth factors VEGF in normoxic condition. Our results showed that the protein expression of PTEN, an upstream regulator of PI3K-Akt signaling, was increased. This was followed by reduced Akt phosphorylation (pAkt) and downregulation of its downstream target mTOR. Rapamycin alone could decrease the protein expression of VEGF, while co-treatment of rapamycin and AST further downregulate these proteins. HIF-1 has been considered to be a pivotal transcription factor linking between the inflammatory and oncogenic pathways. Under CoCl_2 mimicked hypoxia, the induced HIF-1 α and VEGF expression in HCT 116 cells was suppressed by AST or rapamycin alone. Co-treatment of AST and rapamycin could further decrease their protein expression. Preliminary results have shown that CoCl_2 could induce expression of the pro-inflammatory cytokine TNF- α and of the chaperon protein HSP90, which were dropped to control levels after AST treatment. Our data suggest that AST could downregulate the angiogenic factors HIF-1 α and VEGF through inhibition of mTOR signaling and pro-inflammatory mediators in HCT 116 cells under hypoxic condition.

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Inflammation 2010 on one page

Wednesday, January 27th

Keynote session:

9h30 – 10h30: Jurg Tschopp

10h30 – 11h00: Coffee break

Session 1: Cell signaling pathways I

11h00 – 11h30: Young-Joon Surh

11h30 – 12h00: Massimo Locati

12h00 – 12h30: Matthias Gaestel

12h30 – 13h00: Thad Stappenbeck

13h00: Lunch, workshops, posters

14h00 – 15h00: Becton Dickinson

15h00 – 16h00: Promega

Session 2: Cell signaling pathways II

16h00 – 16h30: Véronique Baud

16h30 – 17h00: Rudi Beyaert

17h00 – 17h30: Hye-Kyung Na

17h30 – 18h00: Wouter de Jonge

18h00 – 18h20: Roberto Gambari

18h20 – 18h40: Emmanuel Dejardin

18h40 – 19h00: Hyeyoung Kim

Thursday, January 28th

Session 3: Inflammatory mediators

8h30 – 9h00: Bharat B. Aggarwal

9h00 – 9h30: Sankar Ghosh

9h30 – 10h00: Edward A. Dennis

10h00 – 10h30: Zigang Dong

10h30 – 11h00: Coffee break

Session 4: Epigenetics and transcription

11h00 – 11h30: Ajay Goel

11h30 – 12h00: Guy Haegeman

12h00 – 12h20: Jonathan Turner

12h20 – 12h40: Oliver H. Krämer

12h40 – 13h00: Alexander Remels

13h00: Lunch, workshops, posters

13h00 – 14h00: IBA

14h00 – 15h00: Polyplus-Transfection

15h00 – 16h00: AMS Biotechnology

Session 5: Virus and immunity

16h00 – 16h30: Jacques Piette

16h30 – 17h00: Andrew Bowie

17h00 – 17h30: Christian Münz

17h30 – 18h00: Johannes Bode

18h00 – 18h30: Yong Sang Song

18h30 – 19h00: Stephen J. Galli

Friday, January 29th

Session 6: Pathologies – Part I

8h30 – 9h00: Seth Masters

9h00 – 9h30: Decio L. Eizirik

9h30 – 10h00: Sushovan Guha

10h00 – 10h30: Kapil Mehta

10h30 – 11h00: Coffee break

Session 7: Pathologies – Part II

11h00 – 11h30: Sunil Krishnan

11h30 – 11h50: Franck Morceau

11h50 – 12h10: Małgorzata Rogalińska

12h10 – 12h30: Jenny E. Gumperz

12h30 – 12h50: George Hajishengallis

12h50 – 13h10: Salahaddin Mahmudi-Azer

13h10 – 13h30: Alfonso Pompella

13h30: Lunch, workshops, posters

14h00 – 15h00: Bio-Rad

15h00 – 16h00: GE Healthcare

Session 8: Cell signaling pathways III

16h00 – 16h30: Varsha Gandhi

16h30 – 17h00: Peter Friedl

17h00 – 17h30: Ivana Scovassi

17h30 – 17h50: Claudia Cerella

17h50 – 18h10: Alicia Torriglia

18h10 – 18h30: Béatrice Charreau

18h30 – 18h50: Thomas Luft

18h50 – 19h10: Iris Behrmann

Saturday, January 30th

Session 9: Natural compounds I

8h30 – 9h00: Lina Ghibelli

9h00 – 9h30: De-Xing Hou

9h30 – 9h50: Norbert Latruffe

9h50 – 10h10: Francesco Peri

10h10 – 10h30: Flavia Radogna

10h30 – 11h00: Coffee break

Session 10: Natural compounds II

11h00 – 11h20: Assam El-Osta

11h20 – 11h40: Marc Schumacher

11h40 – 12h00: Veera R. Konda

12h00 – 12h20: Józefa Węsierska-Gądeck

12h20 – 12h40: Dietmar Fuchs

12h40 – 13h00: Stephan Immenschuh

13h00: Lunch and end of the meeting



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LATE ABSTRACT

Session VI

Poster VI, 29

Astragalus saponins exhibit anti-angiogenic effect in colon cancer cells via inhibition of mTOR signaling and pro-inflammatory mediators

PC Law, KKW Au Yeung and JKS Ko.

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Astragalus membranaceus has been demonstrated to induce colonic protection against experimental colitis through modulation of cytokines in our previous study [1]. Further studies indicate that total *Astragalus* saponins extract (AST) in the herb, exhibit anti-inflammatory and anti-carcinogenic effects using different models^[2-3]. In this study, the correlation between inhibiting pro-inflammatory mediators and the anti-angiogenic effects of AST in HCT 116 human colon cancer cells under hypoxic condition was investigated. The protein and gene expression of angiogenic factors was assessed using Western immunoblotting and RT-PCR, respectively. The involvement of mTOR signaling in the anti-carcinogenic effect of AST was investigated by using its inhibitor rapamycin. Expression of tumor necrosis factor-alpha (TNF- α), heat shock protein 90 (HSP 90), hypoxia-inducible factor (HIF-1 α) and vascular endothelial growth factor (VEGF) under hypoxia conditions were examined using cobalt chloride (CoCl_2) as hypoxia-mimetic agent. AST (80 $\mu\text{g}/\text{ml}$) significantly inhibited the protein expression of the key angiogenic growth factors VEGF in normoxic condition. Our results showed that the protein expression of PTEN, an upstream regulator of PI3K-Akt signaling, was increased. This was followed by reduced Akt phosphorylation (pAkt) and downregulation of its downstream target mTOR. Rapamycin alone could decrease the protein expression of VEGF, while co-treatment of rapamycin and AST further downregulate these proteins. HIF-1 has been considered to be a pivotal transcription factor linking between the inflammatory and oncogenic pathways. Under CoCl_2 mimicked hypoxia, the induced HIF-1 α and VEGF expression in HCT 116 cells was suppressed by AST or rapamycin alone. Co-treatment of AST and rapamycin could further decrease their protein expression. Preliminary results have shown that CoCl_2 could induce expression of the pro-inflammatory cytokine TNF- α and of the chaperon protein HSP90, which were dropped to control levels after AST treatment. Our data suggest that AST could downregulate the angiogenic factors HIF-1 α and VEGF through inhibition of mTOR signaling and pro-inflammatory mediators in HCT 116 cells under hypoxic condition.

[1] J.K.S. Ko, et al., [Cytokine](#), 2009, 47(2): p.85-90. [2] K. K.W. Auyeung, et al., [Int. J. Cancer](#), 2009, 125: p.1082–1091. [3] K. K.W. Auyeung, et al., [Int J Mol Med](#), 2009, 23(2): p.189-96.