International Symposium on Mechanisms of Innate Immunity, Cell Death and Inflammation

September 25-26, Het Pand, Ghent, Belgium
Scientific Organizers: Prof. Dr. Mo Lamkanfi and Prof. Dr. Peter Vandenabeele

Program &
Book of abstracts

This conference is organised within the framework of the Flemish Training Network Life Sciences (FTNLS). FTNLS is a joint initiative of KU Leuven, Universiteit Gent, Universiteit Antwerpen, Vrije Universiteit Brussel, Universiteit Hasselt and VIB that takes into account commitments & requirements of a recent 'order of the Flemish Government awarding a grant for the framework of young scientists'. The mission of the network is to jointly organize high-level workshops for researchers active in the life sciences. In concrete, FTNLS is a forum that will offer the regional scientific community a unique chance to attend open keynote lecturers and, on top, to participate in a framework of highly interactive, hands-on training sessions.

This symposium is further sponsored by

- ✓ the **Methusalem grant** awarded to Prof. Peter Vandenabeele. The Methusalem program is a funding tool provided by the Flemish government that is meant to give substantial stable, long-term financial support to the most excellent researchers of each Flemish university.
- ✓ The VIB department for **Medical Protein Research**

Chairs of the conference

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Venue

The symposium will take place at '**Het Pand'**.

Het Pand
Onderbergen 1
9000 Gent
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Internet connection at the venue

During the symposium, free Wireless Internet Connection will be available to all participants. Please follow the instructions below to connect.

Make a wireless connection with "UGentGuest".
If you have set up to request an IP address automatically, you will receive an IP address starting with 193.190.8x.

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Meals and refreshments

Sandwich lunches on Thursday and Friday as well as coffee breaks are included in the registration fee for all participants. Please wear your name badge at all times when attending these events.

The Welcome reception is offered on Thursday evening September 25.

All these events will take place at the conference venue.

Instructions for poster presenters

Posters can be mounted from the beginning of the meeting and will be displayed throughout the meeting. Mounting material will be present on site.
Two fixed poster sessions are scheduled during the meeting.

A poster list in included in this book of abstracts.

- ✓ Poster should be made in portrait, poster board measurements are 200 cm (height) x 100 cm (width). Please prepare your poster accordingly.
- ✓ Posters should be readable by viewers one meter away. The poster should contain the title of the submitted abstract, the author(s)' name(s) and affiliation at the top. The organisers will provide mounting materials to fix posters.
- ✓ Posters should be removed by the end of the last poster session.
- ✓ Delegates are not allowed to take pictures from slides during the presentations, nor from the posters without the consent of the authors.

Thursday September 25, 2014

Morning session: Unraveling immune mechanisms

Chair: Dr. Mathieu Bertrand (VIB-UGhent)

8:30 Registration

9.20 Welcome and Introduction

9:30 **Vishva Dixit,** Genentech, San Francisco, USA
Ubiquitin Modification in Immune Signaling

10.10 **Ting Liu,** University of Tokyo, Tokyo, Japan
Live-imaging of caspase-1 activation reveals an all-or-none response of inflammasome signaling

10.30 **Arun Mankan,** University of Bonn, Bonn, Germany
Activation of AIM2 inflammasome by RNA-DNA hybrids

10.50 **Mo Lamkanfi**, VIB-UGhent, Belgium
Inflammatory caspases: related proteins, different functions

11.30 Break + Posters

13.00 Lunch

Afternoon session: Necroptosis and RIP kinases

Chair: Dr. Andy Wullaert (VIB-UGhent)

14.00 **Douglas Green,** St Jude, Memphis, USA
Balancing Act: The control of necroptosis and apoptosis in development

14.40 **Siddharth Balachandran,** Fox Chase Cancer Center, Philadelphia, USA
Mechanism and Regulation of Interferon-Induced RIP3-Driven Cell Death

15.00 **Nader Yatim,** Institut Pasteur, Paris, France
RIPK1 signaling in the dying cell regulates cross-priming

15.20 Break

15.40 **Manolis Pasparakis,** University of Cologne, Germany
RIP kinases in cell death and inflammation

16.20 **Peter Vandenabeele,** VIB-UGhent, Belgium
Molecular mechanisms of regulated necrosis, an attempt at an overview

17.00 Welcome Reception

Friday September 26, 2014

Morning session: Programmed cell death in disease

Chair: Dr. Anje Cauwels (VIB-UGhent)

9:30 **Seamus Martin,** Trinity College Dublin, Ireland
How Diverse Signal II Agents Promote NLRP3-dependent IL-1beta release

10.10 **Laurent Boyer,** Nice Sophia Antipolis, Nice, France
Bacterial virulence sensing by the innate immune system mediated by imd RIP proteins

10.30 **Lily Boutens,** Wageningen University, Wageningen, The Netherlands
Evidence for the presence of the phagocytic machinery in the adipose tissue

10.50 **Venizelos Papayannopoulos,** MRC, UK
Decision making in neutrophil antimicrobial responses

11.30 Break + Posters

13.00 Lunch

Afternoon session: in vivo mechanisms of inflammation

Chair: Dr. Jo Van Ginderachter (VIB-VUB)

14:00 **Thirumala-Devi Kanneganti,** St Jude, Memphis, USA
Regulators of Inflammatory Responses

14.40 **Geert van Loo,** VIB-UGhent, Belgium
A20 in inflammatory signaling and pathology

15.20 Closing Remarks

Cultural program

15.30 - 15.55: **Maximiliaan Martens,** UGhent, Belgium
"A brief history of the Ghent Altarpiece"

15.55 - 16.20: **Peter Vandenabeele,** UGhent, Belgium
"Spectroscopic analysis of the Ghent Altarpiece"

Short oral communications

Liu Ting SO-1

Live-imaging of caspase-1 activation reveals an all-or-none response of inflammasome signaling

Mankan Arun SO-2

Activation of AIM2 inflammasome by RNA-DNA hybrids.

Balachandran Siddharth SO-3

Mechanism and Regulation of Interferon-Induced RIP3-Driven Cell Death

Yatim Nader SO-4

RIPK1 signaling in the dying cell regulates cross-priming

BOYER LAURENT SO-5

Bacterial virulence sensing by the innate immune system mediated by imd RIP proteins

Boutens Lily SO-6

Evidence for the presence of the phagocytic machinery in the adipose tissue

Live-imaging of caspase-1 activation reveals an all-or-none response of inflammasome signaling

Ting Liu1, Yoshifumi Yamaguchi1,2, Yoshitaka Shirasaki3, Koichi Shikada1, Mai Yamagishi3, Katsuaki Hoshino4,5,6, Tsuneyasu Kaisho5,6, Kiwamu Takemoto2,7, Toshihiko Suzuki8, Erina Kuranaga9, Osamu Ohara3,10, and Masayuki Miura1,11

1Department of Genetics, Graduate School of Pharmaceutical Sciences, The University of Tokyo 2PRESTO, Japan Science and Technology Agency 3Laboratory for Integrative Genomics, RIKEN Center for Integrative Medical Sciences (IMS-RCAI) 4Department of I

Caspase-1 is a member of cysteine protease family caspases and involved in the secretion of some pro-inflammatory cytokines like interleukin-1β (IL-1β). In addition to this process, caspase-1 also regulates the inflammatory cell death termed 'pyroptosis' in macrophages in response to microbial infection as well as non-infectious stimuli. The activation of caspase-1 is regulated by the intracellular multiple protein complexes called 'the inflammasomes'. Disrupted regulation of inflammasome-caspase-1 axis results abnormal secretion of IL-1β, which is associated with various chronic inflammatory diseases. Although the dynamics of caspase-1 activation, IL-1β secretion, and cell death have been examined intently with bulk assays in population-level studies, they remain poorly understood at the single-cell level because of technical limitations. To address this point, we conducted single-cell imaging using a genetically-encoded fluorescence resonance energy transfer (FRET) sensor that detects caspase-1 activation. We determined that caspase-1 exhibits all-or-none (digital) activation at the single-cell level, with similar activation kinetics irrespective of the type of inflammasome or the intensity of the stimulus. Concurrent live-imaging of caspase-1 activation and IL-1β release demonstrated that dead macrophages containing activated caspase-1 release a local burst of IL-1β in a digital manner, which identified these macrophages as the main source of IL-1β within cell populations. Our results highlight the value of single-cell analysis in enhancing understanding of the inflammasome-mediated chronic inflammatory diseases.

Presenting author: Ting Liu

Activation of AIM2 inflammasome by RNA-DNA hybrids.

Arun K. Mankan, Dhruv Chauhan, Tobias Schmidt, Andrew V. Kubarenko, Veit Hornung

Institute of Molecular Medicine, University Hospital, University of Bonn, Sigmund-Freud-Strasse 25, 53127 Bonn, Germany

Abortive HIV replication and the resultant accumulation of nucleic acids has been implicated in IFI-16 mediated CD4 T cell death due to pyroptosis. RNA-DNA hybrids are generated during viral replication and have been shown to accumulate in the cytoplasm and endolysosomes. While TLR9 has been implicated in the recognition of RNA-DNA hybrids in endolysosomes, the detection of cytoplasmic hybrids and the role of inflammasome in this process has so far not been reported. The AIM2 protein detects cytoplasmic dsDNA and its interaction with dsDNA is sequence independent. Hence we hypothesized that AIM-2 could also detect RNA-DNA hybrids. Here we provide evidence that RNA-DNA hybrids can induce caspase-1/AIM2-dependent secretion of processed IL-1β and promote pyroptosis.

Presenting author: Arun Mankan

Mechanism and Regulation of Interferon-Induced RIP3-Driven Cell Death

Roshan J. Thapa1*, Shoko Nogusa1*, Alexei Degterev2, Willam J. Kaiser3, Edward S. Mocarski3 and Siddharth Balachandran1#

Fox Chase Cancer Center

Mechanism and Regulation of Interferon-Induced RIP3-Driven Cell Death Roshan J. Thapa1*, Shoko Nogusa1*, Alexei Degterev2, Willam J. Kaiser3, Edward S. Mocarski3 and Siddharth Balachandran1# 1Fox Chase Cancer Center, Philadelphia, PA 19111 2Department of Biochemistry, Tufts University, Boston, MA 02111 3Department of Microbiology and Immunology, Emory University Interferons (IFNs) are cytokines with powerful immunomodulatory and antiviral properties, but less is known about how they induce cell death. We have found that both type I (α/β) and type II (γ) IFNs induce precipitous RIP3 kinase-dependent necrosis when the adaptor protein FADD is lost or disabled, or when caspases (e.g. caspase 8) are inactivated. IFN-induced necrosis proceeds via progressive assembly of a RIP1-RIP3 'necrosome' complex that requires Jak1/STAT1-dependent transcriptional activation of the RNA-responsive kinase PKR, which then interacts with RIP1/3 to initiate necrosome formation and trigger necrosis. Surprisingly, IFNs also induce cell death in the absence of RIP1; this cell death that is mediated by both caspase 8-driven apoptosis and RIP3/MLKL-dependent necrosis, and is attributable, at least in part, to a kinase-independent role for RIP1 in activating a pro-survival NF-κB transcriptional response. Collectively, these findings outline new mechanisms and regulators of IFN-induced cell death, with ramifications for host defense and therapeutics.

Presenting author: Siddharth Balachandran

RIPK1 signaling in the dying cell regulates cross-priming

Nader Yatim, Helene Saklani, Oliver Schulz, Caetano Reis e Sousa, Doug R. Green, Andrew Oberst and Matthew L. Albert

Laboratory of Dendritic Cell Immunobiology, Institut Pasteur and Inserm U818, Paris France

The danger model predicts that the way a cell dies influences the immune response, and in particular that necrotic cell death releases inflammatory damage-associated molecular patters (DAMPs) that are contained during programmed cell death (PCD). In the past decade, non-apoptotic forms of PCD have been defined, including necroptosis, a form of death morphologically similar to necrosis. Understanding how different forms of PCD influence the immune response has been hampered by the multiple programs intersecting at a molecular level, and as a result the heterogeneous mixture of cell death phenotypes within a bulk cell population. We have used inducible forms of key apoptotic or necroptotic enzymes to specifically control cell death pathways, and test their impact in immunologically relevant settings. Our study demonstrates that RIPK3-induced necroptotic cells can mediate efficient cross-priming of antigen by dendritic cells in contrast to cells undergoing Caspase-8 induced apoptosis, and vaccination with necroptotic cells induced protective immunity to tumor challenge. Surprisingly, secondary necrotic cells or primary freeze/thaw necrotic cells, despite release of DAMPs, did not induce such a potent CD8+ T cell response, indicating that programmed necrosis provide a unique set of signals that control the cross-priming.

Presenting author: Nader Yatim

Bacterial virulence sensing by the innate immune system mediated by imd RIP proteins

Mamady Diabate, Patrick Munro, Arnaud Jacquel, Gregory Michell, Diogo Goncalves, Sandrine Marchetti, Sandrine Obba, Clara Degos, Patrick Auberger, Jean-Pierre Gorvel, Lynda M. Stuart, Luce Landraud, Emmanuel Lemichez and Laurent Boyer

INSERM U1065 NICE

Innate immune signaling pathways are hard-wired networks that result in activation of transcription factors and expression of the immune effectors essential for pathogen defense in many species. These pathways are triggered by pattern recognition receptors (PRRs) that recognize invariant molecular patterns expressed by microbes. Although the ligands that stimulate these receptors are shared between avirulent and virulent strains, the host demonstrates a remarkable capacity to tailor the response to the virulence of the invading microorgansim. However, how the innate immune system recognizes the array of structurally diverse virulence factors to achieve this specificity remains obscure. Using Drosophila as a tractable system, we have set out to identify defense mechanisms that respond to microbial virulence, focusing on those that target the RhoGTPases. We demonstrate that toxin induced activation of Rac is sufficient to initiate defense signals in the absence of other bacterial components and identify a conserved Rac-dependent immune pathway that signals via the adaptor proteins IMD and Rip1/2 to initiate these signals in flies and mammals respectively. Further, we demonstrate the capacity of the host to detect the activity of the RhoGTPase targeting toxin CNF1 of Escherichia coli during mice bacteremia. This mechanism of immune surveillance, based on monitoring the activity of virulence factors, provides a framework for a recognition system able to deal with the large number of highly varied microbial toxins targeting RhoGTPases. We anticipate that other targets of microbial virulence determinants will be guarded and that this is an evolutionarily conserved means by which pathogenicity is detected.

Presenting author: Laurent Boyer

Evidence for the presence of the phagocytic machinery in the adipose tissue

Lily Boutens, Jiska van der Reest, Alexandra Helmke, Rinke Stienstra

Wageningen University

Background: The chronic inflammatory state that develops in adipose tissue (AT) during obesity is generally considered to promote insulin resistance (IR) and Type 2 Diabetes (DMII). Obese, inflamed AT is characterized by the presence of dead adipocytes surrounded by inflammatory macrophages, forming so-called Crown-like structures (CLS). These CLS might be suggestive of ineffective phagocytosis of dead adipocytes by macrophages during obesity. Within healthy tissue, immunologically silent clearance of dead cells by macrophages is known to be an essential process to maintain tissue homeostasis. We hypothesize that the clearance of dead adipocytes by macrophages is ineffective in obese AT and might importantly contribute to the development of AT inflammation. Objective: We aim to unravel the metabolic and immunologic (dys)regulation related to the phagocytic response by macrophages in the AT, in order to better understand the development of AT inflammation during obesity and find novel leads for therapeutic targets to treat obesity-induced AT inflammation. Methods: In vivo, C57Bl/6 mice were fed a High fat diet (HFD) or Low Fat diet (LFD) to promote IR and DMII. (Regulation of) Phagocytosis was studied in vitro using a co-culture system of macrophages and apoptotic adipocytes or AT explants. Flow Cytometry was used to determine phagocytosis of apoptotic adipocytes by macrophages. Quantitative PCR, Western Blotting, ELISA and Microarray were used to unravel the metabolic and immunologic response of macrophages. Results: Microarray analysis of AT from mice revealed differential regulation of genes that are known to play crucial roles in phagocytosis (Mertk, Gas6, Mfge8, Lrp, Gulp1, Elmo1 and Ucp2) upon the development of obesity, accompanied by changes in expression of apoptosis- (Caspases, Bax) and inflammation-related (Il-1β, Nlrp3) genes. In vitro, macrophages co-cultured with apoptotic adipocytes had markedly increased intracellular lipids, suggestive of phagocytosis of apoptotic adipocytes by macrophages. Upon phagocytosis of apoptotic adipocytes, genes involved in lysosomal lipid breakdown (Lipa) and fatty acid oxidation (Ppara, Pgc1a, Cpt1) were higher expressed and macrophages were skewed towards an anti-inflammatory phenotype marked by respectively higher and lower expression and secretion of IL-10 and TNFα. Interestingly, co-culturing macrophages with AT explants from lean mice led to a two-fold increase in their phagocytic capacity, accompanied by a gene expression pattern closely mimicking the regulation found in macrophages that phagocytised dead adipocytes. Conclusion: Our data suggest that the phagocytic machinery is present in AT and is differentially regulated in obese, inflamed AT. Interestingly, macrophages are capable of phagocytising apoptotic adipocytes in vitro and AT-derived factors stimulate the phagocytic machinery of macrophages and enhance their phagocytic capacity.

Presenting author: Lily Boutens

Poster abstracts

Abstracts are in alphabetical order by last name of the presenting author

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An NF-kB-independent role of IKKs in regulating RIPK1 killing potential

Breyne Koen P-2

Non-classical proIL-1beta activation during mammary gland infection is pathogen-dependent but caspase-1 independent.

Chiocchia Gilles P-3

FADD, a new protein with unconventional secretion under the control of the NALP3 inflammasome

De Trez Carl P-4

Trypanosomosis-induced inflammation control requires sequential IL-10 production by multiple cellular sources to avoid a pro-inflammatory cytokine storm leading to premature host and B cell death

Demon Dieter P-5

Caspase-11 is expressed in the colonic mucosa and protects against dextran sodium sulphate-induced colitis

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FAS, but not apoptosis, plays a pivotal role in the in vivo human cutaneous leishmaniasis transcriptome

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Flagellin-induced NLRC4 phosphorylation primes the inflammasome for activation by NAIP5

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Caspase 4 mediates non-canonical activation of the NLRP3 inflammasome in human cells

An NF-kB-independent role of IKKs in regulating RIPK1 killing potential

Yves Dondelinger1,2, Sandrine Jouan-Lanhouet1,2, Tatyana Divert1,2, Emmanuel Dejardin3, Peter Vandenabeele1,2 and Mathieu JM Bertrand1,2

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3Unit of Molecular Immunology and Signal Transduction, University of Liège, Liège, Belgium

TNF is a pleiotropic cytokine that can paradoxically induce both cell survival and cell death. In most cell types, activation of TNFR1 by TNF does not induce cell death but instead leads to the NF-kB-dependent transcriptional upregulation of genes encoding pro-survival molecules that inhibit activation of the death pathway. Accordingly, when the NF-kB response is inhibited, either by expression of a proteasomal degradation-resistant mutant of IkBa or by the use of the general translation inhibitor cycloheximide (CHX), TNFR1 activation switches from a pro-survival to a pro-apoptotic response. Under these conditions, TNF-mediated death was shown not to depend on RIPK1. Cellular inhibitor of apoptosis 1 and 2 (cIAP1/2) are required for TNF-dependent NF-kB activation and, as a consequence, their depletion also induces a switch to apoptosis. However, under these conditions, TNF-mediated death was shown to rely on RIPK1 kinase activity, highlighting existence of another cell death checkpoint in the TNFR1 apoptotic pathway. It has been postulated that the contribution of RIPK1 to the killing process was controlled by its ubiquitylation status at the receptor complex, a process regulated by cIAP1/2. We demonstrate in this study that depleting IKKa/b also induces RIPK1-dependent apoptosis without affecting RIPK1 ubiquitylation status. We show that IKKa/b directly phosphorylate RIPK1, and that inhibiting IKKa/b kinase activities allows assembly of a caspase-8/RIPK1 death complex. Importantly, we demonstrate that the role of IKKa/b in regulating RIPK1 killing potential is NF-kB-independent, therefore demonstrating an unexpected new function of IKKa/b.

Presenting author: Mathieu Bertrand

Non-classical proIL-1beta activation during mammary gland infection is pathogen-dependent but caspase-1 independent.

Koen Breyne1*, Steven K. Cool2, Dieter Demon3, Kristel Demeyere1, Tom Vandenberghe4, Peter Vandenabeele4, Harald Carlsen5, Wim Van Den Broeck6, Niek N. Sanders2, Evelyne Meyer1

1Laboratory of Biochemistry, Faculty of Veterinary Medicine, Ghent University, 9820 Merelbeke, Belgium. 2Laboratory of Gene Therapy, Faculty of Veterinary Medicine, Ghent University, 9820 Merelbeke, Belgium. 3Department of Medical protein research, V

Infection of the mammary gland with live bacteria elicits a pathogen-specific host inflammatory response. To study these host-pathogen interactions wild type mice, NF-kappaB reporter mice as well as caspase-1 and IL-1beta knockout mice were intramammarily challenged with Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus). The murine mastitis model allowed to compare the kinetics of the induced cytokine protein profiles and their underlying pathways. In vivo and ex vivo imaging showed that E. coli rapidly induced NF-kappaB inflammatory signaling concomitant with high mammary levels of TNF-alpha, IL-1 alpha and MCP-1 as determined by multiplex analysis. In contrast, an equal number of S. aureus bacteria induced a low NF-kappaB activity concomitant with high mammary levels of the classical IL-1beta fragment. These quantitative and qualitative differences in local inflammatory mediators resulted in an earlier neutrophil influx and in a more extensive alveolar damage post-infection with E. coli compared to S. aureus. Western blot analysis revealed that the inactive proIL-1beta precursor was processed into pathogen-specific IL-1beta fragmentation patterns as confirmed with IL-1beta knockout animals. Additionally, caspase-1 knockout animals allowed to investigate whether IL-1beta maturation depended on the conventional inflammasome pathway. The lack of caspase-1 did not prevent extensive proIL-1beta fragmentation by either of S. aureus or E. coli. These non-classical IL-1beta patterns were likely caused by different proteases and suggest a sentinel function of IL-1beta during mammary gland infection. Thus, a key signaling nodule can be defined in the differential host innate immune defense upon E. coli versus S. aureus mammary gland infection, which is independent of caspase-1.

Presenting author: Koen Breyne

FADD, a new protein with unconventional secretion under the control of the NALP3 inflammasome

Benjamin Even (1,2,3), Lucas Treps (1,2,3), Alain Schmitt (2,3,4), Sylvie Mistou (1,2,3), Gilles Chiocchia (5) and Léa Tourneur (1,2,3)

1) INSERM U1016, Institut Cochin, Département Infection-Immunité-Inflammation, Paris, France; 2) CNRS UMR 8104, Paris, France; 3) Université Paris Descartes, Sorbonne Paris Cité, Paris, France; 4) Plateforme de microscopi

FADD (Fas-Associated Death Domain) is the key adaptor molecule mediating apoptosis through death receptors. Moreover, FADD is a multifunctional protein implicated in numerous non-apoptotic processes including autophagy, innate immunity, cancer development and inflammation. We have previously shown that FADD protein expression could be lost in cancerous cells in mice and humans, and be used as prognostic factor. Using in vitro-cultured mouse organ models, we have demonstrated that loss of FADD occurred through a new regulatory pathway of FADD expression by secretion. Thereafter, we have shown that FADD protein was released by human biopsies from lung cancer and that FADD secretion correlates positively with both tumor progression and aggressiveness. We then decided to decipher the molecular mechanisms accounting for FADD secretion. We first performed a screening of human cell lines and found that the THP1 human monocytic cell line secreted spontaneously the FADD protein, whereas U937, Jurkat or K562 cell lines did not. Using the THP1 cell line model, we showed that the FADD secretion process is resistant to the ER-Golgi transport inhibitor Brefeldin A. Thus, FADD is a new member of the small family of proteins secreted by unconventional secretion pathway that includes the well characterized IL-1beta. Most of non-classically exported proteins exhibit spontaneous release from the cells. However, a group of them usually require cell stress to be exported. We cultured THP1 cells under hypoxic conditions but no increase in the FADD secretion process was observed. We next treated THP1 with Nigericin, a NALP3 inflammasome activator known to induce unconventional IL-1beta secretion. We showed that Nigericin increased FADD release from THP1 cells in a dose dependent manner. Moreover BAPTA-AM, that block the intracellular calcium increase necessary to the NALP3 inflammasome activation, inhibited by half the FADD secretion by THP1 cells. Thus, the FADD secretion process is under the control of the NALP3 inflammasome in human THP1 monocytic cell line. Until now, 4 non-classical secretory pathways have been described: exosome-mediated secretion; membrane blebbing; endolysosomal pathway; and translocation through the plasma membrane. It was shown that induction of autophagy promotes inflammasome-dependent IL-1beta secretion. We tested the effect of co-treatment of Nigericin and pp242, an inducer of autophagy, on the FADD secretion process. Preliminary results showed no increase of FADD secretion by co-treatment, suggesting that the endolysosomal pathway would not be involved in FADD release by THP1 cells. We used FADD specific immunogold electron microscopy to show the presence of FADD in microvesicles measuring from 100 nm to 1 µm of diameter in the culture medium from THP1 cells. Using differential centrifugation followed by flow cytometry analysis, we showed that THP1 cells' culture medium contained microvesicles that derived from the plasma membrane and exosomes, this second type of vesicles being less abundant. Furthermore, ELISA performed on the microvesicular and exosomal fraction confirmed that FADD was contained within this type of vesicles. In conclusion, we have begun to identify the unconventional secretory pathway accounting for FADD secretion. In human THP1 monocytic cell lines, FADD is mainly secreted within microvesicles that derived from the plasma membrane and perhaps within exosomes. Further analysis will be necessary to confirm these results that could have strong impact as FADD secretion represent a therapeutic target in some cancer and probably inflammatory diseases.

Presenting author: Gilles Chiocchia

Trypanosomosis-induced inflammation control requires sequential IL-10 production by multiple cellular sources to avoid a pro-inflammatory cytokine storm leading to premature host and B cell death

De Trez Carl, Cnops Jennifer, Stefan, Magez

Research Unit of Cellular and Molecular Immunology, Vrije Universiteit Brussel, Brussels, Belgium; VIB Department of Structural Biology, Brussels, Belgium

Nowadays inflammation has been considered as a double-edged sword in many models. In the context of pathogen infections, it mainly plays a beneficial and important role in the initiation of a protective immune response. However, if not controlled, it can be detrimental, causing multiple collateral body damages and potentially lead to death. Trypanosoma brucei (T. brucei) parasites are extracellular protozoan hemoflagellates causing African trypanosomosis in Human and vertebrates animals by the bite of infected tsetse flies (genus Glossina). About 60 million people are at risk and Nagana, the animal disease, causes three million cattle deaths every year due to fever, weight loss and anemia. The associated economic loss in livestock production is estimated at 4 billion USD/year. While the control of inflammation following T. brucei infection is mandatory as IL-10-deficient mice succumb from an inflammatory cytokine storm within 10 days post-infection, the relevant cellular source of IL-10 and the associated molecular mechanisms implicated in its production are still poorly understood. Using an IL-10 reporter mouse line, we traced the early IL-10 production in trypanosomosis and demonstrated that NK cells and both CD8+ and CD4+ T cells are the main cellular sources of IL-10 within the spleen and liver at day 6 post-infection. This early detection of IL-10 coincides with the peak of pro-inflammatory cytokine production occurring in the blood at the peak of parasitemia. Nine days after infection, the cellular source of IL-10 is still similar in the liver, whereas, surprisingly, the main of IL-10-producing cells is constituted of splenic plasma B cells. Similar results were confirmed following physiological infection of IL-10-deficient and IL-10 reporter mice with T. brucei-bearing tsetse flies. Preliminary observations suggest a correlation between the expression of the transcription factor Blimp-1 and IL-10 detection. We also showed that the early production of IL-10 is required to counterbalance inflammation to avoid accelerated immature and mature B cell death, which is a hallmark of trypanosomosis. Together these data suggest that T. brucei-mediated sequential IL-10 production plays a key role in controlling inflammation-mediated collateral damages, which ultimately lead to host survival.

Presenting author: Carl De Trez

Caspase-11 is expressed in the colonic mucosa and protects against dextran sodium sulphate-induced colitis

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Ulcerative colitis and Crohn's disease are major inflammatory intestinal syndromes that affect millions of patients. The inflammatory cysteine protease caspase-11 confers protection against Gram-negative enteropathogens by inducing pyroptotic cell death and by promoting maturation and secretion of the proinflammatory cytokines interleukin (IL)-1beta and IL-18. However, whether caspase-11 plays a role during colitis is not known. Here, we show that caspase-11 was constitutively expressed in the colon, and that caspase-11-deficient (caspase-11-/-) mice were hypersusceptible to dextran sodium sulfate (DSS)-induced colitis, a mouse model that mimics the environmental and genetic aspects of the human pathology. Notably, pro-inflammatory Prevotella species were strongly reduced in the gut microbiota of caspase-11-/- mice. Co-housing with wildtype mice leveled Prevotella contents, but failed to protect caspase-11-/- mice from increased susceptibility to DSS-induced colitis. We therefore addressed the role of caspase-11 in immune signaling. DSS-induced tissue damage and inflammatory cell infiltration in the gut were markedly increased in caspase-11-/- mice, while release of the pyroptosis/necroptosis marker HMGB1 was abolished. Moreover, caspase-11-/- mice showed normal or increased production of mature interleukin (IL)-1β and IL-18, whereas IL-1β and IL-18 secretion was blunted in animals lacking both caspases 1 and 11. In conclusion, we showed that caspase-11 shapes the gut microbiota composition, and that caspase-11-deficient mice are highly susceptible to DSS-induced colitis. Moreover, DSS-induced inflammasome activation relied on caspase-1, but not caspase-11. These results suggest a role for other caspase-11 effector mechanisms such as pyroptosis in protection against intestinal inflammation.

Presenting author: Dieter Demon

FAS, but not apoptosis, plays a pivotal role in the in vivo human cutaneous leishmaniasis transcriptome

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We report the first systemic in vivo gene signature in human cutaneous leishmaniasis, a neglected infectious disease, despite the 1.5-2.0 million new cases every year. Using a systems biology approach, we identified Fas as the most robust and most significantly upregulated mRNA in PBMCs of cutaneous leishmaniasis patients (n=18), as compared to healthy controls (n=12). We introduce Transcriptome-wide Correlation Analysis (TraCA) as a powerful tool to discover linear relationships between molecular and clinical features in transcriptomic data. TraCA revealed both STAT1 and Sp1 transcription factors (known to bind FAS promoter) as positively correlated (following FDR correction) to FAS mRNA levels in cutaneous leishmaniasis, but not healthy controls, validating our approach. Pathway analysis revealed phagosome as significantly associated to FAS mRNA. A significant increase in ex vivo Fas expression was confirmed at the protein level, by flow cytometry for membrane Fas ($p<0.01$ for neutrophils, lymphocytes and monocytes) and by ELISA for soluble Fas protein (sFas), when comparing an independent cohort of patients and healthy controls. Surprisingly, the profound increase in Fas levels occurred in the absence of detectable ex vivo apoptosis and was not correlated to active caspase-3 or cleaved PARP1 protein levels. Finally, sFas TraCA revealed inflammation/tissue destruction, oxidative burst, phagosome-related intracellular machinery and apoptosis as significantly associated pathways. Conclusions: TraCA captures the broader part of the pathologically relevant disease associated pathways, when compared to the actual disease gene signature. Our data confirm a central role for Fas, mostly apoptosis-independent, in the in vivo transcriptome and pathogenesis of human cutaneous leishmaniasis.

Presenting author: Tim Dierckx

Melphalan induced melanoma cell death favors danger signaling and inflammation-associated immunogenicity

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Systemically-applied chemotherapeutics in general are considered to cause immunosuppression and induce tolerogenic apoptosis. Recently, certain agents like anthracyclines, have been showed to increase cancer cell death immunogenicity and evoke danger signaling stimulating a protective anti-tumor immune response. Whether this applies to (loco-)regionally applied therapies, like Melphalan (Mel)-based isolated limb perfusion (Mel-ILP) for extremities-associated melanoma, has not been investigated yet. Here we show that in human melanoma biopsies, Mel-ILP caused upregulation of IL1B, IL8 and IL6, which was associated with the rapid (1 hr) release of IL6 and IL1β detectable in patients' loco-regional sera. In line with the stimulation of a pro-inflammatory microenvironment, in vitro analysis showed that Mel induces melanoma apoptosis associated with endoplasmic reticulum (ER) stress and ROS, intracellular pathways favoring induction of danger signaling. Mel treatment induced caspase-dependent surface exposure of HSP90 also reliant on proximal ER stress and ROS induction, in the absence of calreticulin exposure and ATP secretion. Mel-treated human melanoma cells elicited induction of semi-mature DCs and partial T cell activation. Moreover, in vivo prophylactic immunization showed that Mel-treated cancer cells were able to stimulate a protective anti-tumor response, although to a lesser extent than Hypercin-based photodynamic therapy, a well known strong inducer of immunogenic cell death. All together, these results unravel the ability of Mel as loco-regional treatment to induce a melanoma cell death modality entailing basal pro-inflammatory/immunogenic features both in vitro and in vivo, which could be further augmented by designing appropriate combinatorial immunotherapy.

Presenting author: Aleksandra Dudek-Perić

RIPK1 promotes death ligand-independent caspase-8-mediated apoptosis under unresolved ER stress conditions

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Accumulation of unfolded proteins in the endoplasmic reticulum (ER) causes ER stress and results in the activation of the unfolded protein response (UPR), which aims at restoring ER homeostasis. However, when the stress is too severe the UPR switches from being a pro-survival response to a pro-death one, and the molecular mechanisms underlying ER stress-mediated death have remained incompletely understood. In this study, we identified receptor interacting protein kinase 1 (RIPK1) - a kinase at the crossroad between life and death downstream of various receptors - as a new regulator of ER stress-induced death. We found that Ripk1 deficient MEFs are protected from apoptosis induced by ER stressors, which is reflected by reduced caspase activation and PARP processing. Interestingly, the pro-apoptotic role of Ripk1 is independent of its kinase activity, is not regulated by its cIAP1/2-mediated ubiquitylation, and does not rely on the direct regulation of JNK or CHOP, two reportedly main players in ER stress-induced death. Instead, we found that ER stress-induced apoptosis in these cells relies on death ligand-independent activation of caspase-8, and identified Ripk1 upstream of caspase-8. However, in contrast to RIPK1-dependent apoptosis downstream of TNFR1, we did not find Ripk1 associated with caspase-8 in a death-inducing complex upon unresolved ER stress. Our data rather suggest that RIPK1 indirectly regulates caspase-8 activation, in part via interaction with the ER stress sensor inositol-requiring protein 1 (IRE1).

Presenting author: Yann Estornes

The BCG SapM transposon mutant as vaccine against tuberculosis: Less is more.

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One-third of the world's population is infected with Mycobacterium tuberculosis, the causative agent of tuberculosis (TB). M. bovis Bacille Calmette Guérin (BCG), the only licensed vaccine against TB, shows variable efficacy in protection against adult TB. Development of a better vaccine is hampered by the lack of reliable correlates of protection against TB. We have described a BCG transposon mutant in the secreted acid phosphatase SapM, which leads to prolonged survival of mice infected with M. tuberculosis. Previous observations indicated that more effective recruitment of inflammatory dendritic cells (iDCs) towards the draining lymph nodes might account for superior protection by the SapM::Tn mutant in Balb/c mice. Studies in C57BL/6 mice now show that the kinetics of iDC recruitment differ upon BCG SapM::Tn and wild-type vaccination: iDC recruitment to lymphoid organs starts earlier when mice are vaccinated with the BCG SapM::Tn strain. BCG SapM::Tn is cleared better than wild-type BCG after vaccination and, intriguingly, induces reduced frequencies of IFNγ-producing CD4+ and CD8+ T cells. While the emphasis of TB vaccine research the last 100 years has been mainly on inducing a strong IFNγ cytokine response, recent data weaken the status of IFNγ as hallmark of protection. Our data also support the idea that we need to reevaluate IFNγ induction as read-out of a good TB vaccine. We are currently investigating how enhanced BCG SapM::Tn clearance and induction of lower IFNγ levels lead to improved vaccine efficacy.

Presenting author: Nele Festjens

Vaccination-resistant cancer phenotype is promoted by intrinsic defect in immunogenic cell death regulated by surface exposed calreticulin

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Over last ten years, immunogenic cell death (ICD) has been established as an important paradigm in anticancer immunotherapy. ICD through spatiotemporally-defined emission of 'danger signals', stimulates anticancer vaccination effect (AVE). Recently, mitoxantrone (MTX) and hypericin-based photodynamic therapy (Hyp-PDT) have been characterised as two of the most potent ICD or AVE inducers. Our recent metadata analysis hinted at the possible existence of therapy-resistant phenotypes in cancer capable of subverting ICD/AVE. Despite the high relevance of characterising such phenotypes, no naturally-occurring experimental models of intrinsic AVE-resistant phenotypes have been identified so far. Taking cue of our previous observations that, despite effective Hyp-PDT induced debulking of AY27 rat bladder tumours in vivo these tumours relapsed rapidly (a sign of anticancer immunity failure); we decided to understand whether the AY27 bladder cancer model represents a naturally-occurring phenotype model of vaccination-resistance. To this end, the efficacy of Hyp-PDT or MTX mediated vaccination efficacy was checked in an AY27-Fischer rat model while comparing with CT26-BALB/c mice model (a well-established ICD/AVE model). As expected, a significant number of mice vaccinated with Hyp-PDT or MTX-killed CT26 cells, rejected CT26-tumours upon rechallenge. However strikingly, none of the rats vaccinated with Hyp-PDT/MTX-killed AY27 cells could reject AY27-tumours. Next, we analysed various ICD or AVE relevant danger signals in vitro. Both CT26 and AY27 dying cells exhibited significant secretion of ATP and release of HSPs/HMGB1 in response to Hyp-PDT or MTX. However, in stark contrast, while the murine CT26 cells exhibited surface CRT, yet rat AY27 cells failed to expose surface CRT. This, despite the fact that, the AY27 cells exhibit an intact ER stress response, both in vitro (for MTX or Hyp-PDT) as well as in vivo (for Hyp-PDT). Interestingly, exogenous addition of rCRT, significantly rescued the ability of Hyp-PDT or MTX to induce AVE in the AY27 rat model. Of note, a retrospective clinical metadata analysis for ovarian and lung cancer patients, revealed that under therapeutic contexts known to associate with surface CRT, cancer patients exhibiting high CRT expression show better survival than patients with low CRT expression. In conclusion, we have characterised for the first time, a naturally-occurring experimental model of cancer cell-autonomous resistance to vaccination due to intrinsic defect in surface CRT mediated ICD – an observation with possible clinical implications.

Presenting author: Abhishek D. Garg

α-Galactosylsphingamides as novel NKT-cell ligands

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NKT-cells are a unique subset of T-lymphocytes that exert a crucial role in host immunity. They recognize lipid and glycolipid antigens presented by CD1d on antigen presenting cells. Activation leads to rapid secretion of both Th1 and Th2 cytokines. While Th1 cytokines mediate protective immune functions like tumor rejection, antiviral and antibacterial effects, Th2 cytokines mediate regulatory immune functions to ameliorate autoimmune diseases. The prototypical NKT-cell antigen is α-GalCer, a marine sponge derived galactosylceramide. After α-GalCer administration the secretion of vast amounts of Th1 and Th2 cytokines is observed, which, however only excerts limited clinical benefit. Altering the physicochemical properties of this prototypical antigen or modulating its affinity for CD1d are promising strategies to obtain analogues with a superior cytokine profile. Towards this end, we developed a divergent synthetic pathway towards α-GalCer analogues containing an extra amide bond in the phytosphingosine chain. Since the amide moiety is only formed in the final stages of the synthesis, this pathway opens interesting prospectives to produce α-GalCer analogues with improved properties. [1] P. B. Savage, L. Teyton., A. Bendelac, Chem. Soc. Rev. (2006), 35, 771-779. [2] R. D. Goff, Y. Gao, et al., JACS (2004), 126, 13602-13603

Presenting author: Joren Guillaume

Hantaviruses inhibits two major pathways of apoptosis

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Hantaviruses cause hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardio-pulmonary syndrome (HCPS), two human diseases with high case fatality rates. Endothelial cells are the main targets for hantaviruses and vascular permeability is a hallmark of HFRS/HCPS. An intriguing observation in patients with HFRS/HCPS is that while virus infection leads to strong activation of cytotoxic lymphocytes, CD8 T cells and Natural Killer cells, no obvious destruction of infected endothelial cells occur. Here, we provide a possible explanation for this dichotomy by showing that hantavirus-infected endothelial cells are protected from cytotoxic lymphocyte-mediated killing. Cytotoxic granule dependent pathway is the main way for cytotoxic lymphocytes to kill virus infected cells; we have previously showed that hantavirus infection confers resistance to cytotoxic lymphocyte mediated apoptosis via inhibition of granzyme B and caspase 3 (Gupta et al, PLoS Pathogens 2013). Another key mechanism used by cytotoxic lymphocytes is the death ligand/receptor pathway. Investigation of this pathway showed that hantavirus infection leads to decreased levels of the death receptors DR5, DR3 and TNFR1 on the cell surface. This in turn protected cells from death ligand mediated apoptosis. Our findings provide a tentative explanation for the hantavirus-mediated block of the two major pathways of killing, and hence the protection of infected cells from cytotoxic lymphocytes. These findings may explain why infected endothelial cells in hantavirus-infected patients are not destroyed by the strong cytotoxic lymphocyte response.

Presenting author: Shawon Gupta

Functional characterization of in silico-designed selective probes and inhibitors of mouse caspase-1

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Inflammatory caspases play central roles in inflammatory and host defense responses through the secretion of pro-inflammatory cytokines and induction of pyroptosis. The inflammatory caspase (casp) group comprises casp1, casp11 and casp12 in mice, and their orthologs casp1, casp4 and casp5 in humans. Currently existing caspase probes and inhibitors target multiple inflammatory caspases, indicating that development of specific probes and inhibitors for inflammatory caspases is still a major challenge. Because aberrant activation of inflammatory caspases is associated with inflammatory and autoimmune disorders in humans, the availability of more selective probes and inhibitors would offer tremendous opportunities for diagnostic and therapeutic intervention. In the presented work, we designed novel probes and inhibitors for mouse casp1 by using in silico modeling and computational fitting of amino acids in the caspase binding pockets, combined with biochemical and cell biological validation of derived polypeptide substrates that were predicted to exert high specificity for murine casp1 over other inflammatory caspases. In silico design of selective peptide sequences for murine caspase-1 entailed homology modelling of the mouse inflammatory caspase structures based on the publicly available X-ray structure of human casp1 (PDB entry: ED6M). We subsequently calculated the energy for amino acids binding in the S2-S5 binding pockets of each caspase. Polypeptides assembled from the amino acids with optimal binding energies for the S2-S5 pockets of murine casp1 that simultaneously docked less favourably in the binding pockets of murine casp11 and casp12 were synthesized and conjugated to 7-Amino-4-methylcoumarin (AMC) by click-chemistry. We showed that Ac-FFEMD-AMC was efficiently hydrolysed by recombinant mouse casp1, but not casp11 and casp12. Analysis of the S2-S5 pockets of these inflammatory caspases suggested the P4 position of the peptide to be the critical position determining its specificity for casp1 by causing sterical hindrance in the substrate binding pockets of mouse casp11 and casp12. Unlike the marked specificity noted for mouse casp1 over its murine paralogs, Ac-FFEMD-AMC displayed significant activity towards recombinant human casp1, 4 and 5. Analysis of modelled structures suggested that the P4 position of the peptide to fit well in the corresponding binding pockets of the human inflammatory proteases. Finally, we showed that - in analogy to the AMC-coupled substrate probe – an aldehyde (CHO)-based reversible inhibitor to specifically inhibit recombinant mouse casp1, but not casp11 or casp12 in vitro. Moreover, the CHO-inhibitor significantly reduced casp1 autoprocessing and inflammasome-mediated interleukin-1β and interleukin-18 secretion from nigericin-stimulated bone marrow-derived macrophages. In conclusion, these results suggest rational design of peptide probes and inhibitors as a suitable approach for the development of highly specific caspase probes and inhibitors, and warrant further analysis of the described molecules in in vivo studies.

Presenting author: Daniel Jiménez Fernández

MDSC home to tumor draining lymph nodes and locally modulate the T cell response

Qods Lahmar, Elio Schouppe, Wim Van Grimbergen, Yannick Morias, Damya Laoui, Eva Van Overmeire, Kiavash Movahedi, Patrick De Baetselier, Jo A. Van Ginderachter, and Adelaida Sarukhan

VIB-VUB

Cancer is one of the most important causes of death in industrialized countries. Immunotherapy is a promising therapeutic avenue, but the efficiency of immune-based treatments is severely hampered by the induction of immunosuppressive mechanisms by the tumor. An almost common feature of the tumors is their ability to activate abnormal myelopoeisis, which results in the generation, and accumulation of Myeloid derived suppressor cells or MDSC in different tissues. Although MDSC accumulation has been widely documented in the bone marrow, spleen and tumor microenvironment, very little is known on the presence and function of MDSC in lymph nodes (LN), in particular those draining solid tumors. This question is particularly relevant since T-cell priming occurs within tumor-draining LN (tdLN) and is essential for the initiation and maintenance of effective anti-tumoral responses. Hence, a better understanding of the MDSC interference with T-cell responses would provide novel opportunities for therapeutic applications. Our study aim to explore the suppressive activity of MDSC in tumor bearing mice, first by analyzing their impact on CD8+ T-cell activation and then by addressing the eventual effect of MDSC on dendritic cells function. Here, we show that tdLN contain 20-fold more MDSC than naïve lymph nodes, and moreover, that the MO-/PMN-MDSC (MO-: monocytic/ PMN-: polymorphonuclear MDSC) ratio was completely inverted in tdLN as compared to the spleen of tumor-bearing mice. Although tdLN MO-MDSC phenotypically correspond to splenic MO-MDSC, they are more immunosuppressive. Finally, we also show that the inverted ratio is due a preferential migration of MO-MDSC to LN as compared to PMN-MDSC and that tumor proximity enhances MO-MDSC homing by yet undefined mechanisms. Altogether, our findings open up new perspectives for anti-cancer therapeutic application.

Presenting author: Qods Lahmar

Tumors are infiltrated with ontogenically and functionally distinct tumor-associated dendritic cell subpopulations

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Tumors should be considered as organ-like structures encompassing different intratumoral microenvironments. Hence, a plethora of environmental stimuli regulates the recruitment and differentiation of immune cells inside tumors. We previously described the heterogeneity of tumor-infiltrating macrophages, but up to now no evidence for dendritic cell (DC) heterogeneity in tumors existed. Here, we demonstrated that murine tumors of different histological origins, either heterotopically or orthotopically transplanted or spontaneously developed, harbor discrete tumor-associated DC (TADC) subsets. Using a combination of CD24, CD11b, CD64, Ly6C and MAR-1, we uncovered that tumors contain CD8α+-like conventional DC (cDC), CD11b+-like cDC and monocyte derived DC (Mo-DC). The ontogenic differences between the distinct TADC subsets were further corroborated by growing tumors in mice deficient for CCR2 or Flt3L, which lack Mo-DC and CD8α+-like cDC, respectively. Moreover, the distinct TADC subsets differed at the functional level. Mo-DC were characterized by a high inherent phagocytic capacity and T-cell suppressive potential through their high production of NO, while the cDC subsets were able to stimulate CTL proliferation and displayed migratory potential. Together, our data provide the first evidence for the complexity of the TADC compartment and might prove important for therapeutic interventions targeted at specific TADC subsets or their precursors.

Presenting author: Damya Laoui

Gastric de novo Muc13 expression and spasmolytic polypeptide-expressing metaplasia during Helicobacter heilmannii infection

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Helicobacter heilmannii is a zoonotic bacterium that has been associated with gastric disease in humans. The agent is highly prevalent in cats and dogs which most likely are the main source of infection for humans. In this study, mRNA expression of mucins was analyzed at several time-points in the stomach of BALB/c mice during a one year infection with H. heilmannii in which gastric disease progressed from gastritis to low grade mucosa associated lymphoid tissue (MALT) lymphoma-like lesions and mucous metaplasia. Markers for acid production by parietal cells and mucous metaplasia were also examined. We first tested the induction of inflammation by and colonization capacity of H. heilmannii ASB1 and H. pylori SS1. At all time-points, inflammation was observed mainly in the fundus and inflammation in ASB1.4- and SS1-infected mice was characterized by mononuclear and polymorphonuclear cell infiltration in the lamina propria mucosae, the tunica submucosa or both, depending on the individual animal. From 24 weeks post-infection onwards, large lymphoid aggregates of mononuclear and/or polymorphonuclear cells were mainly seen in a narrow zone in the fundus near the forestomach/stomach transition zone of both Helicobacter-infected mice. In mice infected with ASB1.4 and SS1 for at least 34 weeks, B-cell containing germinal centers were seen in those large lymphoid aggregates. In several mice infected with ASB1.4 and SS1 for 52 weeks, numerous lymphoepithelial MALT-lymphoma like lesions could be detected in the gastric mucosa. These were most abundant in a narrow zone in the fundus near the forestomach/stomach transition zone. In all Helicobacter-infected mice, mild signs of inflammation were also detected in the antrum of the stomach and the duodenum at 52 weeks post-infection. At all time-points, Helicobacter DNA was found in both the antrum and fundus of the stomach from all infected animals. H. pylori and H. heilmannii DNA was found in the duodenum from 3 and 12 weeks post-infection onwards, respectively In the first 9 weeks post-infection, mRNA expression of Muc6 was clearly upregulated in both the antrum and fundus of the stomach. Interestingly, Muc13 was also upregulated in the stomach in this early stage of infection. The positive correlation found between the increased Muc6 and Muc13 expression and the increased number of Helicobacter bacteria in the fundus of the stomach, suggests a potential role for these mucins in H. heilmannii colonization. In addition, the expression level of Muc13 remained high in the stomach over the course of the infection. At 16 weeks post-infection, mRNA expression of H+/K+-ATPase α/β and KCNQ1 decreased whereas the expression of Muc4, Tff2, Dmbt1 and PigR increased suggesting the existence of spasmolytic polypeptide-expressing metaplasia in the fundus of the stomach. Mucous metaplasia present in the mucosa surrounding low grade MALT lymphoma-like lesions was also histologically confirmed. Our findings indicate that H. heilmannii infection causes severe gastric pathologies, alterations in the expression pattern of gastric mucins as well as a disruption in the gastric homeostasis by inducing loss of parietal cells resulting in the development of mucous metaplasia.

Presenting author: Cheng Liu

Role of A20 in the restriction of bacterial infection

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A20 plays a key role in the control of inflammation and immunity and is best known as a negative regulator of NF-κB signaling, where it is believed to function as a dual ubiquitin-modifying enzyme that exerts deubiquitinase as well as E3 ubiquitin ligase activity. To better understand the function and regulation of A20, we performed a yeast two-hybrid screening to identify several novel A20 interacting proteins. This led to the identification of NDP52 as a novel A20 binding protein. Mutation analysis showed that the binding is mediated via the LIR region of NDP52 and the zinc finger containing domain of A20. NDP52 is best known for its role as an autophagy receptor that recognizes ubiquitinated Salmonella bacteria in the cytosol and delivers them to autophagosomes for degradation. The observed A20-NDP52 interaction prompted us to analyze the role of A20 in Salmonella infection in HeLa epithelial cells. Inhibition of A20 expression in these cells by esiRNA mediated RNA interference did not affect the number of intracellular bacteria at early time points post infection but resulted in a significant higher number of bacteria at later time points. Similar results were obtained in mouse embryonic fibroblasts derived from A20 knockout mice. Interestingly, immunostaining revealed a lower number of ubiquitin-coated bacteria in A20-deficient cells. These data indicate that A20 restricts the growth of intracellular bacteria, possibly by increasing their ubiquitination, recognition by an autophagy receptor such as NDP52, and autophagic clearance. Paradoxically, western blotting for the autophagosomal marker LC3-II showed an increased number of autophagosomes in the absence of A20, which may indicate an additional defect in the fusion of autophagosomes to lysosomes. Altogether, our findings demonstrate a complex role for A20 in the combat of intracellular bacteria. Future work will focus on the characterization of the underlying molecular mechanisms.

Presenting author: Marie Lork

Flagellin-induced NLRC4 phosphorylation primes the inflammasome for activation by NAIP5

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The Nlrc4 inflammasome contributes to immunity against intracellular pathogens that express flagellin and type III secretion systems. Both Naip5 and phosphorylation of Nlrc4 at Ser533 mediate flagellin-induced inflammasome activation, but how they converge upon inflammasome activation is not known. Here, we showed that Nlrc4 phosphorylation occurs upstream of Naip5 detection of flagellin because Naip5 deletion in macrophages blocked caspase-1 activation, but not Nlrc4 phosphorylation by S. Typhimurium and cytosolic flagellin. ASC speck formation and caspase-1 expression also were dispensable for Nlrc4 phosphorylation. Interestingly, Helicobacter pylori flagellin induced robust Nlrc4 phosphorylation, but failed to activate caspase-1. This suggests that H. pylori flagellin retained Nlrc4 Ser533 phosphorylation activity despite escaping TLR5 and Naip5 detection. In agreement, Nlrc4 phosphorylation required the flagellin D0 domain, whereas Naip5 detects the flagellin carboxy-terminus. Collectively, this work suggests a biphasic activation mechanism for the Nlrc4 inflammasome in which Ser533 phosphorylation primes Nlrc4 for subsequent activation by the flagellin sensor Naip5.

Presenting author: Magdalena Matusiak

Stabilization of C-type lectin receptors Mincle and MCL through post-transcriptional regulation

Yasunobu Miyake and Sho Yamasaki

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Tuberculosis is a life-threatening infectious disease caused by Mycobacterial tuberculosis. C-type lectin receptor Mincle recognizes mycobacterial glycolipid, TDM (trehalose-dimycolate) and induces anti-mycobacterial responses. We have recently reported that MCL, which shares highly homology with Mincle, is also indispensable for efficient TDM responses. MCL has a lower affinity to TDM and structural analysis of MCL supported the weak affinity to TDM. We therefore speculated that MCL has an important function that is distinct from a direct receptor for TDM. Here we show that the surface expression of Mincle induced by LPS or zymosan was impaired in MCL-deficient mice and enhanced in MCL Tg mice. The stalk region that connects transmembrane domain and carbohydrate recognition domain was necessary and sufficient for the enhancement of Mincle expression. MCL formed a heteromeric complex with Mincle. From these data, MCL may bind to Mincle via stalk region and stabilize it on the cell surface to ensure efficient TDM responses. In conclusion, we have identified a novel function of MCL that modulates Mincle expression post-transcriptionally.

Presenting author: Yasunobu Miyake

The role of apoptotic blebbing in tissue homeostasis and tumour growth

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Efficient apoptotic cell clearance prevents the release of potentially immunogenic intracellular contents into the surrounding environment to maintain tissue homeostasis; however, the importance of membrane blebbing for apoptotic cell recognition and clearance has not been established in vivo. To address fundamental questions regarding the influence of ROCK1 cleavage and consequent apoptotic membrane blebbing in cellular corpse clearance and the maintenance of tissue homeostasis, mice were generated with a single amino acid substitution in the caspase cleavage site (D1113A) that converts ROCK1 to a non-cleavable (ROCK1nc) form. We previously showed that during apoptosis, caspase cleavage of ROCK1 removes an auto-inhibitory carboxy-terminal region to yield a constitutively active kinase fragment that leads to phosphorylation of downstream targets which promote contractile force generation leading to shrinkage, blebbing and nuclear disintegration. When apoptosis was induced in mouse embryonic fibroblasts, the biochemical apoptotic programme itself was unaffected, although cells had significantly impaired morphological alterations. Furthermore, by imaging apoptosis of GFP labelled melanoblasts in ex vivo embryonic skin explants, the effect of the ROCK1nc point mutation was to impair typical apoptotic morphological changes. To understand the biological role of membrane blebbing in the maintenance of tissue homeostasis, we used the liver-selective genotoxic compound diethylnitrosamine (DEN) to induce acute tissue damage. Following DEN treatment, we found that TUNEL positive apoptotic cells were inefficiently cleared in ROCK1nc mice, which resulted in increased neutrophil infiltration in response to the accumulating apoptotic debris. Histologically, the ROCK1nc livers appeared more damaged with evidence of steatohepatitis and perivenular damage, which was accompanied by higher serum alanine transferase levels. We then asked whether defects in apoptotic blebbing in ROCK1nc mice would affect tumour development in a model of DEN-induced hepatocellular carcinoma and a c-myc oncogene driven model of B-cell lymphoma. Interestingly, ROCK1nc mice had reduced liver tumour volumes and numbers, suggesting that defective blebbing may be beneficial for reducing tumour initiation and growth. Liver tumours in ROCK1nc mice also had higher levels of immune cell infiltration. Survival of c-myc expressing mice with B-cell lymphoma was significantly greater for ROCK1nc mutants relative to wild-type mice. Taken together, our results indicate that the influence of apoptotic blebbing on the efficiency of apoptotic cell clearance impacts upon the development and progression of cancer.

Presenting author: Michael Olson

Innate immune response as a novel therapeutic target of brain infarction

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Cerebrovascular disease is the leading cause of death and disability in Japan and most Western countries. Although thrombolytic agents are available until 4.5 hours after the onset of brain infarction, most patients arrive at the hospital after the golden time. Therefore, novel approaches to expand the therapeutic time window are inevitable. An important therapeutic target in brain infarction is the ischemia-induced inflammatory reactions. The inflammatory process occurs in response to the ischemic insult, and the induced expression of cytokines and chemokines, mediators of innate immune response, peaks later than 12 hours. One of the key chemokines is monocyte chemoattractant protein-1 (MCP-1) and overexpression of MCP-1 is reported to exacerbate brain damages. We have revealed that post-ischemic gene transfer of dominant negative MCP-1 inhibits macrophage infiltration into the ischemic area and reduces the infarct volume [1]. The role of lymphocytes in brain ischemia has long been unclear. We have investigated the specific roles of T cell subtypes in brain ischemia using genetically-modified animals. FACS analyses in combination with the adoptive transfer studies revealed that interleukin-17 (IL-17)-producing γδ T cells play a pivotal role in the evolution of brain infarction [2]. In contrast, another study have shown that the regulatory T cells play a protective role in the late stage of brain ischemia and the effect is attributable to the function of IL-10, an anti-inflammatory cytokine [3]. We have found that the early infiltration of macrophages plays important roles in the stimulation of γδT cells via IL-23 production. Furthermore, the upstream activation of macrophages was mediated by the toll-like receptor (TLR) 2 and 4 signaling pathway in the MyD88-dependent manner. Using the dendrite cells from TLR2/4 knock mice, we have identified peroxiredoxin family as a novel damage-associated molecular pattern to stimulate the IL-23 producing macrophage that initiate the early immune responses to exacerbate the ischemic damages [4]. Thus, specific approaches targeting the innate immune responses would lead to the neurovascular protection in brain infarction. References 1. Ooboshi H: Curr Pharm Des 17: 424-33, 2011. 2. Shichita T, Sugiyama Y, Ooboshi H, et al: Nat Med 15: 946-50, 2009. 3. Ooboshi H, Ibayashi S, Shichita T, et al: Circulation 111: 913-9, 2005. 4. Shichita, T et al: Nat Med 18: 911-7, 2012.

Presenting author: Hiroaki Ooboshi

Caspase-1 autoproteolysis is differentially required for NLRP1b and NLRP3 inflammasome function

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Inflammasomes are caspase-1-activating multi-protein complexes. The mouse NLRP1b inflammasome was identified as the sensor of Bacillus anthracis Lethal Toxin (LT) in mouse macrophages from sensitive strains such as BALB/c. Upon exposure to LT, the NLRP1b inflammasome activates caspase-1 to produce mature IL-1β and to induce pyroptosis. Both processes are believed to depend on autoproteolysed caspase-1. In contrast to human NLRP1, mouse NLRP1b lacks a N-terminal PYD domain, indicating that the assembly of the NLRP1b inflammasome does not require the adaptor ASC. LT-induced NLRP1b inflammasome activation was shown to be impaired upon inhibition of potassium efflux, which is known to play a major role in NLRP3 inflammasome formation and ASC dimerization. We investigated whether NLRP3 and/or ASC were required for caspase-1 activation upon LT stimulation in the BALB/c background. The NLRP1b inflammasome activation was assessed in both macrophages and dendritic cells lacking either ASC or NLRP3. Upon LT treatment, the absence of NLRP3 did not alter the NLRP1b inflammasome activity. Surprisingly, the absence of ASC resulted in IL-1β cleavage and pyroptosis, despite the absence of caspase-1 auto-processing. By reconstituting caspase-1/caspase-11-/- cells with a non-cleavable or catalytically mutant version of caspase-1, we directly demonstrated that non-cleavable caspase-1 is fully active in response to the NLRP1b activator LT whereas it is non-functional in response to the NLRP3 activator nigericin. Taken together, these results establish variable requirements for caspase-1 cleavage depending on the pathogen and the responding NLR.

Presenting author: Virginie Petrilli

Breast cancer cells with low Toll-like receptor 9 expression have increased sensitivity to zoledronate

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Toll-like receptor 9 (TLR9) is an innate immunity receptor which recognizes microbial and vertebrate (self) DNA. DNA recognition via TLR9 results in an inflammatory reaction, which eventually leads to Th1-biased adaptive immune attack. In addition of immune cells, TLR9 is widely expressed in several cancers, for example breast cancer, prostate cancer and esophageal cancer.

We have shown that TLR9 has prognostic value in breast cancer, but only among patients that have estrogen receptor, progesterone receptor and HER2-negative, i.e. triple negative tumors (TNBC). Specifically, low tumor TLR9 expression predicts poor breast cancer-specific survival among TNBC patients. Of all breast cancer types, TNBC patients have the worst prognosis as there are no targeted therapies for this group. We have also shown that living cancer cells take up DNA from chemotherapy killed cancer cells and that DNA induces TLR9-mediated invasion. Our preclinical studies suggest that TLR9 expression may affect tumor immunophenotype and contribute to the immunogenic benefit of chemotherapy.

Bisphosphonates (BPs) are widely used inhibitors of bone resorption e.g. in osteoporosis and breast cancer bone metastasis treatment. BPs inhibit the vicious cycle between the bone resorbing osteoclasts and the osteoclast-activating breast cancer cells at the site of bone metastasis. Unlike initially thought, recent studies suggest that BPs may also have direct antitumor effects against breast cancer cells that are residing in soft tissues. Zoledronate (ZOL) has been shown to inhibit angiogenesis, cell invasion, homing of tumor cells to bone marrow, cell adhesion, bone resorption and cell proliferation. ZOL also activates gamma-delta T cells, a T cell subtype which has antitumoral effects.

We have discovered that TNBC cells with decreased TLR9 expression exhibit significantly increased sensitivity to ZOL-induced growth inhibition in comparison to control cells. In vivo, in orthotopic breast tumor-bearing mice, ZOL was more effective against low-TLR9 TNBC tumors than high-TLR9 tumors, indicating a wider possible use for ZOL in addition to bone-related therapy.

ZOL has a very high affinity to bone. Therefore, the bioavailability of ZOL is low. Side-by-side with our free ZOL experiments, we are studying whether tumor-targeted mesoporous silica nanoparticle-bound ZOL would be more effective than the free drug. At the moment, we are comparing free drug and nano-ZOL both *in vitro* and *in vivo*.

Presenting author: Jouko Sandholm

Retinoids mediate the liver X receptor-induced enhancement in apoptotic cell clearance

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Previous work in our laboratory has shown that transglutaminase 2 (TG2) acting as a co-receptor for integrin beta 3 is required for proper phagocytosis of apoptotic cells. In the absence of TG2, SLE-like autoimmunity develops in mice, similarly to other mice characterized by a deficiency in the clearance of apoptotic cells. In the present study we demonstrate that increasing TG2 expression alone in wild-type macrophages is not sufficient to enhance engulfment. However, during engulfment the lipid content of the apoptotic cells triggers the lipid sensing receptor liver X receptor (LXR), which in response upregulates the expression of the phagocytic receptor Mertk and the phagocytosis-related ABCA1, and that of retinaldehyde dehydrogenases leading to the synthesis of a non-classical retinoid. The novel retinoid then contributes to the up-regulation of further phagocytic receptors including TG2 by ligating retinoic acid receptors. Inhibition of retinoid synthesis prevents the enhanced phagocytic uptake induced by LXR ligation. Our data indicate that stimulation of LXR enhances the engulfment of apoptotic cells via regulating directly and indirectly the expression of a range of phagocytosis-related molecules, and its signaling pathway involves the synthesis of a non-classical retinoid. Based on our retinoid analysis this compound might be a dihydro-retinoic acid derivative synthetized via the retinol saturase pathway. Indeed retinol saturase expression is induced following engulfment and our preliminary data indicate that retinol saturase knockout mice develop SLE-like autoimmunity. Supported by by Hungarian grants from the National Research Fund (OTKA K83865, T104228, NK105046), and the TÁMOP 4.2.2.A-11/1/KONV-2012-0023 "VÉD-ELEM" project. The project is implemented through the New Hungary Development Plan co-financed by the European Social Fund and the European Regional Development Fund.

Presenting author: Zsuzsa Szondy

RIPK1 is a guardian of intestinal homeostasis protecting the epithelium against apoptosis

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Receptor interacting protein 1 (RIPK1) plays an essential role in the signaling triggered by Death Receptors and Pattern Recognition Receptors1,2. RIPK1 is believed to function as a node driving NF-κB-mediated cell survival and inflammation as well as caspase-8-dependent apoptotic or RIPK3/MLKL-dependent necroptotic cell death. The physiological relevance of this dual function has remained elusive because of the perinatal death of RIPK1 full knockout mice3. To circumvent this problem, we generated RIPK1 conditional knockout mice, and show that mice lacking RIPK1 in intestinal epithelial cells (IEC, Ripk1IEC-KO mice) spontaneously developed severe intestinal inflammation associated with IEC apoptosis leading to early death. This early lethality was rescued by antibiotics treatment, MyD88 deficiency or TNF receptor 1 deficiency, demonstrating the importance of commensal bacteria and TNF in the Ripk1IEC-KO phenotype. Caspase-8 deficiency, but not RIPK3 deficiency, rescued the inflammatory phenotype completely, indicating the indispensable role of RIPK1 in suppressing caspase-8-dependent apoptosis but not RIPK3-dependent necroptosis in the intestine. RIPK1 kinase-dead knockin mice did not exhibit any sign of inflammation, suggesting that RIPK1-mediated protection resides in its kinase-independent platform function. Depletion of RIPK1 in intestinal organoid cultures sensitized them to TNF-induced apoptosis, confirming the in vivo observations. Unexpectedly, TNF-mediated NF-κB activation remained intact in these organoids. Our results demonstrate that RIPK1 is essential for survival of IECs, ensuring epithelial homeostasis by protecting the epithelium from caspase-8-mediated IEC apoptosis independently of its kinase activity and NF-κB activation.

Presenting author: Nozomi Takahashi

Activation of the NLRP1b inflammasome independently of ASC-mediated caspase-1 autoproteolysis and speck formation

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Despite its clinical importance in infection and autoimmunity, the activation mechanisms of the NLRP1b inflammasome remain enigmatic. Here, we show that deletion of the inflammasome adaptor ASC in BALB/c mice and in C57BL/6 macrophages expressing a functional NLRP1b prevents anthrax lethal toxin (LeTx)-induced caspase-1 autoproteolysis and speck formation. However, ASC-/- macrophages undergo normal LeTx-induced pyroptosis and secrete significant amounts of interleukin (IL)-1β. In contrast, ASC is critical for caspase-1 autoproteolysis and IL-1β secretion by the NLRC4, NLRP3 and AIM2 inflammasomes. Notably, LeTx-induced inflammasome activation is associated with caspase-1 ubiquitination, which is unaffected in ASC-deficient cells. In vivo, ASC-deficient mice challenged with LeTx produce significant levels of IL-1β, IL-18 and HMGB1 in circulation, although caspase-1 autoproteolysis is abolished. As a result, ASC-/- mice are sensitive to rapid LeTx-induced lethality. Together, these results demonstrate that ASC-driven caspase-1 autoprocessing and speck formation are dispensable for activation of caspase-1 and the NLRP1b inflammasome.

Presenting author: Nina Van Opdenbosch

Macrophage dynamics in injured pancreas is regulated by local macrophage proliferation and monocyte recruitment

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Pancreas injury by partial duct ligation (PDL) activates a healing response, encompassing β-cell neogenesis and proliferation. Macrophages were recently shown to promote β-cell proliferation after PDL, but they remain poorly characterized. We assessed myeloid cell diversity and the factors driving myeloid cell dynamics following acute pancreas injury by PDL. In naive and sham-operated pancreas, the myeloid cell compartment consisted mainly of two distinct tissue-resident macrophage types, designated MHC-IIlo and MHC-IIhi macrophages, the latter being predominant. MHC-IIlo and MHC-IIhi pancreas macrophages differed at the molecular level, with MHC-IIlo macrophages being more M2-activated. After PDL, the loss of tissue-resident macrophages was accompanied by an early surge of Ly6Chi monocyte infiltration in the pancreas, followed by a transient MHC-IIlo macrophage peak and ultimately a restoration of the MHC-IIhi macrophage-dominated steady-state equilibrium. These intricate macrophage dynamics in PDL pancreas depended on monocyte recruitment by CCR2 and M-CSFR as well as on M-CSFR-dependent local macrophage proliferation. Functionally, MHC-IIlo macrophages were more angiogenic. A loss-of-function experiment suggested that tissue macrophages, rather than inflammatory monocyte-derived macrophages, contributed to β-cell proliferation. Together, our study fully characterizes the macrophage subsets of the pancreas and clarifies the complex dynamics of macrophages in the pancreas after PDL injury.

Presenting author: Eva Van Overmeire

Virotrap's view on A20's interactome

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Proteins typically exert their function within supra-molecular complexes. In current mass spectrometry (MS)-based strategies to map protein-protein interactions, preparation of a cell or tissue homogenate is an inevitable step. However, the associated lysis and purification steps may induce loss of interactors (false negatives) and artificially gain false interactors (false positives) due to loss of cell integrity. Virotrap is a novel approach for mapping intracellular protein interactions based on fusion of a bait protein to the HIV-1 GAG protein so that interaction partners become trapped within virus-like particles that bud from mammalian cells (Eyckerman et al., submitted). It obviates the need for cell homogenization and protects the abducted protein complexes during purification. The ubiquitin-editing protein A20 has been described as a key player in the termination of NF-κB signaling, both by removing K63-linked polyubiquitin chains from specific NF-κB signaling molecules and promoting K48-linked polyubiquitinylation followed by proteasome-mediated degradation of its target. Knowledge on the exact molecular mechanisms by which A20 controls inflammatory signaling is however still fragmentary. A20 is also recognized as a strong anti-apoptotic factor, but whether similar ubiquitin-editing mechanisms of A20 mediate this anti-apoptotic activity of A20 is still elusive. Here, we applied the Virotrap approach to map the intracellular protein interactions of A20, both without and with TNFα stimulation. Furthermore, we expand the interaction map to proteins that are involved in inflammation and/or apoptosis signaling. In this still evolving interaction map, we observe both known and novel interactions. Further, we are planning to perform Virotrap experiments with mutants of functional domains of A20 and disease-linked single nucleotide polymorphisms (SNPs) in the A20 locus that introduce a non-synonymous mutation in the OTU domain of A20.

Presenting author: Emmy Van Quickelberghe

Negative regulation of the Nlrp3 inflammasome by A20 protects against arthritis

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Rheumatoid arthritis (RA) is a chronic autoinflammatory disease that affects 1-2% of the world population and is characterized by widespread joint inflammation. Myeloid-cell-specific deletion of the RA-susceptibility gene A20/TNFAIP3 in mice (A20myel-KO mice) triggers a spontaneous erosive polyarthritis that resembles RA in patients1. A20 is a ubiquitin-editing enzyme that post-translationally modifies substrates implicated in NF-κB-dependent gene expression. However, the innate immune mechanisms driving arthritis pathology in A20myel-KO mice are not fully understood. RA was not rescued by tumor necrosis factor receptor 1 (TNF-R1) deletion, but we showed it to crucially rely on interleukin-1 receptor (IL-1R) signaling. Macrophages lacking A20 had increased basal and LPS-induced expression levels of Nlrp3 and proIL-1β. As a result, A20-deficiency in macrophages significantly enhanced Nlrp3 inflammasome-mediated caspase-1 activation, pyroptosis and IL-1β secretion by soluble and crystalline Nlrp3 stimuli. In contrast, activation of the Nlrc4 and AIM2 inflammasomes was not altered. Importantly, increased Nlrp3 inflammasome activation contributed to RA pathology in vivo, because deletion of Nlrp3 and caspase-1 markedly protected against RA in A20myel-KO mice. These results reveal A20 as a novel negative regulator of Nlrp3 inflammasome activation, and describe A20myel-KO mice as the first experimental model to study the role of inflammasomes in RA pathology.

Presenting author: Lieselotte Vande Walle

From the graveyard for pharmacy to new challenges: sepsis revisited!

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Rationale: Sepsis is one of the leading causes of death around the world. The failure of clinical trials to treat sepsis demonstrates that the molecular mechanisms are multiple and still insufficiently understood. Objectives: To clarify the long disputed hierarchical contribution of several central inflammatory mediators, viz. IL-1β, IL-18, CASP7, CASP1 and CASP11, in septic shock, and to explore their therapeutic potential. Methods: LPS- and TNF-induced lethal shock, as well as cecal ligation and puncture (CLP), were performed in genetically or pharmacologically targeted mice. Body temperature and survival were monitored closely, and plasma was analyzed for several markers of cellular disintegration and inflammation. Measurements and main results: Interestingly, deficiency of both IL-1β- and IL-18 additively prevented LPS-induced mortality. The detrimental role of IL-1β and IL-18 was confirmed in mice subjected to a lethal dose of TNF, or to a lethal CLP procedure. Although their upstream activator, CASP1, and its amplifier, CASP11, are considered potential therapeutic targets because of their crucial involvement in endotoxin-induced toxicity, CASP11 or CASP1/11 deficient mice were not, or hardly, protected against a lethal TNF or CLP challenge. In line with our results obtained in genetically deficient mice, only the combined neutralization of IL-1 and IL-18, using the IL-1 receptor antagonist Anakinra and anti-IL-18 antibodies, conferred complete protection against endotoxin-induced lethality. Conclusions: Our data point towards the therapeutic potential of neutralizing IL-1 and IL-18 simultaneously in sepsis, rather than inhibiting the upstream inflammatory caspases.

Presenting author: Tom Vanden Berghe

Caspase 4 mediates non-canonical activation of the NLRP3 inflammasome in human cells

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In mice, bacterial lipopolysaccharide (LPS) is recognized in a TLR4 independent manner to induce a Caspase 11-dependent non-canonical inflammasome, leading to cell death and IL-1b release. Up to now, this pathway has not been characterized in human myeloid cells. Using CRISPR/Cas9 genome editing technology, we here demonstrate that cytotoxicity and IL-1b release by the non-canonical inflammasome is dependent on Caspase 4 in THP-1 cells and that, paralleling results from mice, IL-1b secretion upon intracellular LPS sensing depends on the canonical inflammasome components NLRP3, ASC, and Caspase 1, as well as potassium efflux.

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