EMBO
Workshop

The inflammasomes:the next frontier

21 - 24 September 2021 | Martinsried, Germany

Scientific Organizers:

Veit Hornung | Hao Wu | Mo Lamkanfi | Kate Schroder

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We wish to thank EMBO and DFG for the great collaboration. Their support made it possible to host this workshop on the emerging topic "The inflammasomes" and enabled us to offer excellent scientific exchange.

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EMBO Workshop: "The inflammasomes: The next frontier"

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September 21 – 24, 2021
Biomedical Center Munich
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The innate branch of the immune system serves as the first line of defense, with the intricate task of detecting molecules and organisms that are a danger to the organism. Inflammasomes constitute a family of cytosolic sensors that can directly detect microbial molecules, but also indirectly sense damage by detecting the perturbation of cellular homeostasis. In recent years great progress has been made in the characterization of inflammasome components and their pivotal role as surveillance machineries in microbial infection. In addition, it has become evident that many non-communicable diseases are triggered or perpetuated by inflammasomes and a number of studies have uncovered the significant therapeutic potential of targeting these pathways. In that regard, inflammasomes have also become a prime focus for the development of novel anti-inflammatory therapies.

This EMBO workshop provides the unique opportunity to bring together researchers from diverse fields and disciplines to discuss the latest progress on inflammasome biology, ranging from basic molecular mechanisms all the way to clinical applications. Importantly, the workshop will also provide an interactive forum for trainees, junior researchers, established scientists and industry to foster interdisciplinary and interprofessional communication in this emerging field.

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Tuesday, 21 September 2021

14:00 - 16:45	Foyer Ground Floor	**Arrival and Registration**
16:45 - 17:00	Big Lecture Hall	**Welcome address** Veit Hornung
17:00 - 17:45	Livestreaming	**Keynote lecture I:** *Structural insights into NLRP3 signaling* **Hao Wu**
		Session I: Molecular Mechanisms I Chair: Veit Hornung
17:45 - 18:10	Livestreaming	*Structural insights into the NLRC4 inflammasome* **Jijie Chai**
18:10 – 18:35	Livestreaming	*Inflammasome activation and pyroptosis in severe SARS-CoV-2 infection* **Judy Lieberman**
18:35 - 21:00		**Welcome reception and dinner**

Wednesday, 22 September 2021

9:00 - 9:45	Big Lecture Hall	**Keynote lecture II:** *Inflammasome-mediated defense against intracellular bacterial pathogens* **Russell Vance**
		Session II: Molecular mechanisms II Chair: Isabella Rauch
9:45 - 10:10	Big Lecture Hall	*Delineation of a minimal NLRP3 inflammasome sensor* **Iva Hafner-Bratkovič**
10:10 - 10:40		**Coffee Break**
10:40 - 11:05	Big Lecture Hall	*Posttranslational regulation of NLRP3 inflammasome signaling* **Bénédicte Py**
11:05 - 11:30	Big Lecture Hall	*Targeting inflammasomes for therapeutic applications* **Rebecca Coll**
11:30 - 11:55	Big Lecture Hall	*Pyroptosis, a proinflammatory cell death coordinating host defense and inflammation* **Petr Broz**
11:55 - 12:10	Big Lecture Hall	*Short talk: Conformational transitions in NLRP3 signalling* **Matthias Geyer**
12:10 - 12:25	Big Lecture Hall	*Short talk: BTK operates a phospho-tyrosine switch to regulate NLRP3 inflammasome activity* **Alexander Weber**
12:25 - 13:30	Foyer Ground Floor	**Lunch and Meet the PI session I**
13:30 - 15:30	Ground Floor	**Poster Session I**
14:00 - 15:00	Online Platform	**Discussion and Meet the PI session I** Chair: Hao Wu

		Session III: Regulation of inflammasome signaling Chair: Petr Broz
15:30 - 15:55	Big Lecture Hall	*Inflammasome signaling in T cells* **Veit Hornung**
15:55 - 16:20	Livestreaming	*Counter Regulation of Pyroptotic Cell Death* **Kate Fitzgerald**
16:20 - 16:35	Livestreaming	*Short talk: Full-length NLRP3 forms oligomeric cages to enable NLRP3 activation* **Liudmila Andreeva**
16:35 - 16:50	Livestreaming	*Short talk: In-cell cryo-electron tomography of ASC signalling complexes* **Clare Bryant**
16:50 - 17:20		**Coffee Break**
17:20 - 17:45	Livestreaming	*Endogenous lipids in the scope of the inflammasome* **Jon Kagan**
17:45 - 18:10	Livestreaming	*Mechanisms of inflammasome-induced cell death* **Vishva Dixit**
18:10 - 18:25	Livestreaming	*Short talk: Sensing low intracellular potassium by NLRP3 results in a stable open structure that promotes inflammasome activation* **Pablo Pelegrin**
18:25 - 19:00		Free time
19:00 - 21:00		**Social Dinner at Haderner Augustiner**

Thursday, 23 September 2021

9:00 - 9:45	Livestreaming	**Keynote lecture III:** *Molecular Mechanisms of NLRP3 inflammasome activation* **Kate Schroder**
		Session IV: Inflammasomes under microbial attack Chair: Mo Lamkanfi
9:45 - 10:10	Livestreaming	*Pyroptosis and antitumor immunity* **Feng Shao**
10:10 - 10:40		**Coffee Break**
10:40 - 11:05	Big Lecture Hall	*Non-protease based activation of the human NLRP1 inflammasome* **Franklin Zhong**
11:05 - 11:30	Big Lecture Hall	*Regulation of RIPK1/caspase-8 dependent apoptosis and pyroptosis* **Egil Lien**
11:30 - 11:55	Big Lecture Hall	*Microbial grounds that license inflammasome-mediated execution* **Etienne Meunier**
11:55 - 12:10	Big Lecture Hall	*Short talk: IL-1α and IL-1β non-redundant in tolerance versus resistance to infection* **Kevin Eislmayr**
12:10 - 12:25	Big Lecture Hall	*Short talk: Pseudomonas aeruginosa infection reveals a Caspase-1-dependent neutrophil pyroptosis pathway that restrains damaging Histone release* **Karin Santoni**
12:25 - 13:30	Foyer Ground Floor	**Lunch and Meet the PI session II**
13:30 - 15:30	Ground Floor	**Poster Session II**
14:00 - 15:00	Online Platform	**Discussion and Meet the PI session II** Chair: Kate Schroder

	Big Lecture Hall	**Session V: Inflammasomes in the scope of metabolic pathways and beyond** Chair: Rebecca Coll
15:30 - 15:55	Livestreaming	*Metabolic control of inflammasome signaling* **Vishwa Deep Dixit**
15:55 - 16:20	Livestreaming	*Role of inflammasome signaling in ischemic brain injury* **David Brough**
16:20 - 16:35	Big Lecture Hall	*Short talk: From NLRP3 sensing of RhoGTPase-activating toxins during bacteremia to the analysis of the NLRP3 inflammasome signature during COVID-19* **Laurent Boyer**
16:35 - 16:50	Big Lecture Hall	*Short talk: Intestinal tuft cell inflammasome activation leads to prostaglandin D2 release and an antibacterial response* **Isabella Rauch**
16:50 - 17:20		**Coffee Break**
17:20 - 18:05	Livestreaming	**Keynote lecture IV:** *Blocking IL-1 – insights from the CANTOS trial* **Paul Ridker**
18:05 - 18:20	Livestreaming	*Short talk: Bacterial gasdermins* **Alex Johnson**
18:20 - 19:00		Free time
19:00 - 22:00		**Social Dinner at Haderner Augustiner**

Friday, 24 September 2021

9:00 - 9:25	Big Lecture Hall	*Metabolism driving inflammation* **Luke O' Neill**
9:25 - 9:50	Big Lecture Hall	*Negative regulation of the inflammasome signaling* **Jelena Bezbradica**
9:50 - 10:40		**Coffee Break**
		Session VI: Inflammasomes - moving into the clinic Chair: Iva Hafner-Bratkovič
10:40 - 11:05	Big Lecture Hall	*Inflammasome-driven sterile inflammatory diseases* **Mo Lamkanfi**
11:05 - 11:30	Big Lecture Hall	*Epigenetic control of NLRP3 inflammasome signaling* **Eicke Latz**
11:30 - 11:45	Big Lecture Hall	*Short talk: Recent insights into NLRP1 and CARD8 Inflammasome Activation* **Daniel Bachovchin**
11:45 - 12:00	Big Lecture Hall	*Short talk: Inflammasome regulation of auto-inflammation induced by Otulin deficiency* **Giulia Doglio**
12:00 - 12:15	Big Lecture Hall	*Short talk: Endogenous steroid catabolites reveal a novel mechanism of Pyrin inflammasome activation* **Flora Magnotti**
12:15 - 12:30	Livestreaming	*Short talk: Beyond Phenotypic Screening: Direct target assays identify novel NLRP3 inhibitors with diverse structural and pharmacological features including brain penetrance* **Anick Auger**
12:30 - 14:00	Foyer Ground Floor	**Lunch and Meet the PI session III**

14:00 - 14:30	Big Lecture Hall	**Lightning talks** (selected from posters)
14:30 -15:15	Big Lecture Hall	**Moderated Discussion** *t.b.d.*
15:15- 15:45	Big Lecture Hall	**Poster awards & closing remarks** (Organizers)

Invited Speakers

Bénédicte Py
Centre International de Recherche en Infectiologie, FR

David Brough
The University of Manchester, UK

Egil Lien
University of Massachusetts Medical School, US

Eicke Latz
University Hospitals University of Bonn Institute of Innate Immunity, DE

Etienne Meunier
Institut de Pharmacologie et de Biologie Structurale, FR

Feng Shao
National Institute of Biological Sciences, CN

Hao Wu
Harvard Boston Children's Hospital, US

Iva Hafner-Bratkovič
National Institute of Chemistry, SI

Jelena Bezbradica
University of Oxford, Kennedy Institute, UK

Jijie Chai
Max Planck Institute for Breeding Research; University of Cologne, DE

Jonathan Kagan
Harvard Boston Children's Hospital, US

Judy Lieberman
Harvard Boston Children's Hospital, US

Kate Schroder
The University of Queensland, AU

Kate Fitzgerald
University of Massachusetts Medical School, US

Luke O'Neill
Trinity College Dublin, IE

Mo Lamkanfi
Ghent University, BE

Paul Ridker
Brigham and Women's Hospital, US

Petr Broz
Université de Lausanne, CH

Rebecca Coll
Queen's University Belfast, UK

Russell Vance
University of California, Berkeley, US

Veit Hornung
LMU Gene Center, DE

Vishva Dixit
Genentech, US

Vishwa Deep Dixit
Yale School of Medicine, US

In the following you will find the abstract of the short talk and the poster presentations. Please note that we did not include abstracts from the invited speakers. The contributions are ordered by the poster number, whereas on-site presentations are highlighted in blue and online presentations are highlighted in green. An alphabetic index that provides an overview can be found on page 65 and 66.

003 Chen

An Acetylation Switch of the NLRP3 Inflammasome Regulates Aging-associated Conditions
Danica Chen
University of California, Berkeley, Berkeley, United States

It is well documented that the rate of aging can be slowed, but it remains unclear to which extent aging-associated conditions can be reversed. How the interface of immunity and metabolism impinges upon the diabetes pandemic is largely unknown. Here, we show that NLRP3, a pattern recognition receptor, is modified by acetylation in macrophages and is deacetylated by SIRT2, an NAD+-dependent deacetylase and a metabolic sensor. We have developed a cell-based system that models aging-associated inflammation, a defined co-culture system that simulates the effects of inflammatory milieu on insulin resistance in metabolic tissues during aging, and aging mouse models; and demonstrate that SIRT2 and NLRP3 deacetylation prevent, and can be targeted to reverse, aging-associated inflammation and insulin resistance. NLRP3 is also expressed in hematopoietic stem cells and the SIRT2-NLRP3 axis regulates the functional deterioration of hematopoietic stem cell aging. These results establish the dysregulation of the acetylation switch of the NLRP3 inflammasome as an origin of aging-associated chronic inflammation and highlight the reversibility of aging-associated conditions.

005 Gasterich

Estrogens regulate the NLRP3 inflammasome in acute CNS damage
Natalie Gasterich, Clara Voelz, Alexander Slowik, Cordian Beyer, Adib Zendedel
RWTH Aachen, Institute of Neuroanatomy, Aachen, Germany

The NLRP3 inflammasome is specifically activated after acute brain damage (spinal cord injury (SCI), and ischemic stroke). Estrogens (E2) are anti-inflammatory and protective in various brain pathologies. In this report, we investigated the potency of E2 to alleviate inflammasome activation and modulate regulatory microRNAs in SCI and stroke.
Male rats underwent spinal cord contusion or transient middle cerebral artery occlusion (tMCAO). E2 was subcutaneously given immediately after and every 12h after SCI/tMCAO.
NLRP3 inflammasome components were significantly stimulated in a time-dependent way on gene and protein level in both models. E2 effectively attenuated observed induction although to a different extent and specificity, e.g. NLRP3 expression was increased on mRNA but not on protein level after stroke. Regarding the varying results of NLRP3, we found miR-223 plays a critical role in regulation of NLRP3 inflammasome which is increased after stroke and dampened NLRP3. E2 inhibits the miR-223 expression which result in reregulation of NLRP3.
We propose that E2 executes its anti-inflammatory function through the control of NLRP3 activation. This can be by directly regulating their gene expression but also by influencing the expression levels of microRNAs which play a regulatory role for NLRP3 inflammasome mRNA processing.

007 Harioudh

Specific OAS1 isoform protects against intracellular bacterial infection by upregulating IRF1

Munesh Harioudh [1, 2], Joseph Pérez-Otero [1, 2], Lomon So [3], Ram Savan [3], Veit Hornung [4], Vijay Rathinam [5], Saumendra Sarkar [1, 2]

[1] *Cancer Virology Program, UPMC Hillman Cancer Center, Pittsburgh, United States*
[2] *Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, United States*
[3] *Department of Immunology, University of Washington, Seattle, United States*
[4] *Gene Center and Department of Biochemistry, Ludwig-Maximilians-Universität München, Munich, Germany*
[5] *Department of Immunology, UConn Health School of Medicine, Farmington, United States*

The 2′-5′-oligoadenylate synthetases (OAS) proteins are a family of ISGs that catalyze 2′-5′-linked oligoadenylates and can inhibit viral replication. However, the role of OAS proteins during bacterial infection remains unknown. Here we define a unique mechanism of antibacterial activity of OAS1 against intracellular bacteria Listeria monocytogenes and Francisella novicida.
We screened a set of various OAS family protein-deficient THP1 cells and found only OAS1-KO cells significantly enhanced bacteria growth. Expression of OAS1 P46 WT and enzymatically inactive mutant rescued this antibacterial activity. OAS1-KO cells showed defective IFNγ response and the reduced IRF1expression. The antibacterial activity of OAS1 P46 was absent in IRF1-KO cells suggesting its dependence on IRF1. Through RNA-sequencing, SILAC, polysome profiling, and radiolabeling experiments, we found that OAS1 enhanced the translation of IRF1 and a specific set of proteins with known antiviral and antibacterial activity by binding with the respective mRNA. Among various OAS1 genes in mouse, we found Oas1b to be the functional homolog of human OAS1 P46. To validate our results in vivo we used an Oas1b knock-in mice, which showed improved survival and lower bacterial load compared to the WT mice. Together, we reveal that OAS1 inhibits intracellular bacterial growth through upregulating IRF1.

009 Nandi

Reporter nanoparticle for real-time inflammasome monitoring during a disease-progression

Dipika Nandi [1], Anh Nguyen [2], Ashish Kulkarni [1, 2, 3, 4]

[1] *Veterinary and Animal Sciences Department, University of Massachusetts, Amherst, United States*
[2] *Department of Chemical Engineering, University of Massachusetts, Amherst, United States*
[3] *Department of Biomedical Engineering, University of Massachusetts, Amherst, United States*
[4] *Center for Bioactive Delivery, Institute for Applied Life Sciences, University of Massachusetts, Amherst, United States*

Inflammasome activation is critical to variety of chronic inflammatory diseases like Colitis, Non-Alcoholic-Steatohepatitis, Alzheimer's. This innate immune response induced by microbial or sterile invasion activates the caspase-1 enzyme, an important hallmark of inflammasome activation. Active caspase-1 further cleaves pro-IL-1b and pro-IL18, converting them to their active forms which are released outside the cell, resulting in enhanced immune response and pyroptosis. Currently available techniques fail to provide temporal kinetics of inflammasome activation during disease progression, presenting a window of opportunity for the development of inflammasome monitoring tools. To address this gap, we have developed a lipid-nanoparticle encapsulated caspase-1 activatable probe that will emit signals specifically at inflamed sites upon active-caspase-1 cleavage. This biocompatible platform enhances the probe's residence time in circulation by preventing its opsonization and allowing its sustained release over time. Preliminary observations show that the probe is cleaved specifically by recombinant active-caspase-1 and can be encapsulated stably inside lipid nanoparticles. We have also successfully demonstrated its in-vitro testing in inflammasome-activated-bone-marrow-derived macrophages. We further plan to translate this study into relevant animal models. This entire system will help in the precise estimation of caspase-1 activation kinetics during disease progression, hence ensuring early detection of pathogenic inflammation and its timely treatment.

011 Smatlik

ASC specks formation in primary human keratinocytes
Nikola Smatlik, et al.
University Skin Hospital Tuebingen, Tübingen, Germany

Inflammasomes are innate immune sensors involved in response to cytosolic pathogens or stress signals. Most inflammasomes consist of three major components: a NODlike receptor (NLR), the adaptor protein ASC and pro-caspase-1. The major role of this inflammasome is in processing caspase-1, its activated form proteolytically activate IL-1 and IL-18. Inflammasome assembly can be visualized by ASC speck formation. However its impact in primary human keratinocytes remains unknown. In this study we want to reveal inflammasome activation and ASC specks formation and localization in primary human keratinocytes after UVB light exposure. Also we want to investigate which type of the inflammasome (NLRP1 or NLRP3) is activated after UVB light exposure and which of them plays a role in the formation of ASC specks.
Our data clearly show that irradiation with UVB light leads to nuclear ASC speck formation in primary human keratinocytes. These specks seem to reflect inflammasome formation as their formation is dependent on the presence of NLRP1 and NLRP3. These nuclear ASC speck formation might putatively linked apoptosis and cell death where nuclear ASC is involved to inflammsome activation.

013 Devant

Evolution-inspired redesign of the LPS receptor caspase-4 into an interleukin-1 converting enzyme
Pascal Devant, Anh Cao, Jonathan C. Kagan
Division of Gastroenterology, Boston Children's Hospital and Harvard Medical School, Boston, United States

Several inflammatory caspases cleave GSDMD, but differ in their ability to recognize bacterial LPS and cleave IL-1β. No caspase in mice or humans contains all three activities, yet distinct caspases with complementary activities bookend an LPS-induced pathway to IL-1β cleavage. Herein, we present a detailed characterization of caspase-1/4 hybrid proteins from the common dog (Canis lupus familiaris). These enzymes, despite their evolutionary origin as caspase-4 homologues, display all activities of caspase-1, including the ability to directly cleave pro-IL-1β. Comparative analysis revealed a mechanism by which caspases select pro-IL-1β as a substrate, by a mechanism distinct from that which detects GSDMD. This knowledge enabled us to redesign human caspase-4 into a protease exhibiting substantial ICE activity in vitro and in macrophages. Within cells, redesigned human caspase-4 operated as a one-protein signaling pathway that directly binds LPS and cleaves IL-1 and GSDMD, by a process that bypasses the need for inflammasomes. Further analyses revealed that a caspase-4 homologue present in cats operates in a similar manner. These findings therefore reveal mechanisms of caspase substrate specificity and challenge the idea that complexity is a prerequisite for innate immune pathway design.

015 Cordero

NLRP3 inflammasome inhibition improves lifespan in an animal model of Hutchinson-Gilford Progeria

Elisabet Alcocer-Gómez [1], Mario D. Cordero [2]
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[2] *Instituto de Investigación e Innovación en Ciencias Biomédicas de Cádiz, INiBICA, 11009 Cádiz, Spain, Cádiz, Spain*

Inflammation is a hallmark of aging and accelerated aging syndromes. In this context, inflammation has been associated to the pathophysiology of Hutchinson–Gilford progeria syndrome (HGPS). In this study, we report that progeroid skin fibroblasts and animal models present an hyperactivation of the NLRP3-inflammasome complex. High expression of NLRP3 and caspase 1 was also observed in skin fibroblasts from HGPS associated to the nuclei morphology. Lymphoblast from HGPS also showed increased basal levels of NLRP3 and caspase 1 independent to the induction from metabolic factors. Consistent with these results, Zmpste24–/– showed high expression of Nlrp3 and caspase 1 in heart and liver, however these changes were not observed in other inflammasomes. We also show that pharmacological inhibition of NLRP3 using a direct NLRP3 inhibitor, MCC950, improved cellular phenotype, significantly extends the lifespan of these progeroid animals and reduced inflammasome-dependent inflammation. These findings suggest the NLRP3-inflammasome comples as a therapeutic approach for patients with HGPS.

016 Pelegrin

Sensing low intracellular potassium by NLRP3 results in a stable open structure that promotes inflammasome activation

Ana Tapia-Abellán [1,2], Diego Angosto-Bazarra [1], Cristina Alarcón-Vila [1], María Carmen Baños [1], Iva Hafner-Bratkovič [3], Baldomero Oliva [4], Pablo Pelegrin [1]
[1] *Instituto Murciano de Investigación Biosanitaria IMIB-Arrixaca, Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain*
[2] *Interfaculty Institute for Cell Biology, Department of Immunology, University of Tübingen, Tübingen, Germany*
[3] *Department of Synthetic Biology and Immunology, National Institute of Chemistry, Ljubljana, Slovenia*
[4] *Laboratory of Structural Bioinformatics, Universitat Pompeu Fabra, Barcelona, Spain*

The NLRP3 inflammasome is activated in response to a wide range of stimuli and drives diverse inflammatory diseases. The decrease of intracellular potassium concentration is a minimal upstream signal to most of the different NLRP3 activation proposed models. Here we found that cellular potassium efflux induces a stable structural change in the inactive NLRP3 promoting an open conformation as a step preceding activation. This conformational change is facilitated by the specific NLRP3 FISNA domain. Then, an unique flexible linker sequence between the PYD and FISNA domains is important to structurally allow the ensemble of NLRP3-PYD into a seed structure for ASC oligomerization. The introduction of the NLRP3 PYD-linker-FISNA sequence into NLRP6 resulted in a chimeric receptor able to be activated by potassium efflux-specific NLRP3 activators and promoted an in vivo inflammatory response to uric acid crystals. This sequence was also conferring NLRP6 the ability to be activated by the potassium-independent NLRP3 activator imiquimod. Our results establish that the N-terminal sequence of NLRP3 is key to activate the inflammasome induced by potassium efflux-dependent and independent triggers, involving an initial common step associated to a conformational change in the inactive NLRP3 structure.

017 Boyer

From NLRP3 sensing of RhoGTPase-activating toxins during bacteremia to the analysis of the NLRP3 inflammasome signature during COVID-19

Laurent Boyer
Inserm, Université Côte d'Azur, C3M, Nice, France

The consequent inflammasome-triggered Caspase-1 activation is critical for the host defense against pathogens. During infection, NLRP3 triggers the assembly of an inflammasome activating Caspase-1 via ASC and Nek7 recruitment. Using the CNF1 toxin from Escherichia coli, we provide evidence of the role of the NLRP3 inflammasome in sensing the activity of bacterial toxins and virulence factors that activates host RhoGTPases. We demonstrate that this activation relies on monitoring of the toxin's activity on the RhoGTPase Rac2 via a signaling cascade involving the Pak1-mediated phosphorylation of Threonine 659 of NLRP3, which is necessary for the NLRP3-Nek7 interaction and the IL-1ß maturation. Furthermore, inhibition of the Pak1-NLRP3 axis diminishes bacterial clearance of CNF1-expressing E. coli during bacteremia in mice (Dufies et al, 2021 Nature microbiology). Building on this expertise, we set up an assay to quantify the NLRP3 activation in the blood and applied it to COVID-19 patients. The analysis of the blood of 66 patients allowed us to reveal the heterogenous activation of NLRP3 in circulating myeloid cells during COVID-19 (Courjon et al, 2021, Blood Advances). We showed the NLRP3 inflammasome impaired response of immature neutrophils and the increased response of non classical monocytes in correlation with the COVID-19 severity.

018 Talley

Monitoring peripheral-driven CNS inflammasome activation in vivo and ex vivo

Sarah Talley, Mashkoor Choudhry, Edward Campbell
Loyola University Chicago, Maywood, IL, United States

To better understand the spatiotemporal dynamics of inflammasome activation in vivo during the onset and resolution of inflammatory diseases, we generated transgenic mice expressing caspase biosensors that becomes bioluminescent downstream of inflammasome activation. Using these mice, we were able to measure the onset of inflammation using models of acute and chronic inflammatory diseases/infection paradigms. Recently, we have been interested in understanding how peripheral inflammation can trigger neuroinflammation in various disease contexts. In a mouse model of ulcerative colitis, we found that colonic inflammation drives neuroinflammation, characterized by increased caspase-1 activation, inflammatory cytokine/chemokine production and microglia activation. We further identified upregulated DAMPs that can be therapeutically targeted to ameliorate neuroinflammation. In a model of obesity, we were able to monitor the gradual onset of inflammation in mice fed a western diet. Obese mice exhibited significant biosensor activation in some metabolic and neuronal tissues, including the heart and brain. Therapeutic administration of the SCFA butyrate attenuated inflammation in these tissues. Finally, using minimally invasive surgical techniques, we were able to monitor inflammation in the brains of living mice prior to and following systemic LPS administration. In current work, we are using this technique to track inflammation during the development of neurodegenerative diseases.

019 Zhang, Bronowska

Development of small-molecule fluorescent probes for NLRP3 inflammasome imaging

Junya Zhang, Joao Victor de Souza, Agnieszka K. Bronowska, Fehmi Metehan Benli, Tasneem Binte Raheel
School of Natural and Environmental Sciences, Newcastle University, Newcastle Upon Yyne, United Kingdom

The NLRP3 inflammasome, named after its core protein - NACHT, LRR and PYD domains-containing protein 3 (NLRP3), is the most widely studied inflammasome. It has been reported to be closely associated with a variety of chronic or systemic inflammatory diseases. However, the current lack of necessary imaging tools severely limits the understanding of NLRP3 activation in specific diseases and the involvement of targets for NLRP3 inhibitors in in-depth studies. The binding pocket of the inhibitors at the protein aliasing site has been determined by molecular docking and 100ns molecular dynamics simulations of the sulfonamide NLRP3 inhibitors JC171 and JC124. An environmentally sensitive fluorescent probe is designed based on this pocket. The molecular dynamics results indicate that the probe has a stable binding model with less impact on protein activity and has potential for further optimisation and experimental validation.

020 Fattinger

Epithelium-autonomous NAIP/NLRC4 prevents TNF-driven inflammatory destruction of the gut epithelial barrier in Salmonella-infected mice

Stefan Fattinger, et al.
Institute of Microbiology, Department of Biology, ETH Zürich, Zürich, Switzerland
Science for Life Laboratory, Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden

The gut epithelium is a critical protective barrier. Its NAIP/NLRC4 inflammasome senses infection by Gram-negative bacteria, including Salmonella Typhimurium (S.Tm) and promotes expulsion of infected enterocytes. During the first ~12-24h, this reduces mucosal S.Tm loads at the price of moderate enteropathy. It remained unknown how this NAIP/NLRC4-dependent tradeoff would develop during subsequent infection stages. In NAIP/NLRC4-deficient mice, S.Tm elicited severe enteropathy within 72h, characterized by elevated mucosal TNF (>20pg/mg) production from bone-marrow-derived cells, reduced regeneration, excessive enterocyte loss, and a collapse of the epithelial barrier. TNF-depleting antibodies prevented this destructive pathology. In hosts proficient for epithelial NAIP/NLRC4, a heterogeneous enterocyte death response with both apoptotic and pyroptotic features kept S.Tm loads persistently in check, thereby preventing this dire outcome altogether. Our results demonstrate that immediate and selective removal of infected enterocytes, by locally acting epithelium-autonomous NAIP/NLRC4, is required to avoid a TNF-driven inflammatory hyper-reaction that otherwise destroys the epithelial barrier.

021 Liu

Regulation of NLRP3 inflammasome by Staphylococcus aureus within macrophages
Xiao Liu [1, 3], Janina Bayer [2, 3], Alexander Weber [1, 4], Christiane Wolz [2]
[1] *Interfaculty Institute of Cell Biology, Department of Immunology, Tübingen, Germany*
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Staphylococcus aureus is a notorious facultative pathogen that causes a diverse range of illnesses world-wide. S. aureus has evolved strategies to avoid the host immune response, either by hiding inside of the phagocytic cells (here referred as "PersistStaph"), or escaping from within cells by the induction of a so far unknown type of cell death ("ExitStaph"). The extracellular pore-forming, Leukocidin A/B (LukAB), has been identified to potently trigger the activation of the NLRP3 inflammasome, promote IL-1ß secretion and eventually kill primary human monocytes when added exogenously. But the role of LukAB, when expressed endogenously during intracellular S. aureus infection, and its effects on the NLRP3 inflammasome and cell death pathways is not well understood. We found that NLRP3 inflammasome activation induced by PersistStaph within THP-1 macrophages was LukAB-independent, since IL-1ß and IL-18 release could be potently triggered by both USA300WT and an arg/sae mutant lacking LukAB. However, NLRP3 ablation, MCC950 or ZVAD inhibitors did not alter ExitStaph-induced cell death and Gasdermin D cleavage was not observed. This suggests that toxin-mediated inflammasome activation and cell death are decoupled in S. aureus infections of human macrophages and a non-conventional type of cell death may be employed by leukocidins.

022 de los Reyes Jiménez

Antisense oligonucleotides as a new approach to inhibiting the NLRP3 inflammasome
022
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NLR family pyrin domain containing 3 (NLRP3) inflammasome has evolved as an attractive therapeutic target, e.g. for treatment of CAPS (cryopyrin associated periodic syndromes), NASH or neuroinflammatory disorders. Locked nucleic acid (LNA) modified Antisense Oligonucleotides (ASO) specifically degrade their target (pre) mRNA through RNase H mediated cleavage and do not require delivery systems for in vitro and in vivo activity. Targeting NLRP3 expression by ASOs has several advantages as compared to siRNA, small molecule inhibitors or anti-IL-1β antibodies.
We employed our proprietary LNAplus™ platform to screen mouse- or human-NLRP3 specific ASOs in relevant cell lines and primary cells. We found that treatment with NLRP3-specific ASOs reduced NLRP3 mRNA expression by 80% to 90% in macrophages differentiated from human monocytes or murine bone marrow cells. The half maximal inhibitory concentration (IC50) of the best candidates was in the submicromolar range. ASO mediated NLRP3-depletion inhibited inflammasome activation as well as IL-1β cleavage and release in macrophages. Finally, we demonstrated that administration of NLRP3 specific ASOs to healthy mice resulted in potent reduction of NLRP3 mRNA in the kidney, liver and lymph nodes without causing toxicity. Ongoing experiments are investigating the efficacy of ASOs in pre-clinical disease models and human CAPS patient material.

023 Tapia-Abellán

Studying the effect of NLRP3 inhibitors by Bioluminiscent Resonance Energy Transfer (BRET)

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NLRP3 aberrant activation is involved in chronic inflammatory diseases and autoinflammatory disorders. Intense research is conducted to find compounds that target directly and block NLRP3. Bioluminescence resonance energy transfer (BRET) allows the study of protein conformational changes in living cells. In this sense, BRET-based assays can be applied to screen new inhibitors that can result in a change of BRET signal if the inhibitor affects the structure of the protein of interest. We previously found that MCC950 inhibitor was able to change the BRET signal from NLRP3 BRET sensors. Here, we extended the screening to two inhibitors, CY09 and NBC19, shown to inhibit the NLRP3 inflammasome in vitro and in vivo. Upon NLRP3 inflammasome activation, MCC950, NBC19 or CY09 blocked cell death and IL-1β release in BMDM. In cells expressing NLRP3 WT or NLRP3 D305N BRET sensors treatment with CY09 or NBC19 showed that both compounds were able to directly impact on NLRP3 structure by altering the BRET signal. However, whereas MCC950 increased BRET, CY09 and NBC19 showed a significant decrease in the NLRP3 BRET signal, indicating different mechanisms of action in NLRP3. Thus, NLRP3 BRET sensors-based assays could be an easy and effective method for screening new potential compounds.

024 Venegas

Targeting monoamine oxidase dampens NLRP3 inflammasome activation in inflammation

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Inflammasomes represent protective mechanism against pathogens and cellular damage. Several signals activate the NLRP3 inflammasome and a few studies have been reported that mitochondrial reactive oxygen species (ROS) are involved. However, it is still unclear both the source and the specific role of mitochondrial ROS in inflammasome activation. Here, we show that H2O2 produced by the mitochondrial enzyme monoamine oxidase B (MAO-B) plays a non-redundant role in sustaining NLRP3 inflammasome activation. Mechanistically, MAO-B-dependent ROS formation caused mitochondrial dysfunction and triggered NF-kB activation, resulting in NLRP3 and pro-IL-1β overexpression in macrophages. MAO-B inhibition by the clinical-grade drug rasagiline prevented both ASC speck formation and IL-1β secretion upon stimulation with LPS and ATP or calcium pyrophosphate crystals. We show that MAO-B-derived ROS is the trigger for IL1β release both in vitro and in vivo. MAO-B deficient mice showed impaired response to LPS-mediated endotoxemia, confirming the importance of the MAO-B-driven ROS production.
Our findings identify MAO-B as a specific source of mitochondrial ROS fueling NLRP3 inflammasome, thereby providing the basis for repurposing MAOB inhibitors to treat inflammasome-mediated pathologies like sepsis and gout.

026 Gritsenko, Lopez-Castejon

Interleukin-37 is Released in a Pyroptosis and NLRP3 dependant manner from Human Monocytes

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Interleukin-37 is an important anti-inflammatory member of the IL-1 family that plays a key role in dampening inflammation associated with non-communicable diseases. However, mechanisms of its regulation remain understudied. We aimed to investigate the enzymatic cleavage of IL-37 that potentiates extracellular signalling, as well as pathways of its secretion. In human monocytes, we found IL-37 to be released following canonical NLRP3 inflammasome activation. The release of IL-37 was blocked by plasma membrane permeability inhibitor punicalagin and in GSDMD deficient monocyte-like THP-1 cells, suggesting that pyroptosis mediates IL-37 export. As a caspase-1 cleavage site has been suggested, we studied the role of the NLRP3 inflammasome in the maturation and release of IL-37. Whilst the cleavage of IL-37 was found to be constitutive, the release of mature IL-37 was blocked in NLRP3 deficient THP-1s and by NLRP3 inhibitor MCC950. Site directed mutagenesis of the two suggested cleavage sites: D20A (caspase-1) and V46G (unknown enzyme) did not block the processing observed. Therefore, we propose a novel pathway in which IL-37 is cleaved by caspase-1 independent mechanisms and released following NLRP3 inflammasome activation and pyroptosis. Further work is required to uncover novel cleavage sites and enzymes responsible.

028 Bryant

In-cell cryo-electron tomography of ASC signalling complexes

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Inflammasomes induce inflammatory cell death in response to infection and cell damage. NLRP3 activation induces polymerization of ASC into a single, micron-scale perinuclear punctum, which recruits and activates caspase-1. ASC puncta have yet to be imaged at sufficient resolution to resolve their cellular ultrastructure. Here, we apply correlative cryo-light microscopy and cryo-electron tomography to visualize ASC/caspase-1 puncta in NLRP3-activated cells. The puncta are composed primarily of hollow, branched ASC filaments. The variable filament density allows ribosomes and trans-Golgi-like vesicles to permeate the network. Morphometric analysis of the associated mitochondria revealed reduced inter-cristae spacing and 10-20-nm discontinuities in the outer membrane. We propose that the ASC branching filament structure provides structural integrity while allowing macromolecules and small vesicles to diffuse in or bind with the necessary density, timing and subcellular localization

029 Weber

BTK operates a phospho-tyrosine switch to regulate NLRP3 inflammasome activity

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Activity of the NLRP3 inflammasome, a critical mediator of inflammation, is controlled by accessory proteins, post-translational modifications, cellular localization and oligomerization. How these factors relate, is unclear. We show that the well-established drug target, Bruton's Tyrosine Kinase (BTK), affects several levels of NLRP3 regulation: BTK directly interacts with NLRP3 in immune cells and phosphorylates four conserved tyrosine residues upon inflammasome activation, in vitro and in vivo. Furthermore, probably by charge neutralization, BTK modification of a polybasic linker region that directs NLRP3 Golgi association and inflammasome nucleation, BTK promotes NLRP3 re-localization, oligomerization, ASC polymerization and full inflammasome assembly. As NLRP3 tyrosine modification by BTK also positively regulates IL-1β release, we propose BTK as a multi-functional positive regulator of NLRP3 regulation and BTK phosphorylation of NLRP3 as a novel and therapeutically tractable step in the control of inflammation.

030 Kogel

Extracellular ASC specks are released after marathon running and induce pro-inflammatory effects in endothelial cells

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Background: The NLRP3 inflammasome emerged as a new immunological target for different diseases and acute stress conditions. This study aims to characterize the acute effects of exercise on the release of extracellular Apoptosis-Associated Speck-Like Protein (ASC) specks and the effects of ASC specks on endothelial and muscle cells.

Methods: We used flow cytometry to measure extracellular ASC specks in the serum of 46 marathon runners, 24-72 hours before, immediately after, and 24-72 hours after the run. ASC specks were isolated from of a THP1-ASC-eGFP cell line to treat primary human coronary artery endothelial cells (HCAEC).

Results: Athletes showed a significant increase in serum concentration of circulating ASC specks immediately after the marathon (+45.5% compared to baseline, $p<0.05$) and a decrease during follow-up (-11%, $p<0.01$). Confocal microscopy revealed that human endothelial cells were able to internalize extracellular ASC specks. After internalization, these cells showed an inflammatory response with higher expression of proinflammatory proteins (e.g., ICAM1 2.7-fold) and endothelial leakage.

Conclusion: These findings suggest that extracellular inflammasome specks may act as a novel way of cell-cell communication after acute exercise. Their pro-inflammatory effects on vascular cells may contribute to the increased cardiovascular risk mediated by high loads of exercise.

031 Maeder

Extracellular NLRP3 particles are internalized by human vascular cells and exert pro-inflammatory and atherogenic effects

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The pivotal role of the intracellular activated nod-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome in caspase-1 and IL-1β activation is well characterized whereas its extracellular function still remains elusive. We tested the hypothesis that extracellular NLRP3 particles are internalized by primary human vascular cells, such as coronary artery endothelial cells (HCAEC) and smooth muscle cells (HCASMC) inducing atherogenic effects.

Isolated fluorescently labelled NLRP3- YFP inflammasome particles were internalized from HCAEC and HCASMC after 4 hours. Immunhistologic and expressional analyses showed that internalized NLRP3 particles induce caspase-1 (1.9-fold, $p<0.05$).

In conclusion, the experiments show that extracellular NLRP3 particles are internalized into human vascular cells where they induce pro-inflammatory and pro-atherogenic effects representing a novel mechanism of cell-cell communication and perpetuation of inflammation in atherosclerosis.

032 Mateo Tortola

Uncoding the molecular mechanism of NLRP3 exon 3

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The NLRP3 inflammasome has a critical role in inflammation since it has been linked to chronic inflammatory diseases and is responsible for several autoinflammatory pathologies called cryopyrin associated syndromes (CAPS). NLRP3 can be activated by diverse stimuli by so far unknown molecular events. Human NLRP3 contains 10 exons encoding the three main components of the protein: the pyrin domain (PYD), the nucleotide binding and oligomerization domain (NACHT) and the Leucine-rich domain (LRR). Exon 3 encodes a short 40 amino acids segment of the protein that contributes to the linker between the PYD and NACHT domain. This exon seems to be unique for NLRP3 in that is absent from other NLRs, however its role has not been fully established. Our data here show that exon 3 has a critical impact on the pre-activated conformation of NLRP3 CAPS mutants, as measured by BRET. Furthermore, the absence of exon 3 affected the localization of NLRP3 to the Trans-Golgi network, enriched in the phosphoinositide PI(4)P. Thus exon 3 seems to contribute to NLRP3 activation via both conformation as well as localization. Further characterization of the contribution of this unique feature of NLRP3 may help us gain understanding on the mechanism of activation of NLRP3.

033 Rauch

Intestinal tuft cell inflammasome activation leads to prostaglandin D2 release and an antibacterial response

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The intestinal epithelium is exposed to various pathogens and is instrumental as a first line of defense against invasion of the host. The NAIP/NLRC4 inflammasome has emerged as an important innate immune sensor in intestinal epithelial cells (IECs) in bacterial gastrointestinal infection, leading to rapid extrusion of infected cells and release of cytokines and eicosanoids upon pathogen recognition. The role of innate immune sensing and the NAIP/NLRC4 inflammasome at the level of individual subtypes of IEC is not well understood.
We discovered that tuft cells uniquely among IECs release the eicosanoid prostaglandin D2 (PGD2) after inflammasome activation. Tuft cells are a rare subtype of IECs primarily known for their role in anti-parasitic immunity. The role of tuft cells during bacterial infection in general as well as the role of released PGD2 in intestinal immunity is unclear. Our data show that tuft cell inflammasome activation leads to increased tissue levels of IL-22, an antibacterial cytokine that can be produced by TH17 cells or innate lymphoid cells (ILCs), and that tuft cell inflammasome expression protects from small intestinal bacterial colonization. Based on our data and published data on PGD2 receptor expression we propose a tuft cell- ILC3 communication axis via PGD2.

034 Santoni

Pseudomonas aeruginosa infection reveals a Caspase-1-dependent neutrophil pyroptosis pathway that restrains damaging Histone release

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Neutrophils mediate essential immune and microbicidal processes. Consequently, to counteract neutrophil attack, pathogens have developed various virulence strategies. Here, we showed that Pseudomonas aeruginosa (P. aeruginosa) phospholipase ExoU drives pathological NETosis in neutrophils. Surprisingly, inhibition of ExoU phospholipase activity uncovered a fully functional Caspase-1-driven pyroptosis pathway in neutrophils. Mechanistically, activated NLRC4 inflammasome promoted Caspase-1-dependent Gasdermin-D activation, IL-1β cytokine release and neutrophil pyroptosis. Whereas both pyroptotic and netotic neutrophils released alarmins, only NETosis liberated the destructive DAMPs Histones, which exacerbated Pseudomonas-induced lethality in mice. To the contrary, subcortical actin allowed pyroptotic neutrophils to physically limit poisonous inflammation by keeping Histones intracellularly. Finally, murine models of infection highlighted that both NETosis and neutrophil Caspase-1 contributed to P. aeruginosa spreading. Overall, we established the host deleterious consequences of Pseudomonas-induced-NETosis but also uncovered an unsuspected ability of neutrophils to undergo caspase-1-dependent pyroptosis, a process where neutrophils exhibit a self-regulatory function that limit Histone release.

035 Lopez-Rodriguez

Involvement of the inflammasomes in the pathophysiology of traumatic brain injury: use of the selective NLRP3 inhibitor (MCC950) as acute treatment.

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Traumatic brain injury (TBI) is the result of a mechanical insult to the brain that produces hematoma, haemorrhage, contusion, and disruption of the blood-brain barrier (BBB), which leads to brain edema formation and neurological impairments. Most of the cases in humans show closed-head injuries and for this reason, we used the murine weight-drop model, which induces closed-head trauma and mimics some symptoms found in humans.

Neuroinflammation is a key feature of TBI, but the underlying molecular mechanisms are poorly understood. Inflammasomes are intracellular multi-protein complexes that upon activation triggers caspase 1-mediated maturation of interleukin IL-1β and IL-18. Here we show an upregulation of the mRNA levels of Aim2 and NLRP3 inflammasomes at 24h after the lesion and the following microglia and astrocytes activation that led to an increase in the expression of inflammatory cytokines: Il1-beta, Tnf-alpha, Il6, Ccl5, and Ccl2. It is known that NLRP3 is a major driver of neuroinflammation and neurobehavioral deficits after TBI. We used the selective NLRP3 inhibitor (MCC950; 3mg/Kg, i.p.) one hour after TBI and showed an improvement in the BBB integrity, edema formation, and neurological deficit suggesting that inflammasomes play an important role in the sterile inflammatory response in astrocytes and microglia during TBI.

036 Fraser

Investigating the role of deubiquitinases during the mycobacterial infection-driven inflammatory process

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Mycobacterium tuberculosis (Mtb) is the causative agent of tuberculosis and ranks in the top five global causes of death by infectious disease. Increasing drug resistance necessitates the development of new therapeutic avenues. Mtb has evolved multiple mechanisms to manipulate the host's innate immune responses, including targeting the host ubiquitin system. Deubiquitinating enzymes (DUBs) are an important family of enzymes that regulate ubiquitin-mediated pathways. Here, we investigated the contribution of two DUBs, ubiquitin-specific protease (USP) 7 and USP47, to the host response to the M. bovis BCG strain. USP7 is a known regulator of NF-kB and, together with USP47, regulates NLRP3 inflammasome activation, suggesting a role for these DUBs in regulating inflammatory responses. We have found that in BCG infected THP1 macrophages, the USP7/47 inhibitor P22077 reduced BCG growth, indicating a requirement for USP7/47 in mycobacterial growth. P22077 also reduced NLRP3-mediated IL-1β and IL-18 secretion in BCG-infected THP1 macrophages, which is probably due to the inhibition of BCG growth. Ongoing work explores the role of DUBs in BCG infection, as well as their contribution to infection-mediated inflammation. Better understanding of these pathways will allow improvements in vaccination and/or therapeutic interventions for tuberculosis.

037 Díaz-Pino

Understanding the regulation of the inflammasome by type I interferons

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Inflammasomes are macromolecular complexes, essential in the initiation of inflammation. Inflammasomes can be activated upon recognition of a variety of danger signals, leading to activation of the pro-inflammatory cytokines IL-18 and IL-1b. Furthermore, there are other molecules capable of fighting inflammation: interferons (IFNs). Type I IFNs can induce hundreds of interferon-stimulated genes (ISGs) that act in many processes of inflammation. While the mechanisms of inflammasome activation and type I IFNs are well characterized, how type I IFNs regulate the inflammasome is still unclear. Our data shows that type I IFNs trigger the upregulation of CASP1 and GSDMD genes, in THP-1 cells and monocyte-derived macrophages from healthy donors, but no other inflammasome components. Furthermore, we observed an increase in the protein expression of Casp1 and Gsdmd, however, this did not reflect in increased inflammasome activation in our experimental conditions. Using zebrafish as an in vivo model, we found that treatment with poly I:C, a known inducer of type I IFNs, resulted in an increased expression of CASPY, CASPY2, GSDMEa, and GSDMEb, confirming ours in vitro data. Our data points to a regulatory role of type I IFNs in inflammasome mediated responses, nonetheless, more studies are necessary to elucidate the mechanisms involved.

038 Cristaldi

Cigarette smoke induces ASC-independent and caspase-dependent activation of Gasdermin D in human macrophages

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Chronic obstructive pulmonary disease (COPD) is a leading cause of death worldwide. Cigarette smoke is a major risk factor for COPD. We previously demonstrated that cigarette smoke extract (CSE) induces NLRP3-independent activation of caspase-1 leading to impaired activation of macrophages in response to lipopolysaccharide (LPS).
Herein we further investigated the mechanisms and downstream effects of caspase-1 activation in response to CSE, alone or in combination with LPS, by evaluating gasdermin D (GSDMD) cleavage, cell permeability and cell death in human monocyte-derived macrophages (hMDMs) and THP-1 cells.
Using THP1 ASC-/- macrophages, we found that caspase-1 activation by CSE did not require ASC-dependent inflammasomes. In hMDMs CSE did not alter the expression of GSDMD (RT-qPCR and Western Blot) and induced GSDMD cleavage as demonstrated by western blot and immunofluorescence analyses, with stronger effects in combination with LPS. GSDMD cleavage was associated with increased cell permeability (propidium iodide (PI) internalization assay) and occurred in the absence of cell death (assessed by LDH release and Annexin V/PI assay). Noteworthy, these effects were reverted by the caspase inhibitor Z-VAD-FMK.
These findings show that CSE induces caspase-dependent cleavage of GSDMD and increases cell permeability in hMDMs. This may contribute to cigarette smoke-associated lung inflammation and COPD.

039 Narros-Fernández

A role for mitochondrial NCLX in NLRP3 inflammasome activation

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NLRP3 inflammasome can be activated by a wide range of DAMPs and PAMPs. NLRP3 has been proposed as a sensor of intracellular stress events that occur upon these signals. One of them is intracellular ion disbalance, mainly calcium alterations. Calcium homeostasis is regulated by ER and mitochondria through specific transporters. The mitochondrial sodium/calcium exchanger (NCLX) mediates calcium extrusion from the mitochondria. We have observed that NCLX inhibition by the molecule ITH12575 reduces IL1β release in murine BMDMs, after ATP and MSU-induced NLRP3 activation. In these conditions, NCLX inhibition reduced caspase-1 cleavage and ASC speck formation. Mitochondrial dysfunction (mitochondrial network fragmentation and a decay in mitochondrial respiration) happened upon NLRP3 activation. NCLX inhibitor partially rescued this phenotype, while reducing inflammasome activation. We have validated this target in vivo in a mice model of gout: 1 mg MSU crystals were injected subcutaneously in the paw of C57/BL6 mice, and ITH12575 (3mg/kg) or vehicle were administered i.p.. 24 hours later, mice were sacrificed and inflammation was measured. ITH12575 treatment reduced paw inflammation and IL-1β and NLRP3 proteins production in the paw tissue. We can conclude that NCLX inhibition reduces NLRP3 inflammasome activation in macrophages and in vivo in an acute gout mouse model.

040 Strandt

The role of the NLRP3 inflammasome in Langerhans cell for vitiligo pathogenesis

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Vitiligo is an autoimmune disease leading to progressive destruction of melanocytes by autoreactive CD8+ T cells. Elevated ROS levels and an inability to deal with cellular stress in melanocytes are major causes for vitiligo. Damage-associated molecular patterns (DAMP) were found to be upregulated in stressed melanocytes and are involved in vitiligo. However, the role of the innate immune system, especially of dendritic cells (DC) in vitiligo is still unclear.
Due to the fact that epidermal Langerhans cells (LC) express NLRP1 in lesional skin of vitiligo patients and mouse LC express high amounts of NLRP3, we hypothesize that LC are activated by melanocyte-stress and present melanocyte-antigens to induce autoreactive T cells.
In order to investigate the importance of LC and NLRP1/3 for vitiligo, we will establish chemically-induced vitiligo models in mouse and men. Melanocyte stress and early activation of the innate immunity, with a focus on DC, will be determined by using multicolor flow cytometry, microscopy and qPCR. The role of NLRP1/3 in LC will be investigated using a LC-depletion mouse model, bone marrow chimera models lacking NLRP3 in LC and human skin explants.
Taken together, this project will shed light on the role of NLRP1/3 in LC for vitiligo pathogenesis.

041 Shankar

Delineating the mechanism of regulation of NLRP3 by Bruton's Tyrosine Kinase (BTK)

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NLRP3 is an innate immune intracellular danger sensor that detects infection-related and sterile insults. Upon activation it forms an inflammasome complex comprising NLRP3, ASC and caspase-1 that releases mature IL-1β. We previously identified Bruton's Tyrosine kinase, a well-known mediator of B cell signaling and malignancies, as a direct positive regulator of the NLRP3 inflammasome. By its kinase activity, BTK phosphorylates several tyrosine residues in the NACHT domain of NLRP3 promoting inflammasome assembly and IL-1β release. Here we sought to delineate how BTK is activated during NLRP3 priming and stimulation, enabling its interaction and phosphorylation of NLRP3. Using phosphorylation of BTK as a marker of activation, we investigated if BTK recognizes specific motifs in NLRP3 leading to both its phosphorylation and kinase activation. We also studied how the different domains of BTK contribute to NLRP3 interaction and activation. By using KO models of BTK, NLRP3 and ASC in bone marrow macrophages, we investigate how BTK's presence affects NLRP3-ASC interaction and oligomerization. Collectively this study provides a deeper understanding of BTK's post translational regulation of NLRP3 which could be exploited for targeting inflammasome related inflammatory disorders using BTK kinase inhibitors.

043 Pinilla

Development and use of novel cell-based systems to identify NLRC4-inhibitory compounds

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The NLRC4 inflammasome confers host protection against a broad set of bacterial infections; however, NLRC4 gain-of-function mutations also promote severe inflammatory disorders. Regarding this, mutations located either in the NACHT or LRR domains of NLRC4 have been described in some patients with pathology ranging from urticaria-like rashes, to fatal organ failure.

In order to identify NLRC4-targetting compounds, we have developed a reporter cell line based on the human THP1 monocytic/macrophage cells. This cell line expresses the NLRC4 agonist needle, leading to NLRC4 activation, IL1β secretion and pyroptotic cell death. Thanks to this model, we were able to launch an unbiased pharmacological screen and thus found interesting targets with inhibitory action on NLRC4-mediated cell pyroptosis. Out of 10000 compounds, we selected four molecules showing efficient inhibition of NLRC4-induced cell death, IL1B release, gasdermin D and caspase 1 cleavages in primary murine and human macrophages. Among those four compounds, one showed the strongest potency against NLRC4-activated signaling pathways, with minimal effects on the AIM2, NLRP1 and NLRP3 inflammasomes. We now aim at characterizing the specific targets of our identified molecules and to challenge their efficiency in the frame of NLRC4 gain of function mutations both in human and mouse settings.

045 Mangan

NLRP3 is a sensor of macropinosome dysfunction driven by Clostridioides difficile toxin B

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Inflammasomes are a family of cytosolic immune sensors that initiate pro-inflammatory cytokine release and cell death on detection of pathogenic and danger associated stimuli. Of the inflammasomes NLRP3 is the best characterised and is activated by a diverse range of cellular stresses. In this study we demonstrate a novel role for NLRP3 as a general sensor for the large clostridial toxin family, including toxin B (TcdB) from Clostridium difficile. Using primary human macrophages, we determined that TcdB and other large clostridial toxins activated NLRP3 by inhibiting maturation and resolution of macropinosomes, causing aberrant accumulation of macropinosomes in the cytosol. This prevented efficient removal of extracellular fluid, triggering sodium dependent cell swelling and potassium expulsion, resulting in activation of NLRP3. Of note, TcdB driven macropinosome accumulation was entirely independent of its enzymatic activity. Furthermore, we found that overproduction of macropinosomes by bacterial virulence factors could similarly activate NLRP3 in a sodium dependent manner. These findings demonstrate the first pathway described for monitoring dysfunction of macropinocytosis and identifies NLRP3 as a sensor of endosomal function by monitoring efficient egress of endosomal sodium from the cytoplasm.

046 Theofani

TFEB signaling attenuates NLRP3-driven inflammatory responses in severe asthma.

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Background: NLRP3-driven responses by inflammatory monocytes (IMs) are critical drivers of asthma pathogenesis. Stimulation of autophagy and its controller, transcription factor EB (TFEB), restrain NLRP3 activation in IMs. Still, the effects of autophagy on IM responses in human asthma remain unexplored.
Methods: Peripheral blood CD14+ monocytes from asthmatic patients (n=61) and Healthy Controls (HC) (n=37) were stimulated with LPS/ATP and/or rapamycin. NLRP3-associated ASC specks, caspase-1 activation, IL-1β and IL-18 levels, mitochondrial function, ROS release and mTORC1 signaling were examined. Using a SA model, we investigated the effects of TFEB activation on asthmatic disease phenotype through therapeutic administration of trehalose and in mice with myeloid cell-specific TFEB-overexpression.
Results: We observed increased ASC specks, IL-1β and IL-18 secretion, mitochondrial depolarization and ROS accumulation, concomitant with impaired autophagic flux in IMs that correlated with asthma severity. Autophagy induction failed to inhibit NLRP3-driven IMs responses in SA, associated with enhanced mTORC1 and decreased TFEB signaling. In vivo trehalose administration and/or TFEB overexpression in myeloid cells restored impaired autophagy, attenuated NLRP3-driven airway inflammation and ameliorated SA manifestations.
Conclusions: Our studies uncover a crucial role for TFEB signaling in suppressing dysregulated IM responses and linked asthmatic disease, that can be harnessed for the management of SA.

047 Planès

Inflammasome cell-based assays identify SARS-CoV-2 protease-inactivated Gasdermin-D as a way to counteract NLRP1 inflammasome-mediated viral restriction

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Inflammasomes are crucial innate immune complexes that trigger inflammatory and anti-microbial-responses. However, a dysregulated inflammasome-activation promotes pathological inflammation. Regarding this, exacerbated cytokine production and lung tissue damages observed in COVID-19 patients suggest that inflammasomes might regulate various steps of SARS-CoV-2 infection and/or pathology. Studying inflammasome-response is challenging as the majority of cancer cell-lines have lost the expression of various inflammasome-related effectors. To circumvent this limitation, we have reconstituted the inflammasome-pathway in a A549 lung cell-line engineered to be permissive to SARS-CoV-2 (A549-hACE2-TMPRSS2). Surprisingly, we found that NLRP1-reconstituted lung epithelial cells respond to SARS-CoV-2 infection. Specifically, NLRP1 triggers a Caspase-1/Gasdermin D-dependent cell-death. Although the mechanism by which SARS-CoV-2 activates the NLRP1 response is not fully understood, we show that such response restricts SARS-CoV-2 replication. Finally, we found that SARS-CoV-2 protease 3CL (NSP5) counterbalances NLRP1-dependent pyroptosis by cleaving and inactivating Gasdermin-D, hence favoring viral replication. Thus, we have identified that NLRP1 inflammasome promotes early viral restriction in lung epithelial cells, a process lowered by 3CL protease-inactivated Gasdermin D. In addition, we have developed a new cell-based model that allows the study of inflammasome pathway in the context of SARS-CoV-2 infection and could be used to screen molecules inhibitor for therapeutic applications.

048 Oda

Sharpenia, a novel autoinflammatory disorder caused by dysregulation in TNF-mediated cell death

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The LUBAC consists of HOIP, HOIL1, and SHARPIN, and is essential for linear ubiquitination. Patients with HOIP and HOIL1 deficiencies present with immunodeficiency, autoinflammation and glycogen storage; however, the role of SHARPIN in human disease remains unknown. Here we identified a homozygous frameshift mutation c.220dupC in SHARPIN in a boy who presented with polyarthritis, parotitis, colitis and hepatic glycogen deposition. Patient cells demonstrated no detectable SHARPIN and reduction of HOIP and HOIL1, and reduced induction in the canonical NF-κB. We then observed increased apoptosis ex vivo and in vivo in all three LUBAC-deficient samples. Of note, the extent of apoptosis correlated with the severity of the disease. Anti-TNF therapy achieved the complete resolution of inflammation, which further corroborates the role of TNF-mediated cell death in the pathogenesis of autoinflammation. Intriguingly, in the LUBAC patients we further noted a substantial defect of germinal center formation in adenoid and lymph node. We hypothesize that this is due to aberrant B cell death caused by the loss of LUBAC. We identified the first case of human SHARPIN deficiency in a patient with autoinflammation and subclinical immunodeficiency, which we denote as sharpenia. We re-define human LUBAC deficiency as a cell-death mediated disorder.

049 Liu

Discovery and Development of VTX2735: A Novel, Potent, Selective Inhibitor of Wild Type and Mutant NLRP3 inflammasome Activation

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Efforts to discover and develop potent and selective NLRP3 inhibitors having activity against both wild-type (WT) NLRP3 and disease-relevant mutants will be disclosed. The lead compound from the series, VTX2735, inhibits WT NLRP3 in human monocytes with an IC50 of 2 nM and has an IC50 < 100 nM in an ex vivo human whole blood assay measuring IL-1b production in the presence of NLRP3 specific stimuli. VTX2735 is highly specific and shows no effects on related inflammasome pathways (NLRC4, AIM2) or the NF-κB pathway that regulates IL1B and NLRP3 transcription. VTX2735 is also highly efficacious (oral dosing, ED50 < 1 mg/kg) in pharmacologically relevant in vivo rodent models of inflammasome-driven inflammation.
VTX2735 also potently inhibits of all tested mutant strains of NLRP3 from cryopyrin-associated periodic syndrome (CAPS) patients, with IC50 values ranging from 14 to 166 nM. CAPS is a group of rare autoinflammatory disorders with autosomal gain of function mutations in the NLRP3 gene, and broad inhibition of these constitutively active mutants is important for developing a wide-spectrum CAPS therapy. In addition to CAPS, VTX2735 also offers the potential for the treatment of systemic inflammatory diseases such as cardiovascular diseases and fibrotic diseases.

050 Johnson

Bacterial gasdermins

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I will describe our discovery of gasdermin homologs in bacteria and how they execute cell death. Crystal structures of bacterial gasdermins reveal a minimal pore-forming domain that is stabilized in the inactive state by self-palmitoylation. Despite lacking the large autoinhibitory C-terminal domain present in mammalian gasdermins, bacterial gasdermins are activated by dedicated proteases that catalyze site-specific cleavage and removal of a short inhibitory C-terminal peptide. The majority of these proteases are related to caspases and several are fused to repeat or NACHT domains, suggesting that they sense environmental cues to directly initiate proteolysis, in a manner that is functionally homologous to inflammasomes. Release of autoinhibition induces assembly of >200 Å pores that form mesh-like structures and disrupt membrane integrity. Our results demonstrate that caspase-mediated activation of gasdermin pore formation is an ancient form of regulated cell death shared across the tree of life.

051 Auger

Beyond Phenotypic Screening: Direct target assays identify novel NLRP3 inhibitors with diverse structural and pharmacological features including brain penetrance

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NLRP3 is a key inflammasome mediating the maturation of IL-1b and IL-18 that is activated by numerous sterile inflammatory stimuli and for which hyperactivation has been linked to the pathogenesis of many inflammatory diseases. Recently it has become clear that neuroinflammation driven by NLRP3 activation in microglia is a key driver of several neuroinflammatory and neurodegenerative conditions. Since the discovery of the selective inhibitor MCC950, various derivatives have emerged and some of them are currently being tested in clinical trials. However, all publicly disclosed NLRP3 inhibitors being pursued in the clinic have extensive structural similarities. Therefore there is a need to develop compounds that are structurally distinct and have differentiated pharmacological, physiological and/or physicochemical properties, including high levels of brain penetration. To identify structurally distinct NLRP3 inhibitors, we employed the unique approach of screening for compounds that bind directly to purified monomeric NLRP3 protein. Hit triaging using various in vitro and in vivo systems allowed us to identify multiple functional series with fully differentiated structural features and best-in-class pharmaceutical properties including brain penetration. Multiple series demonstrate robust modulation of NLRP3 activation in vivo and are currently being assessed in various pre-clinical efficacy models.

053 Griswold

Mechanisms of DPP8/9 inhibitor-induced NLRP1 and CARD8 inflammasome activation

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NLRP1 and CARD8 are related inflammasome-forming proteins with shared domain architecture. Both proteins undergo constitutive autoproteolysis to generate non-covalently associated fragments, with the autoinhibitory N-terminal fragment (NT) repressing the inflammatory C-terminal fragment (CT). Certain pathogenic and pharmacologic agents, including inhibitors of the serine dipeptidases DPP8/9, stimulate proteasome-mediated NT-degradation, releasing the CT from autoinhibition. However, a second checkpoint exists downstream of the proteasome. DPP9 and full-length NLRP1 (or CARD8) capture and quench free CT, thereby setting a threshold of free CT required for inflammasome formation. These ternary complexes are similar, but only NLRP1-CT interacts with the DPP9 active site and is directly displaced by DPP8/9 inhibitors in vitro. Here we developed and applied chemical biology strategies, including the degron-based dTAG system and unpublished chemical probes, to disentangle the relative contributions of NT-degradation and CT-displacement in inflammasome activation. We found that both degradation and displacement can contribute to activation of the CARD8 and NLRP1 inflammasomes, but that displacement is not required for CARD8 activation.

054 Chen

Allergen protease-induced Gsdmd p40 controls IL-33 secretion

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Interleukin 33, an epithelial cell-derived cytokine that responds rapidly to environmental insult, has a critical role in initiating airway inflammation. However, the molecular mechanism underlying IL-33 secretion following allergen exposure is not clear. Here, we demonstrated that Gasdermin D functions as a conduit for IL-33 secretion following allergen protease exposure. Gsdmd was rapidly cleaved into a functional neo-form, the N-terminal p40 fragment, when cells were exposed to allergen proteases. This generation of the p40 Gsdmd fragment was independent of inflammatory caspases-1/11, as it could not be inhibited by caspase-1 and caspase-11 deficiency in cells. The functional p40 NT-Gsdmd fragment directly contributed to the secretion of both nuclear full-length form and cytosolic mature form of IL-33. Blocking the generation of p40 by amino acid mutation or deletion of residues 308-313 (ELRQQ) in the Gsdmd sequence could efficiently prevent IL-33 release in murine cells. In mice, Gsdmd deficiency prevented IL-33 release and hindered the activation of ILC2s, thus alleviating airway inflammation and lung tissue damage after stimulation with HDMs or papain. Our findings uncovered a mechanism of Gsdmd-mediated IL-33 release under allergen exposure and offer insight into Gsdmd cleavage prevention as a potential approach to reduce allergic airway inflammation.

055 Magnotti

Endogenous steroid catabolites reveal a novel mechanism of Pyrin inflammasome activation

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Pyrin inflammasome activation occurs in a two-step process. The first step is regulated by the dephosphorylation of pyrin downstream of RhoA inhibition while the second step is dependent on microtubule integrity but it is still poorly understood. To better understand the step 2 mechanisms, we screened two chemical libraries to identify compounds triggering the activation of a pyrin variant (p.S242R), constitutively activated for the step 1. We identified two endogenous steroid catabolites as novel Pyrin inflammasome activators. At low doses, they activate pyrin only upon manipulation of pyrin dephosphorylation while at high doses, they are sufficient to trigger WT pyrin inflammasome activation, in the absence of Rho A inhibition. These catabolites specifically activate the human Pyrin inflammasome in a B30.2 domain-dependent manner and represent the first endogenous compounds described to activate the Pyrin inflammasome. Furthermore, they reveal a novel mechanism of pyrin inflammasome activation and may shift the paradigm of the pyrin inflammasome from a microbial toxin/effector sensor to a sensor of steroid homeostasis-altering molecular processes. Importantly, low doses of these catabolites activate the inflammasome in patients with Pyrin-Associated Autoinflammation with Neutrophil Dermatosis (PAAND) suggesting that they could participate in the severe inflammation observed in these patients.

056 Rastogi

Mycobacterium tuberculosis inhibits the NLRP3 inflammasome activation via its phosphokinase PknF.

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Mycobacterium tuberculosis (Mtb) has evolved to evade host innate immunity by interfering with macrophage functions. IL-1β is secreted by macrophages after the activation of the inflammasome complex and is crucial for host defense against Mtb infections. Here we show that Mtb is able to inhibit NLRP3 inflammasome activation. We identified the serine/threonine kinase PknF as one protein of Mtb involved in the NLRP3 inflammasome inhibition, since the pknF deletion mutant of Mtb induces increased production of IL-1β in BMDMs. The increased production of IL-1β was dependent on NLRP3, the adaptor protein ASC and the caspase-1, as revealed by studies performed in gene-deficient BMDMs. Additionally, infection of BMDMs with the pknF deletion mutant resulted in increased pyroptosis, while the IL-6 production remained unchanged compared to Mtb-infected cells, suggesting that the mutant did not affect the priming step of inflammasome activation. In contrast, the activation step was affected since potassium efflux, chloride efflux and the generation of reactive oxygen species played a significant role in inflammasome activation and subsequent pyroptosis mediated by the Mtb pknF mutant strain. In conclusion, we reveal here that the PknF of Mtb plays an important role in innate immune evasion through inhibition of the NLRP3 inflammasome.

057 Zhu

Priming of NLRP3 inflammasome activation by Msn Kinase MINK1 in Macrophages

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The NLRP3 inflammasome is essential in inflammation and inflammatory disorders. Activation of the inflammasome is differentially regulated by phosphorylation at various sites on NLRP3. Ser 725 is a phosphorylation site on NLRP3 depicted in multiple inflammasome activation scenarios, yet the importance as well as the regulation of this site has not been clarified. In this study, we reveal that the phosphorylation of Ser 725 is an essential step for the priming of NLRP3 inflammasome in macrophages. We also show that Ser 725 is directly phosphorylated by Misshapen (Msn) / NIK-related kinase 1 (MINK1), depending on the direct interaction between MINK1 and NLRP3 LRR domain. MINK1 deficiency leads to reduced NLRP3 activation and dampened inflammatory responses in mouse models of acute sepsis and peritonitis. Moreover, reactive oxygen species (ROS) upregulate the kinase activity of MINK1 and subsequently increase inflammasome priming through NLRP3 Ser 725 phosphorylation. Eliminating ROS suppressed NLRP3 activation and reduced both sepsis and peritonitis symptoms in a MINK1 dependent manner. Altogether, our study unveils a direct regulation of the NLRP3 inflammasome by Msn family kinase MINK1 and suggests that modulating the kinase activity of MINK1 could be a potential intervention strategy for inflammasome-related diseases.

058 Chauhan

Canonical inflammasomes promote caspase-1 and gasdermin D-mediated membrane permeabilization and interleukin-1β secretion in neutrophils

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Neutrophils are the most prevalent immune cells in circulation. Despite emerging evidence suggesting a role for the non-canonical inflammasome in neutrophils, a systematic analysis of the repertoire of functional neutrophilic inflammasomes has not been reported. Here, we show that neutrophil-restricted (MRP8/S100A8-Cre-driven) expression of the CAPS disease-associated Nlrp3A350V mutant in mice suffices for inflammatory pathology in vivo. We demonstrate that caspase-1 is dispensable for mitogen-induced classical NETosis, but it was required for maturation and secretion of interleukin (IL)-1β and IL-18 in response to stimuli of the canonical NLRP1b, NLRC4, NLRP3, Pyrin and AIM2 inflammasomes in neutrophils. Similarly, Salmonella Typhimurium induced caspase-1-dependent cell death and IL-1β release in human neutrophils, while PMA-induced NETosis was not affected by caspase-1 inhibition. Conversely, genetic inactivation of NADPH oxidase activity in murine neutrophils abolished mitogen-induced classical NETosis, whereas it increased neutrophil pyroptosis by canonical inflammasomes. Notably, we found that gasdermin D mediated plasma membrane permeabilization and IL-1β secretion in neutrophils in response to other canonical inflammasome stimuli. By establishing that canonical inflammasomes promote GsdmD-dependent cell permeabilization and IL-1ß secretion in neutrophils, this work reveals novel mechanisms by which neutrophils contribute to anti-microbial host defense and inflammasome-associated diseases.

060 Defourny

Picornavirus security proteins promote the activation of the inflammasome along with the release of extracellular vesicle-enclosed virions

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During virus infection, infected cells can communicate danger to surrounding (immune) cells via the release of soluble factors, as well as the production of lipid-bilayer enclosed extracellular vesicles (EV) that can contain virus material and host inflammatory mediators. Currently, it remains largely unknown whether/how inflammasome activation plays a role in the release and molecular composition of EVs produced by virus infected cells. We previously showed that during infection with the picornaviruses EMCV and CVB3 cells incorporate entire virions within their EVs, a process that alters virus-host interactions that can affect disease severity and antiviral immune responses. Here, we show that both EMCV and CVB3 infection leads to inflammasome activation in Hela cells and that this activation depends on the activity of the functionally related viral security proteins CVB3 2A and the EMCV Leader. Inflammasome activation induced by these proteins coincided with induction of pyroptosis-like cell death, induction of secretory autophagy, and the release of EV-enclosed virus, which was strongly promoted by the induction of specific EV subsets. These findings provide novel insights into the viral factors involved in inflammasome activation during infection, and its functional consequences for virus spread and virus-induced inflammation.

061 Bienvenu

Manipulation of intracellular microbial sensors by the stealth pathogen Coxiella burnetii

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Coxiella burnetii is a highly infectious pathogen causing the zoonosis Q fever. Coxiella can invade and develop in alveolar macrophages and trophoblasts. During infection, Coxiella forms an intracellular replicative niche named Coxiella-Containing Vacuole (CCV). This is driven by effector proteins secreted by the bacterium into the host cell cytoplasm via a Type 4b Secretion System (T4SS). Coxiella can dampen, in a T4SS-dependent manner, the inflammatory response of infected cells to promote intracellular persistence. Analysis of our Coxiella mutant library led to the identification of 4 mutants displaying defects in cytoprotection of the infected cells .
We focused on icaB::Tn mutant, as this gene encodes a hypothetical protein with features corresponding to secreted effectors. We confirmed that Coxiella secretes IcaB in a T4SS-dependent manner. Bioinformatics analysis indicated that IcaB possesses partial structural homology with NLRs, which is unprecedented for bacterial effectors. We thus tested the localisation and interaction of IcaB with a set of NLRs. Interestingly, IcaB colocalised with NLRP1, NLRP3, NLRP5 but not with NLRP2, NLRP10 or NLRP11. Furthermore, we could identify a direct interaction between IcaB and several NLRs by co-immunoprecipitation. This opens new perspectives in the inflammasome control by Coxiella

062 Ryan

Dimethyl fumarate and 4-octyl itaconate are anticoagulants that suppress immunothrombosis via inhibition of the non-canonical inflammasome

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There is an urgent requirement for therapeutics to treat bacterial sepsis and disseminated intravascular coagulation (DIC), a life-threatening condition characterized by immunothrombosis. Recently, the non-canonical inflammasome signalling complex which involves caspase-11 has been shown to be required for macrophage tissue factor activation and release, providing an important mechanistic link between innate immunity and coagulation. We have found that dimethyl fumarate (DMF), a derivative of fumarate used to treat multiple sclerosis and psoriasis, and the itaconic acid derivative, 4-octyl itaconate (4-OI), inhibit immunothrombosis activated by the gram-negative bacterial product lipopolysaccharide (LPS). DMF and 4-OI block LPS-induced expression of caspase-11 via inhibition of type I interferon production. They also inhibit tissue factor induction and activation via inhibition of caspase-11-mediated pyroptosis limiting thrombin generation. In vivo, DMF and 4-OI protect against thrombosis, lung damage, and lethality induced by LPS. Our results identify the clinically approved DMF and the itaconate tool compound 4-OI as anti-immunothrombotic agents that could have clinical utility in treating sepsis, DIC, and COVID-19, a syndrome of dysregulated immunothrombosis.

063 Tastan

Regulatory role of lncRNA NEAT1 in microglial NLRP3 inflammasome activation

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Inflammasome activation, an evolutionarily conserved mechanism, is one of the innate immune system's responses. The NLRP3 inflammasome is most studied among several inflammasome complexes as it contributes to various autoimmune diseases, metabolic disorders, and neuropsychiatric diseases. Once activated, the NLRP3 inflammasome complex leads to Caspase-1 activity and eventually cleavage and secretion of pro-inflammatory cytokines, IL-1β and IL-18.
Regulation of inflammasome activation occurs at many levels. Long non-coding RNAs whose length is over 200 nucleotides regulate inflammasome activation during both priming and activation steps. Nuclear Enriched Abundant Transcript 1 (NEAT 1) and other proteins NONO, SFPQ form a protein complex called paraspeckle regulating gene expression by preventing translocation of mRNAs and miRNAs into the cytoplasm.
In this study, we showed the regulatory role of NEAT1 on microglial NLRP3 inflammasome activation by utilizing the LPS and ATP model in N9 microglia. We used different molecular biological tools, including Western Blotting, RIP, FISH, and NEAT1 siRNA. Determining the role and mechanism of NEAT1 in the regulation of microglial NLRP3 inflammasome activation will lead to the development of LncRNA-targeted innovative therapeutic strategies and will shed light on the mechanisms of many neurological and neuropsychiatric diseases in which NLRP3 inflammasome activation participates in the pathogenesis.

064 Andreeva

Full-length NLRP3 forms oligomeric cages to enable NLRP3 activation

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NLRP3 is an intracellular inflammasome sensor that detects disturbances in cellular homeostasis by responding to a broad range of stimuli. Association of NLRP3 with genetic auto-inflammatory diseases, as well as systemic chronic inflammation underlies its high importance as a clinical target. Upon stimulation, inflammasome sensors often convert from an autoinhibited monomeric state to an activated oligomeric state to nucleate formation of a multiprotein inflammasome complex, which activates caspase-1 to induce cytokine maturation and pyroptosis. Here we report that even prior to activation full-length NLRP3 is not monomeric, but forms a 12-16 mer double ring cage held together by LRR-LRR interactions with the pyrin domains shielded within the assembly to avoid premature activation. Structure-guided mutagenesis reveals that double-ring cage is required for NLRP3 activation as judged by inflammasome punctum formation, caspase-1 processing and cell death. Thus, the cage formation pre-assembles NLRP3 into a "ready-to-go" oligomeric precursor, which serves as a gatekeeper for NLRP3 transport and activation.

065 Gokce

Aging drives brain inflammation in white matter

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Aging results in both grey and white matter degeneration, but the specific microglial responses to the damage is unknown. Using single-cell RNA sequencing from aged white and grey matter separately, we identified white matter associated microglia (WAM), which share parts of the disease-associated microglia (DAM) gene signature. WAM states depend on triggering receptor expressed on myeloid cells 2 (TREM2) signaling. In the aged brain, WAM form independently of apolipoprotein E (APOE), which is in contrast to mouse models of Alzheimer's disease (AD), in which microglia with WAM gene signature are generated prematurely and in an APOE-dependent pathway similar to DAM. Within the white matter, microglia frequently cluster in nodules, where they are engaged in clearing degenerated myelin. Thus, WAM may represent a potentially protective response required to clear degenerated myelin accumulating during white matter aging and disease.

066 Kopitar-Jerala

Dysregulation of AMPK/mTOR signaling in stefin B deficient macrophages results in impaired mitophagy and increased NLRP3 inflammasome activation

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Stefin B (cystatin B) is an endogenous cysteine cathepsin inhibitor, and the loss-of-function mutations in the stefin B gene were reported in patients with Unverricht-Lundborg disease (EPM1). In past study we reported that stefin B -deficient mice were significantly more sensitive to the lethal LPS-induced sepsis. We further showed increased caspase-11 gene expression and enhanced processing of pro-inflammatory cytokines IL-1β and IL-18 in stefin B KO bone marrow-derived macrophages (BMDM) upon NLRP3 inflammasome activation. Upon LPS stimulation, stefin B was targeted into the mitochondria, and the lack of stefin B resulted in the increased destabilization of mitochondrial membrane potential and mitochondrial reactive oxygene species generation. Stefin B -deficient BMDMs showed dysfunctional autophagy, as the autophagy induction via mammalian target of rapamycin (mTOR) as and AMP-activated protein kinase (AMPK) signalling pathway was suppressed in stefin B KO BMDMs. In addition, we determined decreased Ulk1 phosphorylation and impaired mitophagy in sefin B KO BMDMs. Our study demonstrated that the excessive inflammatory response to the LPS-induced sepsis in stefin B deficient mice and NLRP3 inflammasome activation is dependent on increased caspase-11 expression and impaired mitophagy, but is not associated with the increased cysteine cathepsin activity determined in stefin B KO BMDMs.

067 Dufies

Escherichia coli Rho GTPase-activating toxin CNF1 mediates NLRP3 inflammasome activation via p21-activated kinases-1/2 during bacteraemia in mice

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It is crucial for the innate immune system to detect microbes and more precisely to sense pathogens. Many pathogens target the host Rho GTPases to invade the host and hijack the immune response. Among them, the CNF1 toxin form UPEC constitutively activates the host Rho GTPases. We have previously shown that the CNF1 activity is detected and triggers an immune response depending on Caspase-1 and IL-1ß, suggesting the involvement of an inflammasome (Boyer et al., Immunity, 2011; Diabate et al., PLoS Pathogens, 2015). In our study, we aimed to identify which inflammasome is responsible for the detection of CNF1 and to decipher the signaling pathway leading to inflammasome activation (Dufies et al., Nature Microbiology, 2021).

We found that the NLRP3 inflammasome detects the Rac2 constitutive activation by CNF1. This signaling pathway involves the Rac2 effector Pak1, which phosphorylates NLRP3 on Thr659 allowing NLRP3-Nek7 interaction and inflammasome activation. Furthermore, inhibition of the Pak–NLRP3 axis decreases the bacterial clearance of CNF1-expressing UTI89 E. coli during bacteraemia in mice.

Our results reveal the importance of Pak1 and NLRP3 in controlling the bacterial burden during bacteraemia in mice.

069 Eeckhout

Dissecting the role of different cell death modes during gastrointestinal infections with attaching an effacing enteropathogens.

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Citrobacter rodentium is an attaching and effacing enteropathogen used as the principle mouse model for the clinically important human gastrointestinal pathogens enteropathogenic and enterohemorrhagic Escherichia coli, which cause a lot of morbidity and mortality in children of the developing world. Immunocompetent wild-type mice resolve a C. rodentium infection in about three weeks. The course of this transient infection is associated with inflammatory and cell death responses in the colon. However, the nature of the specific cell death pathways involved is unclear, as apoptosis, necroptosis and pyroptosis constitute three different cell death modes that may impact on gastrointestinal infections. Several reports showed that inflammasome-deficient mice are hypersusceptible to C. rodentium infection, suggesting that inflammasome-mediated pyroptosis contributes to pathogen clearance. Furthermore, C. rodentium uses a type III secretion system to inject bacterial effectors in host cells that manipulate different processes including cell death pathways, suggesting that the pathogen can curb the different cell death pathways to its advantage. Since the exact roles of these programmed cell death modes during C. rodentium infection have not been elucidated yet, we are using genetic mouse models to dissect the specific roles of these cell death modes in bacterial clearance and inflammatory pathology upon this infection.

070 Faaß, Hauke

Contribution of heptose metabolites and the cag Pathogenicity Island to the activation of monocyte/macrophage cells by Helicobacter pylori

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The gastric pathogen Helicobacter pylori activates human epithelial cells by a particular combination of mechanisms, including NOD1 and ALPK1-TIFA activation, and evading TLR activation. These mechanisms are characterized by a strong participation of the bacterial cag pathogenicity island, which forms a typeIV secretion system that enables bacteria to transport proteins and metabolites into human host cells. Building on previous findings, we determined the activity and contribution of lipopolysaccharide inner core heptose metabolites (e.g. ADP-heptose) in the activation of human phagocytic cells by H. pylori. Using human Thp-1 cells and primary human monocytes, we found that a substantial part of early phagocytic cell activation, including NF-kB activation and IL-8 production, by live H. pylori is triggered by bacterial heptose metabolites. This effect was very pronounced in lymphocytes exposed to bacterial purified lysates or pure ADP-heptose. Comprehensive transcriptome analysis of Thp-1 cells co-incubated with live H. pylori or pure ADP-heptose confirmed a signature of ADP-heptose-dependent transcript activation in monocytes/macrophages. Active bacterial heptose biosynthesis or pure ADP-heptose is required and sufficient for early innate response and NF-kB activation and has consequences for antigen presentation to T-cells. We partially reconstituted the H. pylori heptose biosynthesis pathway and confirmed the immune-activating potential of heptose metabolites.

071 Geyer

Conformational transitions in NLRP3 signalling

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NLRP3 is an intracellular sensor protein whose activation by a broad spectrum of exogenous and endogenous stimuli leads to inflammasome formation and pyroptosis. We present the cryo-EM structures of full-length NLRP3 in its native form and complexed with the inhibitor CRID3. Inactive, ADP-bound NLRP3 is a decamer composed of homodimers of intertwined LRR domains that assemble back-to-back as pentamers. The NACHT domain is located at the apical axis of this spherical structure. Molecular contacts between the concave sites of two opposing LRRs are mediated by an acidic loop extending from an LRR transition segment. Binding of CRID3 significantly stabilizes the NACHT and LRR domains relative to each other. CRID3 binds into a cleft, connecting four subdomains of the NACHT with the transition LRR. Upon activation, the PYDs of oligomeric NLRP3 are thought to form nucleation seeds that serve as the effectors for inflammasome formation. Using cryo-EM we determined the NLRP3 PYD filament structure and the directionality of ASC filament elongation. ASC adaptor elongation on NLRP3 PYD filament seeds is unidirectional, associating exclusively to the B-side of the filament. Knowing the directionality of filament growth, we derive a molecular model of an ASC speck consisting of NLRP3, ASC, and caspase-1 proteins.

072 Doglio

Inflammasome regulation of auto-inflammation induced by Otulin deficiency

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Linear ubiquitination is a post-translational modification of proteins that is counteracted by the deubiquitinase Otulin, which specifically cleaves linear ubiquitin chains. Mutations in the human OTULIN gene that inactivate the enzyme lead to increased levels of linear ubiquitinated proteins, resulting in the 'Otulin-related autoinflammatory syndrome' (ORAS), a rare and life-threatening disease. However, how the enhanced linear ubiquitination seen in these patients leads to such a widespread inflammatory disease is not clear. To investigate the underlying mechanisms, we are using a mouse model with a myeloid-specific deletion of Otulin. These mice develop a strong auto-inflammatory phenotype characterized by systemic neutrophilia, high levels of inflammatory cytokines in serum and splenomegaly, resembling human ORAS pathology. Interestingly, serum levels of interleukin (IL)-18 were elevated in these mice. Release of bioactive IL-18 depends on activation of the inflammasome, a cytosolic multiprotein complex that matures IL-18 through its caspase-1 protease activity. Remarkably, caspase-1 deletion diminished splenomegaly in Otulin-deficient mice, indicating that excessive linear ubiquitination caused by Otulin deficiency causes auto-inflammation in part by activating inflammasome pathways. Therefore, we are studying how Otulin restrains inflammasome activation and how Otulin deficiency impacts on the functions of inflammasomes in provoking auto-inflammation and in providing host defense against infectious pathogens.

073 Benli

Targeting NLRP3 inflammasome using small molecules for development of future therapeutics.

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NLR pyrin domain-containing protein 3 (NLRP3) inflammasome, is one of the most well-known inflammasomes and widely participates in various immune and cellular death pathways. This makes NLRP3 at attractive target for immune, cardiovascular, and neuropsychiatric diseases. Even though several small molecule inhibitors have been reported for NLRP3, most notably MCC950, their exact binding site and mechanism of action remain unknown. This hampers drug development focused on NLRP3.
The NLRP3 is a large and very flexible protein, which makes it very challenging for structural biology studies. The only reported experimental structure of NLRP3 is a recently solved cryo-EM structure of inactive human NLRP3 in complex with the mitotic kinase NEK7 (PDB code: 6NPY, resolution of 3.8 Å). This structure contains the only experimental structure of the nucleotide-binding NACHT domain reported to date. Using this structure, we have assessed dynamics of human NLRP3 using atomistic MD simulations and mapped several reported NLRP3 inhibitors to the binding sites. We also assessed reactivity of NLRP3 cysteine residues and their suitability for targeting with covalent inhibitors and identified several reactive cysteines, which is supported by recently published results reported for oridonin, a natural product, which is showed to target Cys279.

074 EisImayr

IL-1α and IL-1β non-redundant in tolerance versus resistance to infection

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IL-1α and IL-1β are potent pro-inflammatory cytokines, indispensable in restricting bacterial replication, limiting tissue damage and re-establishing homeostasis. Notwithstanding low sequence similarity and different regulation mechanisms, both cytokines engage the same receptor. Stimulation of cells elicit comparable cellular responses; however, for poorly understood reasons, they are not redundant in vivo. In this study, we decoupled IL-1α and IL-1β functions that drive protective responses against invasive infection with Streptococcus pyogenes. IL-1β was central for efficient bacterial containment and recruitment of neutrophils to the infection site by inducing G-CSF and establishing emergency granulopoiesis. On the contrary, IL-1α seems to be utterly dispensable for pathogen elimination but governed reprogramming of liver metabolic pathways associated with adaptation to infection. The IL-1α-dominated hepatic regulation corresponded to high IL-1α induction in the liver during infection. Conversely, IL-1β was critical for transcriptome changes in the spleen which correlated with ample IL-1β and low IL-1α expression in this tissue. Our results implicate a spatial restriction of IL-1α and IL-1β expression and bioavailability as a mechanism of their non-redundant functions in resistance to bacterial infection.

075 McKee

Priming is a fundamental mechanism of inflammasome regulation in myeloid cells

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NLRP3 inflammasome activation generally requires two signals (priming and activation), but how different priming stimuli affect NLRP3 signalling is unclear. While priming is closely associated with NLRP3, the impact of priming on other inflammasomes such as NLRP1 has not been characterised. Caspase-recruitment domain-only proteins (COPs) and pyrin domain-only proteins (POPs) regulate inflammasome activation, but our knowledge of their contribution to inflammasome priming is incomplete.
Using primary mouse and human macrophages and human induced pluripotent stem cell-derived macrophages, we investigated the effectiveness of different priming stimuli on inflammasome activation and COP/POP expression. The role of type I interferon (IFN) signalling was characterised using the JAK inhibitor Tofacitinib and Ifnar1-/- macrophages.
Bacterial and viral stimuli induced transcription-dependent and -independent priming of NLRP3, respectively, but all stimuli triggered formation of ASC specks, indicating inflammasome formation. In contrast, long-term LPS priming limited NLRP3 and NLRP1 activation which was dependent on type I IFN signalling. Microbial stimuli and IFN signalling also increased COP/POP expression, suggesting COPs and POPs potentially act in a negative feedback mechanism to prevent excessive inflammasome activity.
These findings highlight the importance of priming in inflammasome regulation and the role of IFN signalling, which drives tolerance to long-term LPS priming.

076 McElwain

Investigating a Role for the NLRP3 Inflammasome in the Pathophysiology of Gestational Diabetes Mellitus

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Aim:
To investigate Gestational Diabetes Mellitus (GDM)-mediated NLRP3 inflammasome activation in placental and omental adipose tissue.
Methods:
IL-18 and IL-1β release were quantified in placental and visceral omental tissue explant cultures from GDM (n=8) and matched controls (n=7). GDM explant cultures were treated with 1μM MCC950 and 1mM L-Ergothioneine. Infiltrating macrophage populations generating mitochondrial reactive oxygen species (mROS) were characterised by flow cytometry. Statistical analysis was performed with GraphPad Prism 8.
Results:
There was no significant difference in the concentration of IL-1β and IL-18 released from placental or omental adipose tissue explant cultures from GDM and matched controls. Treatment of GDM placental explants with 1μM MCC950 significantly reduced both IL-1β (169.8pg/ml ± 63.41pg/ml vs. 242.1pg/ml ± 64.06pg/ml, p=0.002) and IL-18 (7.25pg/ml ± 1.07pg/ml vs. 14.87pg/ml ± 2.90pg/ml p=0.01). Treatment of GDM omental explants with 1μM MCC950 (6.98pg/ml ± 1.14pg/ml vs. 11.45pg/ml ± 1.97pg/ml) or 1mM L-Ergothioneine (5.57pg/ml ± 0.99pg/ml vs. 11.45pg/ml ± 1.97pg/ml) significantly reduced IL-18 release respectively. While M1 and M2 macrophage phenotypes were evident in both placental and omental tissue there was no significant differences in mROS production between study groups.
Conclusion:
NLRP3 activation can be attenuated by MCC950 and L-Ergothioneine treatment in both placental and omental tissue.

077 Hochheiser

Transition of NLRP3 PYD nucleation seeds to ASC filament elongation reveals the directionality of ASC speck growth

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Inflammasomes sense intrinsic and extrinsic danger signals to trigger inflammatory responses and pyroptotic cell death. Homotypic pyrin domain (PYD) interactions of inflammasome forming Nod-like receptors with the adaptor protein ASC mediate oligomerization into helical filamentous assemblies. These supramolecular organizing centers recruit and activate caspase-1, which results in cytokine maturation and pyroptotic cell death. The molecular details of the critical step in signal transduction of inflammasome signaling, however, remain ill-defined. We describe the cryo-EM structure of the human NLRP3 PYD filament at 3.6 Å resolution. We identify a unique pattern of highly polar interface residues that form the homomeric interactions leading to characteristic filament ends that we designate as A- and B-side, respectively. Coupling a titration polymerization assay to cryo-EM, we demonstrate that the ASC adaptor protein elongation on NLRP3 PYD filament seeds is unidirectional, associating exclusively to the B-side of the NLRP3 filament. Notably, NLRP3 and ASC PYD filaments exhibit the same symmetry in rotation and axial rise per subunit, allowing for a continuous transition between NLRP3 as the nucleation seed and ASC as the elongator. Integrating the directionality of filament growth, we present a molecular model of an ASC speck.

078 Bachovchin

Recent insights into NLRP1 and CARD8 Inflammasome Activation

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A number of pathogen- and danger-associated signals stimulate the formation of inflammasomes, which recruit and activate caspase-1 and trigger pyroptosis. The human NLRP1 and CARD8 inflammasomes are related and share a number of important features. For example, NLRP1 and CARD8 both bind to the serine proteases DPP8 and DPP9 (DPP8/9) and are activated by the DPP8/9 inhibitor Val-boroPro (VbP). Although VbP has served as an important research tool to characterize CARD8 and NLRP1, no chemical probes have yet been discovered that selectively modulate only one of these inflammasomes. Here, we will describe an overview of our recent insights into key differences between these inflammasomes, including in the regulation of their autoinhibitory N-terminal regions and their specific interactions with DPP8/9. Further, we will also describe the discovery and characterization of a small molecule called CQ31 that exploits one of these differences to selectively activate CARD8 without simultaneously activating NLRP1. We expect CQ31 will now become a valuable tool to further characterize the specific biological functions and therapeutic potential of the CARD8 inflammasome.

079 Milner

The molecular mechanisms underpinning inflammasome-driven Alzheimer's disease

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Alzheimer's disease (AD) is the most common neurodegenerative disease worldwide. Despite being first observed over 100 years ago, the precise triggers and drivers of AD are still unclear and effective AD treatments are not available. Many hypotheses for AD pathology focus on amyloid plaque accumulation and tau hyperphosphorylation. However, clinical trials targeting these proteins have shown limited success in treating AD. Emerging research suggests that age-related inflammation may be a key driver in AD onset and pathology, and that anti-inflammatory drugs may provide therapeutic benefit. In particular, the NLRP3 inflammasome is identified as a primary driver of age-related neuroinflammation. This project aims to elucidate the molecular mechanisms of NLRP3-driven AD pathogenesis and progression. We present preliminary project findings for how age affects microglial NLRP3 inflammasome signalling, how microglial inflammasome signalling alters the function of neighbouring neurons, and how targeting the NLRP3 inflammasome pathway may alter AD pathogenesis and progression in murine models. With NLRP3 inhibitors currently entering Phase 2 clinical trials, this project addresses the important and timely question of whether such drugs may be beneficial for treating human AD.

080 Pamo Lawrence

Mitochondrial fission in glial cells: a potential regulator for Parkinsonian neuroinflammation

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An estimated 10 million individuals suffer from Parkinson's Disease (PD) worldwide. Although several current therapies alleviate symptoms, treatments are non-curative and lose efficiency over time. Mounting evidence suggests chronic neuroinflammation drives PD-associated neurodegeneration, however the mechanisms involved are poorly characterised. A phenomenon known as mitochondrial fission may be a critical element in this host-mediated inflammation, and remains largely unexplored in the context of neurodegeneration. A fragmented mitochondrial network is frequently associated with enhanced pro-inflammatory pathways in innate immune cells, such as microglia. For example, mitochondrial fragmentation is associated with NLRP3 inflammasome signalling in myeloid cells. Previous studies demonstrated that pharmacological inhibition of either mitochondrial fragmentation or the NLRP3 inflammasome alleviates disease pathology in animal models of PD. Therefore, we aim to further characterise the molecular mechanisms by which mitochondrial fragmentation and NLRP3 inflammasome signalling contributes to pathological inflammation in glial cells. Defining this inflammatory pathway may provide novel therapeutic targets for future treatment of human PD.

081 Burgener

Targeting inflammasomes in disease – is there a trade-off?

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Inflammasome inhibitors offer tremendous promise as new disease-modifying therapeutics. While inflammasomes mediate host defence against microbes, they also induce pathological inflammation in human diseases. Inhibitors of one inflammasome (NLRP3) are now entering Phase 2 clinical trials while broader-spectrum inhibitors that block multiple inflammasomes (pan-inflammasome inhibitors) are currently under development for clinical use in diseases that involve pathological signalling by multiple inflammasomes. But such beneficial functions of these new therapeutics might come as a trade-off, as inflammasome signalling also prevents infections. We used a suite of genetic and pharmacological approaches to modulate the activity of NLRP3 inflammasome versus multiple inflammasomes. We confirmed recent reports that blocking NLRP3 using MCC950 ameliorates the progression of chronic liver disease. We used a genetic approach (Caspase-1 genetic inactivation versus Nlrp3 knockout) to mimic the actions of pan- versus NLRP3-specific inflammasome inhibition. We discovered that, pan-inflammasome inhibition is likely to confer greater disease protection than NLRP3-specific inhibition. Inactivating one or many inflammasomes in disease is, however, likely to have the unwanted therapeutic trade-off increasing susceptible to infections, such as Salmonella typhimurium. Together, our research findings address the important question of whether inflammasome-modulating drugs have therapeutic trade-offs that are likely to impact on their clinical use.

082 Jenster

Nanobodies selectively activate the human NLRP1 inflammasome by designating it for N-terminal degradation

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The human inflammasome sensor NLRP1 exhibits a unique structure containing both an N-terminal PYD, a C-terminal CARD, as well as a FIIND that has to be autoproteolytically processed. NLRP1 is thought to be activated by N-terminal degradation resulting in the release of the CARD-containing C-terminus which is able to recruit ASC and pro-capase-1. This enables NLRP1 to detect proteolytically active pathogen factors like enteroviral proteases that cleave and destabilize its N-terminus.

We developed a system to test if N-terminal ubiquitination is sufficient to induce activation of human NLRP1: We generated and evaluated nanobodies against the PYD of human NLRP1 and linked them to the Cullin 2 E3 ubiquitin-ligase adaptor Von-Hippel-Lindau (VHL), thus designating NLRP1 for ubiquitination and likely proteasomal degradation. Expression of these VHL-nanobody fusion proteins in NLRP1/ASC-EGFP HEK 293T cells induced robust ASC-speck formation, which was not observed for control nanobody constructs. The VHL-nanobody constructs also enable us to selectively activate NLRP1 in human primary cell types evading the need of drug treatments or infection with specific pathogens.

This tool for precise NLRP1 activation will be useful to analyse the regulation and mechanism of NLRP1 activation, as well as the consequences of inflammasome formation in different cell subsets.

083 Orehek

Antitumor immunity boosted by GSDMD-induced necrosis

083

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Pyroptosis is a programmed mechanism of cellular self-destruction, stimulated by divergent pathogens and endogenous stimuli. Also known as caspase-1 dependent cell death due to gasdermin D (GSDMD) pore formation, it accommodates immunogenic properties, whose outcome results in release of activated inflammatory cytokines IL-1β and IL-18. GSDMD contains two defined domains, N-terminal pore-forming and C-terminal auto-inhibitory domain, separated by a linker region. Upon caspase-1 cleavage, N-terminal GSDMD initiates pyroptotic cell death and release of IL-1β through the GSDMD pores. The mechanism of GSDMD induced pyroptosis could efficiently be used for modulating immune response against tumor. Properly controlled formation of GSDMD pores would not only evoke cancer cell destruction but also promote T-cell infiltration into the tumor and stimulate response against tumor neo-antigens. Additionally, GSDMD treatment can be further improved when combined with clinically established cancer remedies such as checkpoint inhibitors, or mediators of the inflammatory response.

We designed various GSDMD variants and tested their pore forming ability in vitro. The foremost was further used for release of cytokines and tumor antigens in vivo in murine melanoma model.

084 Dilucca

GBP-dependent non-canonical inflammasome activation prevents B. thailandensis-induced multi-nucleated giant cell formation

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Inflammasomes are cytosolic multiprotein signaling complexes that are activated upon pattern-recognition receptors (PRRs)-mediated recognition of PAMPs or DAMPs. Their assembly activates the inflammatory caspase-1 and caspase-4/-5 (human) or caspase-11 (mouse) that induce pyroptotic cell death through the cleavage of gasdermin D. Pathogen detection also results in the production of interferons (IFNs), which fine-tune inflammasome-mediated responses. IFN-induced guanylate-binding proteins (GBPs) control activation of the non-canonical inflammasome by recruiting caspase-4 on the surface of cytosolic Gram-negative bacteria and promoting its interaction with LPS. The Gram-negative pathogen Burkholderia thailandensis infects epithelial cells and macrophages, and hijacks the host actin polymerization machinery to spread into neighboring cells. This process causes host cell fusion and formation of multinucleated giant cells (MNGCs). Here, we report that IFNgamma-priming of human epithelial cells restricts B. thailandensis-induced MNGCs formation, in a GBP1-dependent manner. Mechanistically, GBP1 does not promote bacteriolysis or impair actin-based bacterial motility, but acts by inducing caspase-4-dependent pyroptosis of the infected cell, allowing the infected cells to be quickly eliminated before bacterial spread and formation of MNGCs. This study provides evidence that interferon-induced innate immune activation, through GBP1 and caspase-4, confers protection against Burkholderia infection, potentially opening new perspectives for therapeutic approaches.

085 Hoyle

Itaconate and fumarate derivatives inhibit priming and activation of the canonical NLRP3 inflammasome in macrophages

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The NLRP3 inflammasome regulates caspase-1 activation and subsequent IL-1β release from cells of the innate immune system in response to infection or injury. Derivatives of the metabolites itaconate and fumarate, dimethyl itaconate (DMI), 4-octyl itaconate (4OI) and dimethyl fumarate (DMF), limit both expression and release of IL 1β following NLRP3 inflammasome activation. However, the direct effects of these metabolite derivatives on NLRP3 inflammasome responses in macrophages require further investigation. Using murine BMDMs, mixed glia and organotypic hippocampal slice cultures (OHSCs), we demonstrate that DMI, 4OI and DMF pre-treatment inhibit cytokine production in response to LPS, as well as inhibiting subsequent NLRP3 inflammasome activation induced by nigericin. DMI, 4OI, DMF and monomethyl fumarate (MMF) also directly inhibited biochemical markers of NLRP3 activation in LPS-primed macrophages, mixed glia and OHSCs in response to nigericin and imiquimod. The metabolite derivatives inhibited NLRP3 activation in macrophages in response to lysophosphatidylcholine, which is used to induce demyelination, suggesting a possible mechanism for DMF in multiple sclerosis through NLRP3 inhibition. The derivatives also reduced IL-1α cleavage in response to the calcium ionophore ionomycin. Together, these findings reveal the immunometabolic regulation of both the priming and activation steps of NLRP3 activation in macrophages.

086 Guy

Innate immune recognition of dsRNA in respiratory epithelial cells induces IL-1 and IL-18 secretion, cell death and GSDME cleavage

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Inflammasomes respond to infection or cellular stress to activate caspase-1 which leads to the maturation and secretion of interleukin 1 beta (IL-1β) and IL-18, and to pyroptotic cell death. A key substrate for caspase 1 is gasdermin D, whose cleavage leads to membrane pore formation to facilitate cytokine release and pyroptosis. However, the involvement of inflammasomes in RNA virus- and dsRNA-stimulated cytokine release and cell death are still being elucidated, especially with regard to epithelial cells which have a key role in early innate immune responses to respiratory viruses. In this study we demonstrate that the transfection of poly (I:C), a synthetic analog of double-stranded RNA (dsRNA), induces caspase-dependent but NLRP3-independent IL-1β and IL-18 secretion, and cell death in primary human bronchial epithelial cells. Using pharmacologic inhibition, we showed that unlike cytokine secretion, cell death is dependent on caspase-3, caspase-8 and caspase-9 but not on caspase-1. Interestingly dsRNA induces caspase-3/8/9-dependent gasdermin D inactivation but generates a gasdermin E pore forming fragment. Thus, dsRNA induces inflammasome-dependent cytokine secretion, and cell death, in human epithelial cells. Further cytokine secretion and cell death are mediated by multiple caspases and likely involve gasdermin E rather than gasdermin D pores.

087 Glück

Nanoscale organization of the endogenous ASC speck

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Classical NLRP3 inflammasome activation leads to the formation of a supramolecular assembly of ASC molecules denoted as the "ASC speck". Different models of the overall structure of the NLRP3 inflammasome and the ASC speck are reported. However experiments that involve overexpression or in vitro reconstitution may not represent the endogenous complex since central inflammasome proteins have the intrinsic tendency to form multimers.
We applied widefield, confocal and high-throughput dual-color 3D super-resolution fluorescence imaging (dSTORM and DNA-PAINT) to visualize the endogenous ASC speck following NLRP3 inflammasome activation. We observe that the complex varies in diameter between ~700 and 1000 nm and is composed of intertwined filaments which assemble into an overall spherical structure characterized by a dense core from which filaments reach out into the periphery. Using antibody as well as nanobody staining, we show that certain characteristics of the determined structure vary depending on the choice of the label. By imaging whole cells using dSTORM, we are able to sort the imaged structures into a quasi-temporal sequence which strikingly suggests that the endogenous ASC speck becomes mainly denser but not larger during its formation.
The reported results are an important contribution towards a comprehensive understanding of the endogenous inflammasome complex.

088 Marleaux

Crystal structure of the human NLRP9 pyrin domain suggests a distinct mode of inflammasome assembly

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Inflammasomes are cytosolic multimeric signaling complexes of the innate immune system that induce activation of caspases. The NOD-like receptor NLRP9 recruits the adaptor protein ASC to form an ASC-dependent inflammasome to limit rotaviral replication in intestinal epithelial cells, but only little is known about the molecular mechanisms regulating and driving its assembly. Here, we present the crystal structure of the human NLRP9 pyrin domain (PYD). We show that NLRP9-PYD is not able to self-polymerize nor to nucleate ASC specks in HEK293T cells. A comparison with filament-forming PYDs revealed that NLRP9-PYD adopts a conformation compatible with filament formation, but several charge inversions of interfacing residues might cause repulsive effects that prohibit self-oligomerization. These results propose that inflammasome assembly of NLRP9 might differ largely from what we know of other inflammasomes.

089 Singh

A Crohn's disease-associated pathobiont synergise with NSAID to promote inflammation and cell death in susceptible host via caspase-8/NLRP3 axis

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Non-steroidal anti-inflammatory drugs (NSAIDs) are believed to exacerbate inflammation in patients with inflammatory bowel disease (IBD), but the mechanisms regulating NSAID-induced symptoms are unknown. Pathobionts such as adherent-invasive Escherichia coli (AIEC) are widely prevalent in Crohn's disease (CD)-mucosa, activate the inflammasome and are considered relevant to CD pathogenesis. Caspase-8 is a protein regulating programmed cell death, intestinal homeostasis and inflammation. We hypothesise that the presence of AIEC might explain the NSAID-induced worsening in IBD. Using IL-10-/- mice, we show an aggravation of colitis in AIEC-colonised mice subsequently fed with NSAID due to reduction in barrier function and activation of pro-inflammatory cytokines, NLRP3 inflammasome, caspase-8 and the cell death executors, like caspase-3, PARP and Gasdermin D. Mice with either AIEC colonisation or NSAID feeding alone did not develop colitis. Using small-molecule inhibitors targeting NLRP3 and caspase-8, we show an amelioration in colitis due to a reduction in pro-inflammatory cytokines, M1 macrophages, cell death (apoptosis/pyroptosis) and improved barrier function. In conclusion, our findings provide evidence and mechanistic insights into how NSAID and an opportunistic CD-associated pathobiont can synergise to worsen IBD symptoms. The data suggest that caspase-8 and NLRP3 can be a potential therapeutic strategy for IBD patients with NSAID-worsened inflammation.

091 Boršič

The effect of types of NLRP3 assembly on inflammasome activation

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NLRP3 inflammasome is an important component of innate immunity that responds to a plethora of PAMPs and DAMPs. Its oligomerization enables the binding of ASC and pro-caspase-1, which becomes active and leads to the maturation of IL-1β and IL-18 and the cleavage of gasdermin D. N-terminal part of the protein forms pores, through which mature cytokines are released into the extracellular space where they cause local inflammation and pyroptosis.
Upon NLRP3 activation ASC molecules form large aggregates in the perinuclear region. It is currently unclear what kind of assembly NLRP3 forms upon activation. Similar proteins tend to form pentamers and higher order oligomers. We have previously shown that a trimer of NLRP3PYD domains is sufficient for robust activation of inflammasome. We were interested how the type of PYD domain assembly influences inflammasome activation. For this purpose, NLRP3PYD were fused to proteins with known aggregating properties. The ability of these engineered proteins to support inflammasome activation and their characteristics were tested in immortalized mouse macrophages. Our current results suggest that the type of NLRP3PYD assembly influences the kinetics of inflammasome activation.

092 Chan

Molecular activation mechanisms of the non-canonical caspase-4 inflammasome

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The non-canonical inflammasome is a critical signalling complex for immune defence against cytosolic Gram-negative bacteria. A key step in this pathway is the activation of caspase-4 proteolytic activity within the human non-canonical inflammasome complex. Caspase-4 senses bacterial lipopolysaccharide, and modulates inflammatory responses by cleaving gasdermin-D to initiate pyroptosis. The molecular mechanisms facilitating in caspase-4 activation, and the molecular determinants of protease activity, are unclear. Previous studies reported that caspase-4 activation produced a cleavage fragment (p32), but the nature and function of this cleavage event is unknown. Here, we show that caspase-4 activation requires dimerisation and auto-processing. We also determined that caspase-4 auto-cleavage regulates its substrate repertoire. Overall, our study elucidates the key molecular events underpinning signalling by the caspase-4 inflammasome.

093 El-Sayed

Computational analysis of the solution structure and dynamics of NLRP3

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NLRP3 (NLR family pyrin domain containing 3) inflammasome is a cytoplasmic complex, often observed in macrophages, that regulates the activation of inflammatory cytokines. NLRP3 inflammasome activation is implicated in the pathogenesis of a diverse range of serious conditions including Alzheimer's disease, atherosclerosis, type 2 diabetes, and cancer. There is therefore interest in inhibition of NLRP3 activation as a potential treatment. The current available structural data for NLRP3 is a cryo-EM structure (PDB code: 6NPY), released in 2019 at a 3.8 Å resolution. Based on this cryo-EM structure, we have modelled the full length NLRP3 protein using homology modelling to account for unresolved regions and the deleted pyrin domain. Microsecond molecular dynamics (MD) simulations have been used to study the structure and dynamical features of this NLRP3 monomer in water. The implications of this analysis for function and potential inhibition of NLRP3 will be discussed.

094 Kopp

Biochemical and structural characterization of GSDMD targeting nanobodies

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Gasdermin D (GSDMD) is key mediator of pyroptosis, a pro-inflammatory form of cell death that occurs upon microbial infection or tissue damage as part of the innate immune defence. Human GSDMD is activated downstream of canonical and non-canonical inflammasomes via cleavage by Caspase-1, -4 or -5. Upon caspase cleavage, the N-terminal domain of GSDMD is released, and oligomerizes to form pores in the cell membrane. As a consequence, intracellular contents including pro-inflammatory cytokines are released and eventually, pore formation results in pyroptosis. We generated GSDMD targeting nanobodies and characterized their function in cells. Importantly, two of the nanobodies prevent pyroptosis, which can be considered a strategy to dampen inflammation. Both nanobodies bind to GSDMD with high affinities and exert a stabilizing effect on the protein. Further, we determined the crystal structure of GSDMD in complex with two nanobodies at 3.0 Å resolution, providing detailed insights into the GSDMD-nanobody interaction and epitope binding. The mechanism of pyroptosis inhibition was analyzed using a fluorescence-based liposome pore formation assay. Our biochemical and structural findings open the possibility for rational optimization, including the generation of bivalent nanobodies, and may be ultimately used to target chronic inflammation.

095 Cohen

Activation of inflammasome-mediated cell death by a Vibrio T6SS

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Cell death mechanisms, such as inflammasome-mediated pyroptosis, are central to combat infections and drive inflammation. Inflammasome activation occurs in response to infection with a variety of pathogens, therefore, studying the mechanisms by which those pathogens activate the inflammasome has great immunological importance.
Many Gram-negative bacteria use a spear-like apparatus, called the type VI secretion system (T6SS), to deliver toxic effector proteins into neighboring cells. Although it was originally identified as a virulence mechanism, only few T6SSs were shown to target eukaryotic cells. Here, we set an ex vivo model using bone marrow-derived macrophages (BMDMs) to study the inflammasome activation by the marine bacterium Vibrio proteolyticus (V. proteolyticus), which encodes three clusters of T6SS. Remarkably, we found, that T6SS3 activity-induced cell death and the secretion of the pro-inflammatory cytokine IL-1b in BMDMs. Using chemical genetics and genetic tools, we revealed that activation of the NLRP3 inflammasome was responsible for this phenotype. Furthermore, we identified two novel T6SS3 effectors that were required to activate the inflammasome-mediated cell death. Systems similar to T6SS3 are found in other Vibrios, often within transposable elements, thus underscoring the yet-to-be-uncovered virulence potential of T6SSs in this genus of emerging pathogens.

096 Zhong

Non-protease based activation of the human NLRP1 inflammasome

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The human NLRP1 inflammasome is unique among inflammasome sensor proteins in terms of tissue distribution, domain arrangement and functional divergence from its rodent counterparts. Recently, several groups including ours uncovered a specialized role of NLRP1 in cell autonomous antiviral immunity in the skin and airway epithelia. With remarkable versatility, NLRP1 can sense viral proteases, long dsRNA and chemical inhibitors of cytosolic peptidases, DPP8 and DPP9. In this presentation, I will summarize the recent discoveries related to the human NLRP1 inflammasome and share the latest findings from my laboratory on protease-independent mechanisms of activation of the human NLRP1 inflammasome.

097 Ball

Oxidized Thioredoxin-1 Restrains the NLRP1 Inflammasome

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The danger signals and molecular mechanisms that control the NLRP1 inflammasome have not yet been fully established. NLRP1, like the well-studied inflammasome sensor NLRP3, has PYD, NACHT, and LRR domains, but how these domains contribute to the regulation and activation of NLRP1 remain poorly characterized. NLRP3's NACHT-LRR region directly interacts with the mitotic kinase NEK7 to license inflammasome activation, and we reasoned that an endogenous protein might similarly bind to NLRP1's N-terminal domains and modulate inflammasome activation. Here, we report that NLRP1's NACHT-LRR region directly binds the oxidoreductase thioredoxin-1 (TRX1) in its oxidized form. This interaction represses inflammasome activation, suggesting that reductive stress is a danger signal that activates NLRP1. Patient-derived and ATPase-inactivating mutations weaken this interaction and cause inflammasome hyperactivation. Overall, our results demonstrate that TRX1 integrates information about the cellular redox state to control NLRP1, revealing a new fundamental mechanism that connects cell metabolism to innate immunity.

098 Konaka

Caspase-1–mediated secretion of mitochondrial DNA-rich exosomes causes pathological inflammation in a human chronic inflammatory disorder

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Cell death defends against invasive pathogens by releasing cytoplasmic contents to induce inflammation and send warning signals to neighboring cells. However, it remains unclear how warning signals are transmitted to surrounding cells in response to noxious stimuli. Here, we show that pyroptotic cells secrete mitochondrial DNA (mtDNA) from cells via exosomes, which induce sterile inflammation to transmit warning signals. Activated Caspase-1 induces mtDNA leakage from mitochondria to cytoplasm via Gasdermin-D, as well as generation of intraluminal membrane vesicles that take up the leaked mtDNA and are secreted as exosomes, which further promote leukocyte mobilization and cytokine production via NLRP3 and TLR9. We also found that high levels of serum mtDNA-containing exosomes due to hyper-activation of Caspase-1 cause the pathological manifestations of Behçet's syndrome (BS). Collectively, this mechanism of inflammation induced by exosomes containing mtDNA provides new insights into transmission of warning signals and explains the etiology of BS.

099 Moecking

Biochemical characterization of the common NLRP1 variant M1184V

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The inflammasome sensor protein NLRP1 has recently drawn much attention as a potent facilitator of inflammation. While no directly activating ligand was described for a long time, multiple stimuli like viral proteases, dsRNA and inhibitors of the negative regulator DPP9, are now known to activate human NLRP1. In addition, a number of different mutations are described as the cause of autoinflammatory syndromes by causing hyper-activation of NLRP1. A common single-nucleotide polymorphism resulting in an amino acid substitution of methionine 1184 to valine has been described to increase autolytic cleavage within the FIIND domain, a prerequisite for NLRP1 activation. Although it does not increase inflammasome activity per se, it is known to be a risk factor for several autoinflammatory diseases. In previous work we demonstrate that this variant changes the level of inflammasome activation depending on the respective stimulus, and increases binding to DPP9. To understand the underlying molecular mechanisms, we produced recombinant NLRP1-FIIND protein and characterized its biochemical and biophysical properties. Interestingly, the exchange of M1184 for V significantly alters the oligomerization behavior of this domain. Moreover, structural modelling of the FIIND domain in its cleaved and uncleaved state provides first insights into the molecular basis of the observed effects.

100 Tsujimoto

Ragulator complex regulates inflammasome activation through interaction with HDAC6

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Inflammasomes are signaling platforms coordinated by various organelles for detecting intracellular danger signals. Inflammasome can be activated via several distinct mechanisms including lysosomal destabilization. Recently, several groups have reported the relationship between the Ragulator complex and pyroptosis, a cell death induced by inflammasome activation. However, the precise mechanisms of Ragulator complex, or lysosomes itself in activating inflammasomes are still unknown. Here we show lysosomes expressing Ragulator complex play an essential role in inflammasome activation by interacting with histone deacetylases 6 (HDAC6). We found that Lamtor1, the essential component of Ragulator complex, deficiency abrogates NLRP3 inflammasome activation in vitro and in vivo. Lamtor1 interacts with HDAC6 on the lysosomal membrane and this interaction is essential for activating inflammasome. Inflammasomes are not activated by a mutant form of Lamtor1 that can bind HDAC6, but cannot anchor the Ragulator complex on lysosomes. Furthermore, DL-all-rac-α-Tocopherol, synthetically produced form of vitamin e, inhibit Lamtor1 and HDAC6 interaction and it can alleviate inflammasome activation in vitro and in vivo. Our result demonstrates the essential role of lysosome in activating inflammasome and interaction between Ragulator complex and HDAC6 can be the good therapeutic target of inflammasome related diseases.

101 Hochheiser

Cryo-EM structure of the NLRP3 decamer bound to the cytokine release inhibitory drug CRID3

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NLRP3 is an intracellular sensor protein whose activation by a broad spectrum of exogenous and endogenous stimuli leads to inflammasome formation and pyroptosis. The mechanisms leading to NLRP3 activation and the way how antagonistic small molecules function remain poorly understood. Here we report the cryo-electron microscopy structure of full-length NLRP3 complexed with the inhibitor CRID3. Inactive, ADP-bound NLRP3 is a decamer composed of homodimers of intertwined LRR domains that assemble back-to-back as pentamers with the NACHT domain located at the apical axis of this spherical structure. Molecular contacts between the concave sites of two opposing LRRs are mediated by an acidic loop extending from an LRR transition segment. Binding of CRID3 significantly stabilizes the NACHT and LRR domains relative to each other, allowing structural resolution of 3.9 Å. CRID3 binds into a cleft, connecting four subdomains of the NACHT with the transition LRR. Its central sulfonylurea group interacts with the Walker A motif of NLRP3 and is sandwiched between two arginines from opposing sites, explaining the specificity of NLRP3 for this chemical entity. With the determination of the CRID3 binding site, specific targeting of NLRP3 for the treatment of autoinflammatory and autoimmune diseases and rational drug optimization is within reach.

102 Schiffelers

Nanobodies against GSDMD abrogate inflammatory cell death

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Nanobodies (VHHs) are single-domain antibodies that can serve as valuable tools to disentangle the steps leading to inflammasome-induced inflammation. In this project, VHHs are used to study the mechanism of GSDMD pore formation and the localization of endogenous pores. Six VHHs against human GSDMD have been identified by phage display and analysed for cytosolic binding by LUMIER assay. This yielded three VHHs capable of binding the N-terminal domain of GSDMD in the cytosol. Two VHHs were particularly interesting due to their capability to prevent pyroptosis and release of IL-1β after inflammasome activation. Biochemical analyses revealed that this was due to perturbed N-terminal oligomerisation. Inhibition of GSDMD by these VHHs initiated caspase-1/-3-dependent apoptosis as alternative cell death pathway. The structure of GSDMD in presence of two VHHs (poster Anja Kopp) nicely corroborates the findings of our cellular systems. Meanwhile, a new collection of GSDMD VHHs was generated and epitopes were binned to identify informative novel VHHs. We aim to decipher the molecular organisation of GSDMD pore formation and to determine in which order membrane insertion and oligomerisation of the N-terminal domains occurs. In addition, VHH-EGFP-expressing cell lines and fluorescent GSDMD VHHs are used to study pore formation using live cell microscopy.

103 Boucher

Cell-specific inhibition of the non-canonical inflammasome by statins

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Statins are one of the most-prescribed drugs to control cholesterol level in various medical conditions. Statins have been reported to have a protective effect during sepsis in human in various studies. Here, we explore whether the protective effect of statins in sepsis could be attributed to an indirect or direct inhibition of the non-canonical inflammasome signalling. Using murine and human cells of various origins, we measured immune responses in cells transfected with LPS or infected with Salmonella in presence and absence of statins. We found that under specific conditions, statins inhibits the non-canonical inflammasome and prevent the activation of caspase-4/11 while having no impact on canonical inflammasome signalling. Our work suggest a novel therapeutical avenue to target the non-canonical inflammasome in health and diseases.

104 Steiner

Recessive NLRC4-autoinflammatory disease reveals an ulcerative colitis locus

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[6] *Division of Clinical Immunology and Allergy, Departamento de Clínica Médica, Universidade de São Paulo, Sao Paulo, Brazil;* [7] *Center for Autoinflammatory Diseases and Primary Immunodeficiencies, IRCCS G. Gaslini, Genoa, Italy;* [8] *Center for Rare and Immunological Diseases, Hospital 9 de Julho, Sao Paulo, Brazil*

NLRC4-associated autoinflammatory disease (NLRC4-AID) is an autosomal dominant condition presenting with a range of clinical manifestations which can include macrophage activation syndrome (MAS) and severe enterocolitis. We report the first recessive mutation in NLRC4 (c.478G>A, p.A160T) causing autoinflammatory disease with immune dysregulation and find that heterozygous carriers in the general population are at increased risk of developing ulcerative colitis (UC).
With an onset in childhood, a now 60-year-old Brazilian female patient experienced recurrent episodes of systemic inflammation, chills, oral ulceration, uveitis, arthralgia, abdominal pain, diarrhea and variable skin rash. High doses of corticosteroids were somewhat effective and anti-IL-1β therapy partially controlled symptoms. While on treatment, serum IL-1β and IL-18 levels remained elevated. Genetic investigations identified a homozygous mutation in NLRC4 (A160T).
Increased ASC specking, pyroptosis and IL-1β/IL-18 secretion confirmed activation-induced pathogenicity of NLRC4 (A160T) analysed in human cell lines.
Genome wide association of NLRC4 (A160T) with UC was examined using data from the IBD exomes portal and revealed this allele to be significantly enriched in patients with UC: OR 2.546 (95% 1.778-3.644), P = 0.01305.
The novel mutation NLRC4 (A160T) can either cause recessively inherited autoinflammation and immune dysregulation or function as heterozygous risk factor for the development of UC.

105 Bauernfried

Infection of N/TERT-1 keratinocytes with Staphylococcus aureus leads to inflammasome-independent IL-1β maturation

Stefan Bauernfried [1], Katja Brezovar [1], Maria Tanzer [2], Matthias Mann [2], Veit Hornung [1, 2]
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The skin and as such keratinocytes represent one of the first lines of defense against pathogens. They not only provide passive immunity to the environment as a physical barrier, but also actively participate in host protection by triggering immune responses. One class of genetically encoded pattern-recognition receptors (PRRs), which sense pathogen-associated molecular patterns (PAMPs), are inflammasomes. Inflammasome activation leads to cleavage of the proinflammatory cytokine interleukin-1β (IL-1β) into its active form.
To investigate bacterial infections of the skin, we used the inflammasome-competent N/TERT-1 keratinocyte model. In doing so, we identified Staphylococcus aureus (S. aureus) infection as an activator of IL-1β maturation. However, the identified IL-1β cleavage product had a slightly higher molecular weight than its canonical caspase-1 cleavage product. To further narrow down the IL-1β-processing activity in the supernatant of S. aureus infected cells, we used size exclusion chromatography coupled to mass spectrometry. This led to the identification of a secreted S. aureus protease that was able to cleave IL-1β in an inflammasome-independent manner. The cleavage site was in close proximity to the canonical cleavage site used by caspase-1. This resulted in the production of bioactive and thus signaling-competent IL-1β after S. aureus infection that was independent of inflammasome activation.

106 Bertheloot

Targeting extracellular inflammasomes using ASC-specific nanobodies

Damien Bertheloot [1], Carlos W. de Souza Wanderley [2], Fraser Duthie [1], Cornelia Rohland [1], Lucas S. Ribeiro [1], Lea Jenster [1], Salie Maasewerd [1], Jennifer D. Wuerth [1], Fernando de Queiroz Cunha [2], Florian I. Schmidt [1], Bernardo S. Franklin [1]
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Inflammasomes are essential sentinels for intracellular clues of infection or cell stress and attract increasing interest as targets to treat a plethora of inflammatory diseases. We have previously discovered that ASC-specks are released among the cytosolic content of pyroptotic cells. These extracellular ASC-specks are found in the sera of patients and accumulate in tissues, where they propagate inflammation. However, the in vivo relevance of extracellular inflammasomes for disease and their potential as therapeutic targets remain unexplored. Here, we describe a single-domain anti-ASC nanobody, VHHASC that targets ASC-specks in vitro and in vivo. VHHASC disaggregates extracellular ASC-specks neutralizing their inflammatory and prion-like properties. Since they don't cross membranes, ectopic treatment with VHHASC does not block intracellular activation of the inflammasome, an important step to fight infections. However, following pyroptosis, GSDMD pores allow VHHASC access to the cytosol, where it disrupts already assembled inflammasomes. In a in vivo gout model, both systemic and local administration of VHHASC dampens the inflammation induced by intra-articular injection of MSU crystals. Together, our data provide compelling evidence that extracellular ASC specks actively contribute to inflammation in vivo and raise the possibility to target these complexes in established chronic inflammatory diseases while preserving physiological responses to infection.

107 Linder

CARD8 inflammasome activation triggers pyroptosis in human T cells

Andreas Linder [1, 2], Hannah Fischer [1], Niklas Kuhl [1, 2], Stefan Bauernfried [1], Yiming Cheng [1], Manuel Albanese [3], Christophe Jung [1], Oliver T. Keppler [3], Veit Hornung [1]
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Inflammasomes execute a unique type of cell death known as pyroptosis. Mostly characterized in myeloid cells, caspase-1 activation downstream of an inflammasome sensor results in the cleavage and activation of gasdermin D (GSDMD), which then forms a lytic pore in the plasma membrane. Recently, CARD8 was identified as a novel inflammasome sensor that triggers pyroptosis in myeloid leukemia cells upon inhibition of dipeptidyl-peptidases (DPP). Here, we show that blocking DPPs using Val-boroPro triggers a lytic form of cell death in primary human CD4 and CD8 T cells, while other prototypical inflammasome stimuli were not active. This cell death displays morphological and biochemical hallmarks of pyroptosis. By genetically dissecting candidate components in primary T cells, we identify this response to be dependent on the CARD8-caspase-1-GSDMD axis. Moreover, DPP9 constitutes the relevant DPP restraining CARD8 activation. Interestingly, this CARD8-induced pyroptosis pathway can only be engaged in resting, but not in activated T cells. Finally, we show that pyroptotic human T cells as well as myeloid cells release an as to yet uncharacterised cleavage product of Interleukin-16 (IL-16) that is distinct from the canonical IL-16 fragment.

108 Schmacke

LPS-mediated Golgi recruitment primes NLRP3 for NEK7 independent inflammasome activation

Niklas Schmacke, Moritz Gaidt, Veit Hornung
Gene Center and Department of Biochemistry, Ludwig-Maximilians-University Munich, Munich, Germany

The cytosolic protein NLRP3 forms an inflammasome in response to a large variety of sterile and non-sterile activators. Despite two decades of intensive research, the mechanisms governing this process remain incompletely understood. We and others have found the protein NEK7 to directly interact with and mediate the activation of NLRP3. However, the role of NEK7 and its position in the NLRP3 signal transduction network have not been clarified so far. We recently discovered that human cell lines and human iPS cell-derived macrophages activate the NLRP3 inflammasome independently of NEK7 when treated with LPS. This NEK7-independent pathway requires the kinase activity of TAK1, but proceeds independently of de novo protein synthesis. TAK1 activation instead leads to the recruitment of NLRP3 to the Golgi apparatus, a recently described site of inflammasome formation. Consequently, tethering NLRP3 to PtdIns4P, a lipid known to be enriched in the Golgi apparatus, bypasses NEK7 and enables priming-independent inflammasome activation in mouse and human cells. These data indicate that NEK7 plays a non-essential role in NLRP3 inflammasome priming preceding activation, possibly by inducing a conformational change in NLRP3 that renders it responsive to activating stimuli.

110 Zhou

Poly(dA:dT) activates the NLRP1 inflammasome in unprimed human keratinocytes

Jeffrey Zhou [1], Mrinal Sarkar [2], Stefan Bauernfried [3], Veit Hornung [3], Johann Gudjonsson [2], Kate Fitzgerald [1]
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Miscompartmentalized intracellular DNA is capable of activating innate immune sensors known as inflammasomes, which drive the release of IL-1 family cytokines and proinflammatory cell death known as pyroptosis. In murine BMDMs and THP-1 cells, the AIM2 inflammasome is a critical mediator of IL-1β secretion and cytotoxicity upon sensing DNA. However, AIM2 is not required for the DNA inflammasome in human primary monocytes, and human cells can initiate DNA inflammasomes through AIM2-independent mechanisms involving STING and NLRP3.

In unprimed human N/TERT immortalized keratinocytes, we observed that transfection of a synthetic DNA analog known as poly(dA:dT) can elicit the NLRP1 inflammasome independent of AIM2 and STING. Poly(dA:dT) is an AT-rich nonlinear DNA with complex branching structure, and other linear dsDNAs were incapable of activating the NLRP1 inflammasome in unprimed keratinocytes. Poly(dA:dT) activates NLRP1 through an indirect mechanism at delayed kinetics compared to dsRNA transfection, raising the possibility that a poly(dA:dT)-derived RNA intermediate is activating the NLRP1 inflammasome. Given that NLRP1 is an established sensor for long dsRNA, these findings suggest that human keratinocytes may encode mechanisms that detect a broader range of pathogenic nucleic acid patterns in the setting of viral infection, genotoxic stress, or genomic instability.

112 Palomino-Antolin

Time-dependent dual effect of NLRP3 inflammasome in brain ischemia

Alejandra Palomino-Antolin [1], Paloma Narros [1], Víctor Farré-Alins [1], Javier Sevilla Montero [2], Celine Decouty-Pérez [1], Ana Belen Lopez-Rodriguez [1], Nuria Fernández [3], Luis Monge [3], Ana Isabel Casas [4], María José Calzada [2], Javier Egea [1]
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[3] *Fluorescence Imaging Group, Departamento de Fisiología, Facultad de Medicina, Universidad Autónoma de Madrid, Madrid, Spain*
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Post-ischemic inflammation contributes to worsening of ischemic brain injury and in this process, the inflammasomes play a key role. However, participation of the NLRP3 inflammasome in ischemic stroke remains controversial. Our aims were to determine the role of NLRP3 in ischemia and to explore the mechanism involved in the potential protective effect of the neurovascular unit. WT and NLRP3 knock-out mice were subjected to ischemia by middle cerebral artery occlusion (60 minutes) with or without treatment with MCC950 at different time points post-stroke. We identified a time-dependent dual effect of NLRP3. While neither the pre-treatment with MCC950 nor the genetic approach (NLRP3 KO) proved to be neuroprotective, postreperfusion treatment with MCC950 significantly reduced the infarct volume in a dosedependent manner. Importantly, MCC950 improved the neuro-motor function and reduced the expression of different pro-inflammatory cytokines (IL-1β, TNF-α), NLRP3 inflammasome components (NLRP3, pro-caspase-1), protease expression (MMP9) and endothelial adhesion molecules (ICAM, VCAM). We observed marked protection of the blood-brain barrier (BBB), which was also reflected in the recovery of the tight junctions proteins (ZO-1, Claudin-5). Additionally, MCC950 produced a reduction of the CCL2 chemokine in blood serum and in brain tissue, which lead to a reduction in the immune cell infiltration.

113 Fernandes

Metabolic reprogramming of Mycobacterium tuberculosis infected cells is uncoupled from differential inflammasome activation

Ana Isabel Fernandes [1, 2, 3], Rute Gonçalves [1, 4], Carolina Ferreira [5, 6], Ulrike Zedler [7], Ricardo Silvestre [5, 6], Anca Dorhoi [7], Margarida Saraiva [1, 2]
[1] *i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal*
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[3] *Doctoral Program in Molecular and Cell Biology, ICBAS-Instituto de Ciências Biomédicas Abel Salazar, University of Porto, Porto, Portugal*
[4] *FCUP – Faculdade de Ciências da Universidade do Porto, Porto, Portugal*
[5] *Life and Health Sciences, Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal*
[6] *ICVS/3B's-PT Government Associate Laboratory, Guimarães, Portugal*
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Metabolic reprogramming of macrophages during infection is intimately linked to the macrophage functions required to activate immune responses and contain the pathogen. How this process occurs in the context of Mycobacterium tuberculosis (Mtb) infection is not fully understood. Mtb is the causative agent of tuberculosis, a disease associated with high heterogeneity at different levels, attributed in part to the pathogen. Previous studies from our group have linked severe forms of tuberculosis with lower cytokine responses induced by Mtb clinical isolates in macrophages. In this study, we aimed to reveal the impact of Mtb diversity on the metabolic reprogramming of immune cells. Mouse macrophages were infected with two distinct clinical isolates, one associated with severe forms of tuberculosis and low IL-1β induction (Mtb 6C4) and another with mild forms of disease and high IL-1β induction (Mtb 4I2). Metabolic reprogramming of macrophages induced by these two distinct isolates was assessed. For that, the concentrations of different metabolites and transcript abundances of metabolic enzymes were evaluated in infected macrophages through different assays. Our preliminary data indicate that the ability of the different isolates to differentially activate the inflammasome and modulate IL-1β release is surprisingly uncoupled from the metabolic reprogramming of the infected cell.

114 Pal

Mycobacterial protein-PPE2: A blessing in disguise

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Centre for DNA Fingerprinting and Diagnostics, Hyderabad, India

There is an increasing need for developing biological anti-inflammatory agents that are targeted, effective and more importantly with lesser side effects as compared to conventional drugs. In the present study a biological molecule was identified that can suppresses inflammation by inhibiting mast cells population. We found that PPE2 protein of Mycobacterium tuberculosis can suppress mast cell population and inhibit several vasoactive and fibrogenic mediators and pro-inflammatory cytokines induced by mast cell in mouse model of PAW inflammation. PPE2 was found to inhibit transcription of stem cell factor/kitl by binding to promoter region of stem cell factor, important for mast cell maintenance and migration. We also confirm that unlike NSAIDs, PPE2 administration does not cause any hepato- or renal-toxicity and remain effective for a longer duration. We have also designed and a synthetic peptide (derived form PPE2) that offers similar anti-inflammatory property by suppressing mast cell population. Thus, PPE2/peptide can be used as a potent non-steroidal therapeutic agent for treatment of inflammation and related disorders.

115 Sontyana

Mycobacterial PPE18 protein inhibits inflammasome activation by blocking caspase-1-mediated IL-1β maturation

Brahmaji Sontyana, Sangita Mukhopadhyay
Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad, India

The whole-genome sequence of Mycobacterium tuberculosis (Mtb) has identified PE/PPE family proteins which are known for mycobacterial virulence and modulation of host immune responses. A study from our laboratory has shown that PPE18 protein of Mtb interacts with TLR2 and induces IL-10 (anti-inflammatory cytokine) along with downregulation pro-inflammatory cytokines (TNF-α and IL-12). Induction of IL-10 favours a non-protective Th2-type response which enhances bacterial survival inside host. In addition, recombinantly purified PPE18 (rPPE18) administration lowered TNF-α, IL-1β, and IL-12 levels and reduce organ damage in a mouse model of septic peritonitis. All these results suggest a strong anti-inflammatory activity of PPE18. Also, IL-10 inhibits inflammasome activation by inhibiting IL-1β maturation to favour bacterial survival. Hence, we hypothesize that Mtb PPE18 may inhibit inflammasome activation by downregulating IL-1β secretion. In the present study, we observed decreased levels of mature IL-1β in macrophages infected with M. smegmatis expressing PPE18 (Msmeg-PPE18) as compared to macrophages infected with M. smegmatis harbouring pVV16 vector alone (Msmeg-pVV). In contrast, pro-IL-1β levels were higher in Msmeg-PPE18-infected macrophages compared to Msmeg-pVV-infected macrophages. Msmeg-PPE18 infection blocks activation of caspase-1 that ultimately inhibits cleavage of IL-1β. Together, we show that PPE18 inhibits inflammasome activation and helps in bacterial survival.

116 Gastaldi

In the search for new NLRP3 inhibitors: chemical modulation of the 1-(piperidin-4-yl)-1,3-dihydro-2 H-benzo[d]imidazole-2-one scaffold

Simone Gastaldi [1], Valentina Boscaro [1], Eleonora Gianquinto [1], Christina F. Sandall [2], Marta Giorgis [1], Elisabetta Marini [1], Federica Blua [1], Margherita Gallicchio [1], Francesca Spyrakis [1], Justin A. MacDonald [2], Massimo Bertinaria [1]
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The NLRP3 inflammasome is a cytosolic complex that plays a fundamental role in immune system activation. Different signals can lead to inflammasome activation. Once activated, NLRP3 undergoes a conformational change and then oligomerizes triggering the auto-proteolytic cleavage of pro-caspase-1 into the active caspase-1. The activated caspase-1 can activate IL-1β, IL-18 and to cleave the protein gasdermin-D (GSDMD) involved in the pyroptotic cell death.
In this study, we used a pharmacophore-hybridization strategy by combining the structure of the acrylic acid derivative INF39 with the 1-(piperidin-4-yl)1,3-dihydro-2H-benzo[d]imidazole-2-one substructure present in HS203873, a recently identified NLRP3 binder. A series of differently modulated benzo[d]imidazole-2-one derivatives were designed and synthesised from 2. The obtained compounds were screened in vitro to test their ability to inhibit NLRP3-dependent pyroptosis and IL-1β release in PMA-differentiated THP-1 cells stimulated with LPS/ATP. The selected compounds were evaluated for their ability to reduce the ATPase activity of human recombinant NLRP3 using a newly developed assay. Finally, computational simulations were applied for building the first complete model of the NLRP3 inactive state and for identifying possible binding sites available to the tested compounds. The analyses led us to suggest a mechanism of protein-ligand binding that might explain the activity of the compounds.

117 Pizzuto

Mitochondrial Cardiolipin Inhibits the Non-Canonical NLRP3 Inflammasome through Preventing LPS Binding to Caspase-11

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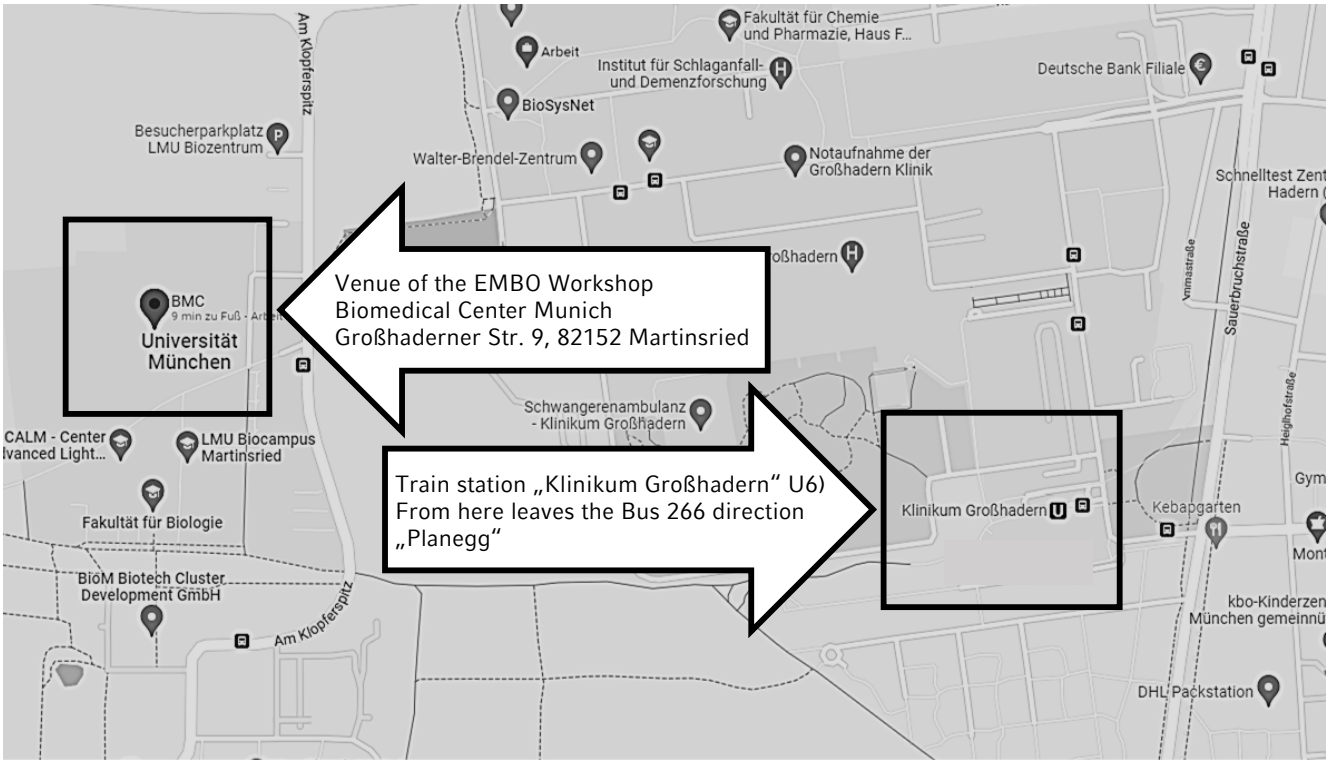
Molecular Inflammation Group, Biomedical Research Institute of Murcia (IMIB-Arrixaca), Murcia, Spain, MURCIA, Spain

Besides the canonical pathway, NLRP3 can also be activated as a consequence of caspase 11 activation by intracellular detection of bacterial lipopolysaccharides (LPS). Active casp-11 cleaves gasdermin D (GSDMD) to induce an NLRP3-independent pyroptosis, which is accompanied by a K+ efflux, with consequent NLRP3 activation and IL1β release defined as non-canonical inflammasome activation. In murine macrophages, we have observed that extracellular administered mitochondrial cardiolipin reaches the intracellular space without affecting LPS uptake but inhibits the binding of LPS to casp-11 and strongly blocked the consequent GSDMD cleavage, IL-1β processing and secretion as well as cell death. GSDMD inhibition occurred in macrophages from both WT and Nalp3-/- mice demonstrating that the action of cardiolipin is upstream of NLRP3. Cardiolipin is a lipid found in the inner mitochondrial membrane. Signals such as lipopolysaccharide priming can cause the translocation of cardiolipin to the outer mitochondrial membrane, making cardiolipin exposed to cytosolic proteins. The new highlighted ability of cardiolipin to inhibit the inflammasome suggests an unsuspected role of cardiolipin on the negative regulation of bacterial induced inflammation and might open the way for new understanding and treatment of sepsis.

Poster Author Index by Page Number

Map of the surrounding area



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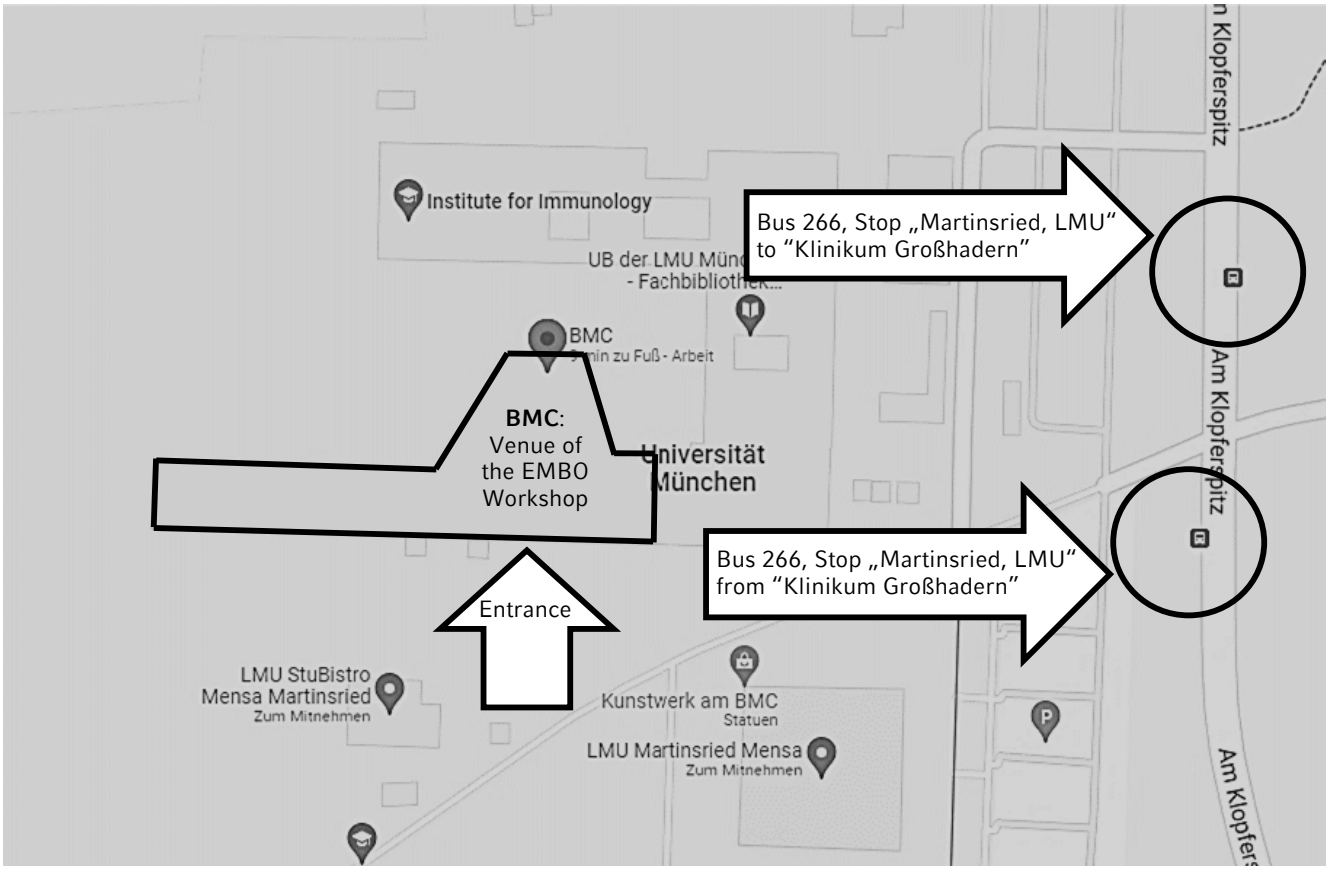

Gültig ab 13. Dezember 2020

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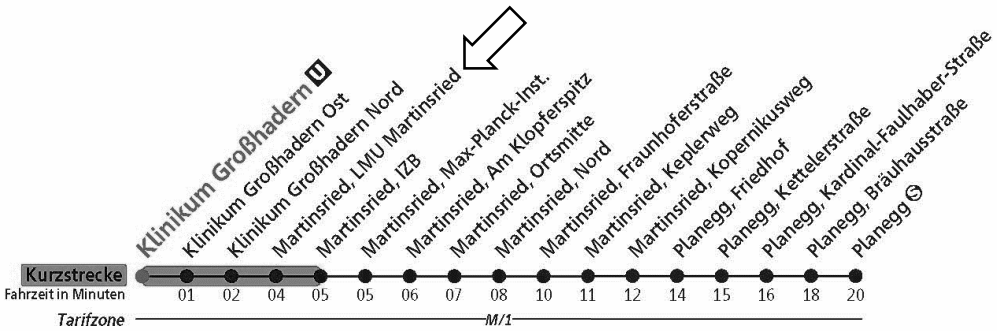

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Am 24. und 31. Dezember Betrieb wie Samstag

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Änderungen vorbehalten

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Gültig ab 13. Dezember 2020

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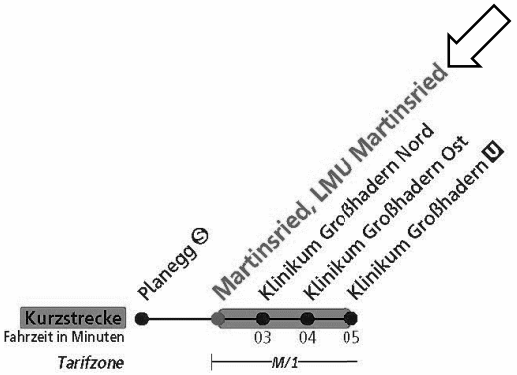
Martinsried, LMU Martinsried



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V59=Nacht Freitag auf Samstag, nicht am Karfreitag
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Subway Schedule from City Center

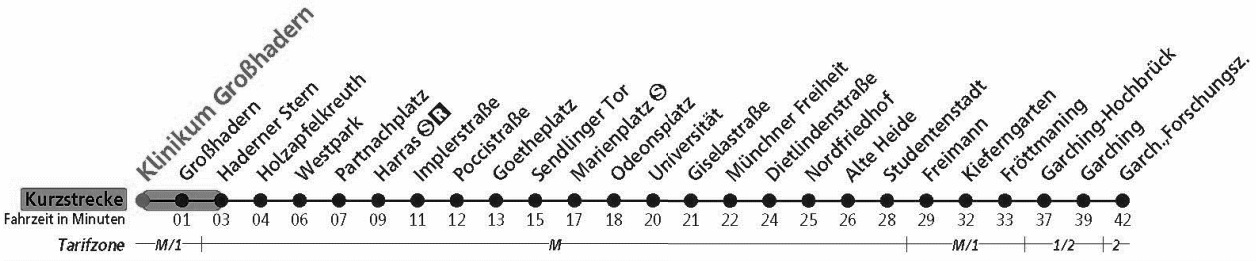
Gültig ab 13. Dezember 2020

U6

Klinikum Großhadern - Harras Ⓢ Ⓡ - Sendlinger Tor - Marienplatz Ⓢ - Odeonspl. - Münchner Freiheit - Fröttmaning - Garching-Forschungsz.

MVV

Klinikum Großhadern



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1 = bis Implerstraße
2 = bis Münchner Freiheit
3 = bis Kieferngarten
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☾ = nur Nächte Fr/Sa, Sa/So und Nächte vor Feiertagen
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In der Silvesternacht sowie den Faschingsnächten gelten Sonderfahrpläne

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Änderungen vorbehalten

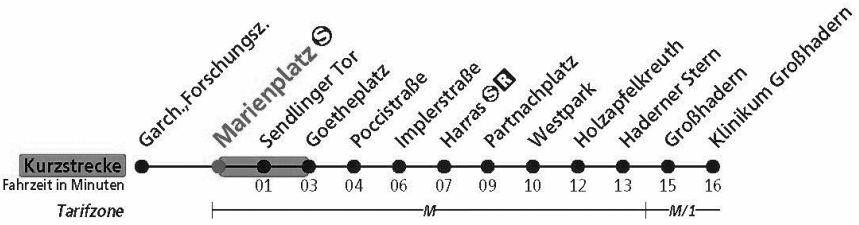
MVG, 0800 344 22 66 00 Tel.: gebührenfreie Nummer

Gültig ab 13. Dezember 2020

U6

Garching-Forschungsz. - Fröttmaning - Münchner Freiheit - Odeonspl. - Marienplatz Ⓢ - Sendlinger Tor - Harras Ⓢ Ⓡ - Klinikum Großhadern

Marienplatz Ⓢ



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6	02 12 22 32 42 46 52 56	02 12 22 32 42 46 52 56	19 39 59	6
7	02 06 12 16 22 26 32 36 42 46 52 56	02 06 12 16 22 26 32 36 42 46 52 56	19 39 52	7
8	02 06 12 16 22 26 32 36 42 46 52 56	02 06 12 16 22 26 32 36 42 46 52 56	02 12 22 32 42 52	8
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13	02 06^2 12 16^2 22 26^2 32 36^2 42 46^2 52 56^2	02 06 12 16 22 26 32 36 42 46 52 56	02 06^2 12 16^2 22 26^2 32 36^2 42 46^2 52 56^2	13
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15	02 06^2 12 16 22 26 32 36 42 46 52 56	02 06 12 16 22 26 32 36 42 46 52 56	02 06^2 12 16^2 22 26^2 32 36^2 42 46^2 52 56^2	15
16	02 06 12 16 22 26 32 36 42 46 52 56	02 06 12 16 22 26 32 36 42 46 52 56	02 06^2 12 16^2 22 26^2 32 36^2 42 46^2 52 56^2	16
17	02 06 12 16 22 26 32 36 42 46 52 56	02 06 12 16 22 26 32 36 42 46 52 56	02 06^2 12 16^2 22 26^2 32 36^2 42 46^2 52 56^2	17
18	02 06 12 16 22 26 32 36 42 46 52 56	02 06 12 16 22 26 32 36 42 46 52 56	02 06^2 12 16^2 22 26^2 32 36^2 42 46^2 52 56^1	18
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20	02 06^2 12 19 29 39 49 59	02 06^2 12 19 29 39 49 59	02 12 19 29 39 49 59	20
21	09 19 29 39 49 59	09 19 29 39 49 59	09 19 29 39 49 59	21
22	09 19 29 39 49 59	09 19 29 39 49 59	09 19 29 39 49 59	22
23	09 19 29 39 49 59	09 19 29 39 49 59	09 19 29 39 49 59	23
0	09^2 19 29^2 39 59	09^2 19 29^2 39 59	09 19 29^2 39 59	0
1	27☾ 57☾	27 57	27 57	1
2		15^1		2

1 = bis Implerstraße 2 = bis Harras Ⓢ Ⓡ ☾ = nur Nächte Fr/Sa, Sa/So und Nächte vor Feiertagen

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Notes

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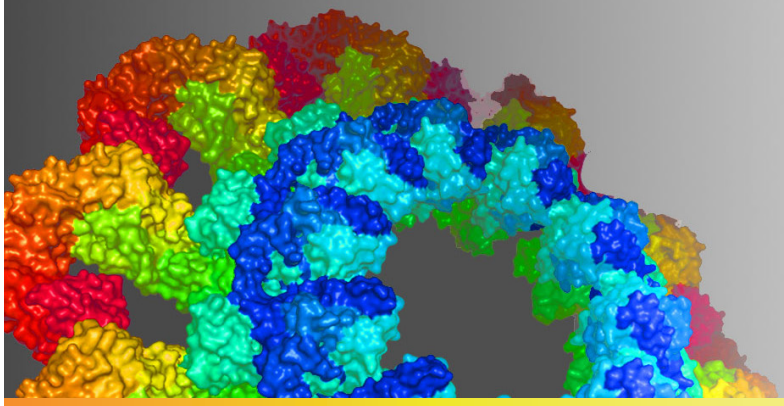

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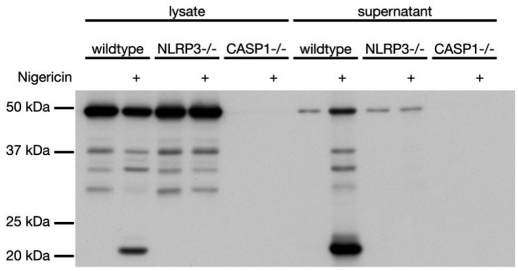
AG-25B-0006 **anti-Asc, pAb (AL177)**

AG-20B-0010 **anti-ZBP1, mAb (Zippy-1)**



METHOD: Caspase-1 was analyzed by Western blot in cell extracts and supernatants of differentiated bone marrow-derived dendritic cells (BMDCs) from wild-type, NLRP3-/- and caspase-1-/- mice activated or not by 5 µM Nigericin.

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MSU (crystals or ready-to-use)

Nigericin . Na

Inhibitors

Dapansutrile

MCC950 . Na

Priming Reagents

LPS (Broad Panel)

PMA

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