SFB / Transregio 237

Nucleic Acid Immunity

International Conference on Nucleic Acid Immunity

Dresden
Germany

October 26-28, 2023

TECHNISCHE UNIVERSITÄT DRESDEN

LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

UNIVERSITÄT BONN

DEUTSCHES HYGIENE-MUSEUM DRESDEN

Carl Gustav Carus Management GmbH

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Nucleic Acid Immunity

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Message from the organizers

Welcome!

Dear friends and colleagues,

It is our great pleasure to welcome you to the International Conference Nucleic Acid Immunity in Dresden!

Together with our co-organizers, Andrea Ablasser, Nicolas Manel, and Zhijian "James" Chen, we have compiled an exciting program on the topic of nucleic acid immunity focusing on immune sensing of nucleic acids, regulation of innate immune signaling pathways, and disease mechanisms rooted in inappropriate activation of nucleic acid sensor pathways.

The conference is sponsored by the DFG-funded Collaborative Research Center 237 (CRC237) "Nucleic Acid Immunity" based at Munich, Bonn, and Dresden (www.trr237.uni-bonn.de).

The Deutsches Hygiene Museum (www.dhmd.de) is not only a science museum, but also an open forum for the discussion of science and society, art and culture. Its permanent exhibition is dedicated to the human being and offers an experiential journey to our body and self, our thoughts and feelings.

In this unique setting, we hope that this meeting will allow for in-depth scientific exchange and spark new scientific collaborations, both in basic research and in translation.

We wish you all a successful and inspiring conference.

Best wishes,

Min Ae Lee-Kirsch, Veit Hornung and Gunther Hartmann

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We would like to thank our Sponsors supporting this conference.

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We would like to thank the German Research Foundation (DFG) for funding the Collaborative Research Center TRR237 enabeling us to organize this conference and Carl Gustav Carus Management GmbH for their assistance.

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SFB / TRR 237 Nucleic Acid Immunity

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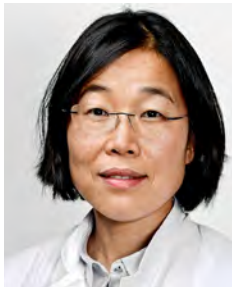
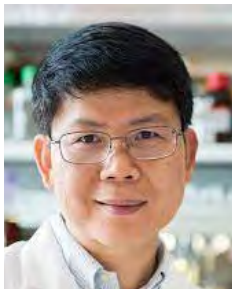
Organizing committee



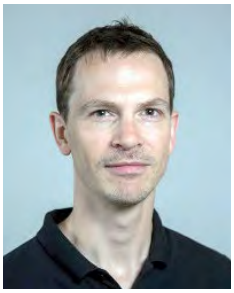
Prof.
Min Ae Lee-Kirsch

Prof.
Veit Hornung

Prof.
Gunther Hartmann



Prof.
Zhijian ‘James’ Chen



Prof.
Nicolas Manel

Prof.
Andrea Ablasser

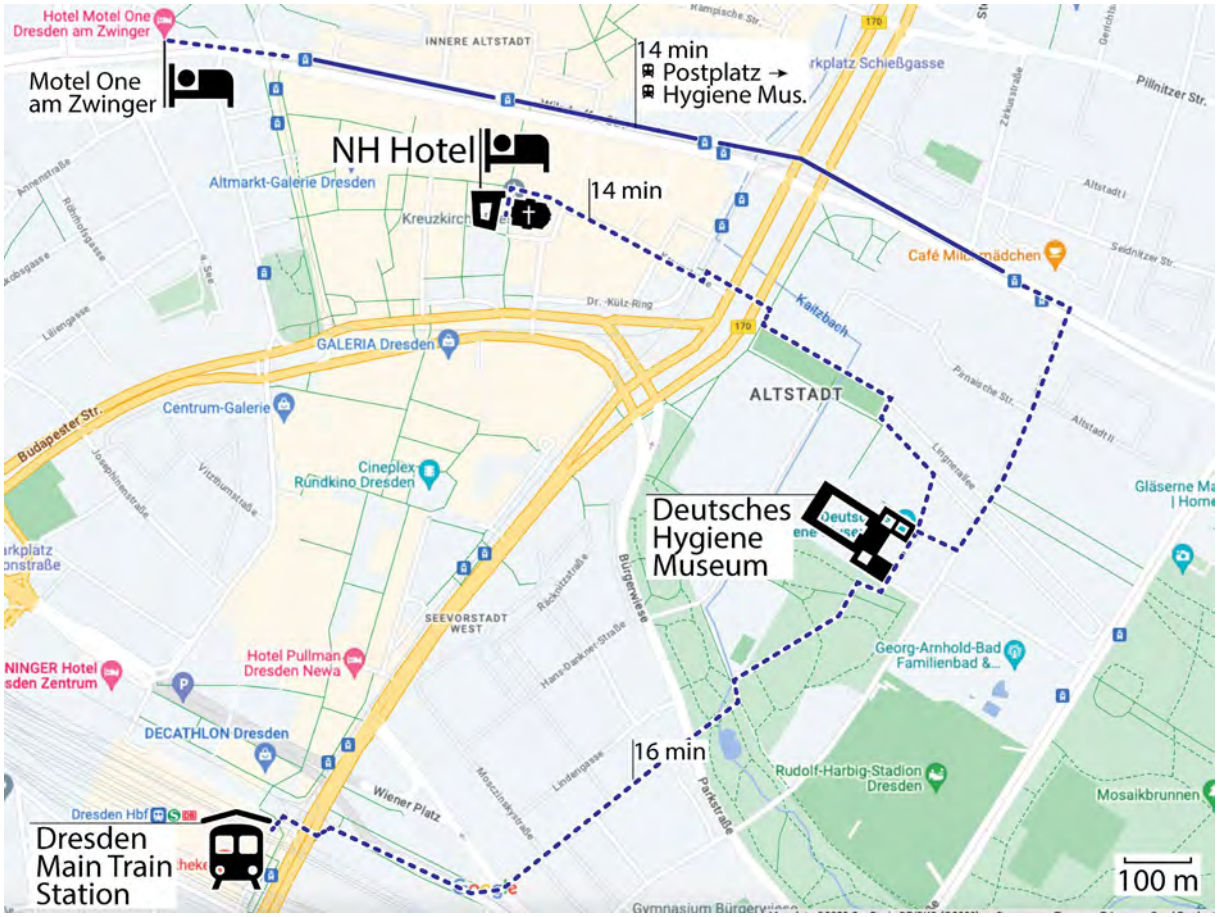
Admin team

Bianca Weissbach, TU Dresden — Conference
Dr. Simon Görgen, Uni Bonn — Registration / ConfTool
Dr. Sabine Bergelt, LMU Munich — TRR237 Nucleic Acid Immunity

Location

Deutsches Hygiene Museum Dresden
Lingnerplatz 1
01069 Dresden
+49 351 48 46 400

Visit the Exposition on Saturday afternoon. Reduced fee for Conference Participants **of 7€.**
https://www.dhmd.de/en/



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110 - Police

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Conference Program

International Conference on

Nucleic Acid Immunity

German Hygiene Museum Dresden, Germany

26th - 28th October 2023

Thursday, October 26, 2023		
08:00 - 08:45	**Registration**	
08:45 - 09:00	Welcome	
SESSION I - Mechanisms of nucleic acid sensing I		
09:00 - 09:45	**Sun Hur | Keynote Lecture**	Innate immune mechanism for self vs. non-self RNA discrimination
09:45 - 10:10	**Michaela Gack**	Two-signal concept of RIG-I-like receptor activation
10:10 - 10:35	**Veit Hornung**	Detection of endogenous RNA by Toll-like receptors
10:35 - 11:05	Coffee & Refreshments	
11:05 - 11:20	**Mauro Castello Sanjuan**	Deciphering the interactions between RNA viruses and retrotransposons in Drosophila melanogaster
11:20 - 11:45	**Simon Alberti**	Biomolecular condensates as sensors of nucleic acids
11:45 - 12:10	**Toshiyuki Shimizu**	Structural study of nucleic acid sensing Toll-like receptors
12:10 - 13:40	Lunch	incl. Poster viewing
SESSION II - Mechanisms of nucleic acid sensing II		
13:40 - 14:05	**James Chen**	Igniting an immune response with cGAS
14:05 - 14:20	**Pengbiao Xu**	Mechanism and structure of cGAS nuclear degradation
14:20 - 14:45	**Jean-Luc Imler**	cGAS-like receptor mediated antiviral immunity in Drosophila flies
14:45 - 15:10	**Glen Barber**	Identification of key nucleic acid dependent, innate-inducible genes required for cellular immunogenicity
15:10 - 15:40	Coffee	
15:40 - 16:05	**Nan Yan**	STING trafficking and signaling activities
16:05 - 16:20	**Hachung Chung**	Long 3'UTRs predispose neurons to inflammation by promoting dsRNA formation
16:20 - 16:45	**Andreas Linkermann**	Mechanisms of siRNA-mediated sensitization of ferroptosis
16:45 - 17:10	**Cynthia Sharma**	CRISPR/Cas immune systems in bacterial pathogens - from biology to applications
17:10 - 17:35	**Leonie Unterholzner**	The innate immune response to nuclear DNA damage and replication stress
17:35 - 19:30	Poster session I	
20:00	**Speakers Dinner**	

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Friday, October 27, 2023

SESSION III - Discrimination of self and non-self nucleic acids		
08:45 - 09:10	**Nicolas Manel**	Activation of intracellular innate sensors
09:10 - 09:35	**Christian Zierhut**	Innate immune self-DNA sensing and cell fate during genotoxic stress
09:35 - 09:50	**Amiram Ariel**	The BiST of burden: Harnessing biased STING agonists to enhance the resolution of inflammation and limit tissue fibrosis
09:50 - 10:15	**Jan Rehwinkel**	MDA5 guards against infection by surveying cellular RNA homeostasis
10:15 - 10:45	Coffee & Refreshments	
SESSION IV - Disorders of nucleic acid immunity		
10:45 - 11:10	**Paul Lehner**	How immunosurveillance by HUSH protects your genome from reverse genetic flow
11:10 - 11:35	**Raphaela Goldbach-Mansky**	When misspellings provide new insights: ... Lessons from patients with autoinflammatory diseases
11:35 - 12:00	**Hiroki Kato**	RIG-I-like receptors and type I interferonopathies
12:00 - 13:30	Lunch	incl. Poster viewing
13:30 - 13:55	**Simone Caielli**	An unconventional novel mechanism of IL-1b secretion that requires type I IFN in SLE monocytes
13:55 - 14:20	**Min Ae Lee-Kirsch**	UNC93B1 variants cause TLR7-dependent autoimmunity
14:20 - 14:35	**Michael Gantier**	3-base long 2'-O-Methyl oligonucleotides are potent TLR7 and TLR8 modulators
14:35 - 15:00	**Olivia Majer**	Disrupting the degradative sorting of TLR7 triggers human lupus
15:00 - 15:30	Coffee	
15:30 - 16:15	**Jean-Laurent Casanova Keynote Lecture**	From second thoughts on the germ theory to a full-blown host theory
16:15 - 16:40	**Filomeen Haerynck**	GTF3A: A novel player in viral sensing by transcribing host-derived RIG-I ligands
16:40 - 17:05	**Claudia Günther**	Chronic ER stress promotes cGAS/mtDNA-induced autoimmunity via ATF6 in myotonic dystrophy type 2
17:05 - 18:45	Poster session II	
18:45 - 19:15	**International Society of Nucleic Acid Immunity**	Establishing Meeting (public)
19:15 - 19:25	TRR237 PI Meeting (exclusively TRR237)	
19:30	**Conference Dinner**	Location: Conference Venue / Hygiene Museum

Saturday, October 28, 2023

SESSION V - Nucleic acid driven immunopathology		
08:45 - 09:10	**Eva Bartok**	Nucleic acid sensing and trained immunity
09:10 - 09:35	**Yanick Crow**	'Type I interferonopathies' - a useful concept or not?
09:35 - 09:50	**Martin Schlee**	A conserved isoleucine in the binding pocket of RIG-I controls immune tolerance to mitochondrial RNA
09:50 - 10:15	**Moritz Gaidt**	Virulence factor-triggered innate immune sensing
10:15 - 10:40	**Gunther Hartmann**	Application of RIG-I oligonucleotide ligands in immunotherapy of cancer and of viral infection
10:40 - 10:55	**Manuele Rebsamen**	SLC15A4 and TASL form a druggable signalling complex required for TLR7-9-induced proinflammatory responses
10:55 - 11:25	Coffee & Refreshments	
11:25 - 11:50	**Anne Krug**	Coordinated activation of human dendritic cell and monocyte subpopulations by yellow fever vaccination
11:50 - 12:15	**Søren Paludan**	Activation of NF-kB through the cGAS-STING pathway
12:15 - 12:30	**Jonathan Maelfait**	ZBP1 forms amyloidal signalling complexes upon recognition of Z-RNA/DNA
12:30 - 12:55	**Katrin Paeschke**	Viral hijacking of hnRNPH1 unveils a G-quadruplex driven mechanism of stress control
12:55 - 13:10	Closing remarks & Poster Prices	
13:10	End of meeting	
Optional social activity - Exhibition at the Hygiene Museum		

Keynote speaker
Talk
Short talk

Carl Gustav Carus Management GmbH

DEUTSCHES HYGIENE-MUSEUM DRESDEN

Speakers

Keynote

Talk

Short Talk

Jean-Laurent Casanova

The Rockefeller University

Sun Hur

Harvard Medical School

Simon Alberti

Technical University Dresden

Glen Barber

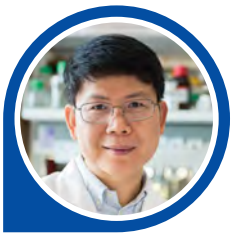
University of Miami

Eva Bartok

University Hospital Bonn

Simone Caielli

Cornell University



Zhijian 'James' Chen

UT Southwestern

Yanick Crow

University of Edinburgh

Michaela Gack

Cleveland Clinic

Moritz Gaidt

IMP - Research Institute of Molecular Pathology

Raphaela Goldbach-Mansky

National Institute of Allergy and Infectious Diseases

Claudia Günther

Technical University Dresden

Filomeen Haerynck

University of Gent

Gunther Hartmann

University Hospital Bonn

Veit Hornung

LMU Gene Center Munich

Jean Luc Imler

University of Strasbourg

Hiroki Kato

University Hospital Bonn

Anne Krug

Biomedical Center Munich

Min Ae Lee-Kirsch

Technical University Dresden

Paul Lehner
University of Cambridge

Andreas Linkermann
Technical University Dresden

Olivia Majer
MPI for Infection Biology

Nicolas Manel
Institut Curie

Katrin Paeschke
University Hospital Bonn

Søren Riis Paludan
Aarhus University

Jan Rehwinkel
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Institut Pasteur

Hachung Chung
Columbia University

Michael Gantier
Hudson Institute of Medical Research

Jonathan Maelfait
University of Gent

Manuele Rebsamen
University of Lausanne

Martin Schlee
University Hospital Bonn

Pengbiao Xu
École polytechnique fédérale de Lausanne

Table of Contents

Table of Abstracts

Abstract 1

Neurons are a unique cell type enriched for immunostimulatory dsRNA structures.

Chung, Hachung
Columbia University, United States of America

Viral double-stranded RNA (dsRNA) structures are well known to trigger innate immune responses when sensed by host pattern recognition receptors (PRRs). However, aberrant sensing of endogenous dsRNAs by PRRs can cause Aicardi-Goutières syndrome (AGS), a neuroinflammatory disorder with spontaneous interferon production. Why the brain is especially susceptible to inflammation in AGS is an undetermined long-standing question. Recently, we discovered that neurons carry exceptionally high levels of endogenous dsRNA structures that constantly stimulate PRR activation in homeostasis and disease. I will discuss the identity, regulation, and function of these neuronal dsRNAs.

Abstract 2

TLR7/8 stress response drives histiocytosis in SLC29A3 disorders

Shibata, Takuma (1); Taoka, Masato (2); Shimizu, Toshiyuki (3); Miyake, Kensuke (1)
1: The Institute of Medical Science, The University of TOKYO, Japan;
2: Graduate School of Science, Tokyo Metropolitan University;
3: Graduate School of Pharmaceutical Sciences, The University of Tokyo

Loss-of-function mutations in the lysosomal nucleoside transporter SLC29A3 cause lysosomal nucleoside storage and histiocytosis: phagocyte accumulation in multiple organs. However, little is known about the mechanism by which lysosomal nucleoside storage drives histiocytosis. Herein, histiocytosis in Slc29a3–/– mice was shown to depend on Toll-like receptor 7 (TLR7), which senses a combination of nucleosides and oligoribonucleotides (ORNs). TLR7 increased phagocyte numbers by driving the proliferation of Ly6Chi immature monocytes and their maturation into Ly6Clow phagocytes in Slc29a3–/– mice. Downstream of TLR7, FcRγ and DAP10 were required for monocyte proliferation. Histiocytosis is accompanied by inflammation in SLC29A3 disorders. However, TLR7 in nucleoside-laden splenic monocytes failed to activate inflammatory responses. Enhanced production of pro-inflammatory cytokines was observed only after stimulation with ssRNAs, which would increase lysosomal ORNs. Patient-derived monocytes harboring the G208R SLC29A3 mutation showed enhanced survival and proliferation in a TLR8 antagonist-sensitive manner. These results demonstrated that TLR7/8 responses to lysosomal nucleoside stress drive SLC29A3 disorders.

Abstract 3

Human MCTS1-dependent translation reinitiation is essential for IFN-g immunity to mycobacteria

Bohlen, Jonathan (1,2,3,4); Zhou, Qinhua (5,6); Philippot, Quentin (1,2); Ogishi, Masato (5); Rinchai, Darawan (5); Conil, Clement (1,2); Nieminen, Tea (7); Momenilandi, Mana (1,2); Seyedpour, Simin (8,9); Parvaneh, Nima (8,10,11); Rezaei, Nima (8,12); Yazdanpanah, Niloufar (8,12); Neehus, Anna-Lena (1,2); Arango Franco, Carlos Andres (1,2); Le Voyer, Tom (1,2); Khan, Taushif (13,14,15); Yang, Rui (5); Puchan, Julia (16); Erazo, Lucia (1,2); Roiuk, Mykola (3,4); Vatovec, Taja (1,2,4); Janda, Zarah (1,2,4); Bagaric, Ivan (1,2,4); Materna, Marie (1,2); Gervais, Adrian (1,2); Li, Hailun (1,2); Rosain, Jeremie (1,2); Peel, Jessica (5); Seeleuthner, Yoann (1,2); Han, Ji Eun (5); L'Honneur, Anne-Sophie (17); Moncada-Vélez, Marcela (5); Martin-Fernandez, Marta (18,19,20); Espino, Michael (18,19,20); Kochetkov, Tatiana (5); Schmidt, Monika (23); AlShehri, Mohammed A. (24); Salo, Eeva (7); Harri, Saxen (7); ElGhazali, Gehad (25); Schmidt, Carltin (5,26); Soudee, Camille (1,2); Sallusto, Federica (16,27); Ensser, Armin (23); Marr, Nico (13,14); Zhang, Peng (5); Bogunovic, Dusan (20,21,22); Cobat, Aurélie (1,2); Shahrooei, Mohammad (28,29); Béziat, Vivien (1,2,5); Boisson-Dupuis, Stéphanie (1,2,5); Abel, Laurent (1,2,5); Wang, Xiaochuan (6); Teleman, Aurelio A. (3,4); Bustamante, Jacinta (1,2,5,30); Zhang, Qian (1,2,5); Casanova, Jean-Laurent (5,31,32)

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31: Howard Hughes Medical Institute, 10032 New York, NY, USA;
32: Department of Pediatrics, Necker Hospital for Sick Children, 75015 Paris, France, EU

Human inherited disorders of IFN-g immunity underlie severe mycobacterial diseases. We report X-linked recessive MCTS1 deficiency in men with mycobacterial disease from kindreds of different ancestries (from China, Finland, Iran, and Saudi Arabia). Complete deficiency of this translation re-initiation factor impairs the translation of a subset of proteins, including the cytosolic kinase JAK2 in all cell types tested, including T lymphocytes and phagocytes. JAK2 translation is sufficiently low to impair cellular responses to IL-23 and, to a lesser extent, IL-12, but apparently not other JAK2-dependent cytokines. Defective responses to IL-23 preferentially impair the production of IFN-g by innate-like MAIT and gd adaptive T lymphocytes upon mycobacterial challenge. Surprisingly, the lack of MCTS1-dependent translation re-initiation and ribosome recycling seems to be otherwise physiologically redundant in these patients. These findings suggest that X-linked recessive human MCTS1 deficiency underlies isolated mycobacterial disease by impairing JAK2 translation in innate-like adaptive T lymphocytes, thereby impairing the IL-23-dependent induction of IFN-g.

Abstract 4

A-to-I editing of Filamin A affects colitis severity by causing changes in the inflammatory response and shifts in the microbiome

Varada, Rajagopal (1); Pereira, Fatima (2); Tanzer, Andrea (1); Knapp, Syvia (1); Jantsch, Michael (1); Gawish, Riem (1); Vesely, Cornelia (1)

1: Medical University Vienna;
2: University of Vienna / University of Southampton

A -to-I RNA editing is an essential post-transcriptional modification of RNA that is performed by editing enzymes ADAR1 and ADAR2 in mammals. While editing of non-coding RNAs plays and important role in autoimmunity and cancer, the targeted editing of codons within exons of protein coding genes has important functions in the nervous system. Also, A-to-I editing of Filamin A (Flna), which leads to a Glutamine/Q to Arginine/R change in an interactive domain of the protein, plays an important role in the cardiovascular system and tumor angiogenesis. Besides endothelial cells and the heart, mice also have high levels of FLNA editing in their distal colon. Utilizing a colitis model (chemical induction) we find that pre-edited FLNAR mice are protected from severe DSS-induced colitis. Closing in on the underlying mechanism(s), we analyzed epithelial barrier tightness, transcriptome and microbiome. We find differences in the microbiome between FLNAQ and FLNAR contributing the severity of symptoms in FLNAQ. Expression profiling showed differences in the early inflammatory response. This was manifested by differences in numbers of tissue resident/attracted immune cells of in the colon during colitis. FLNAR mice had more NK cells and less neutrophiles in their inflamed colons than the sicker FLNAQ mice. Finally, the protective effect of FLNAR mice was to some extent transplantable in BMT experiments. Since Filamin A plays an important role in cell migration, angiogenesis and the switching of a cytotoxic to a cytokine secreting state of NK cells we are currently identifying the cell type, that needs highly edited FLNA.

Abstract 5

NINJ1 acts as a ‘gate-keeper’ for viral entry

Hartenian, Ella; Broz, Petr

University of Lausanne, Switzerland

Antiviral restriction factors are a set of diverse proteins that target many different steps of the viral lifecycle, acting in cellular compartments where host and viral gene products reside. By contrast, few restriction factors target viral entry directly, the very first step of the viral lifecycle. Here we show that NINJ1, a plasma membrane resident protein that was recently found to execute plasma membrane rupture downstream of programmed cell death pathways, reduces HSV-1 replication in mouse macrophages and 3T3 mouse fibroblasts. NINJ1-deficient cells display an accelerated viral lifecycle, with an earlier expression of early and late viral genes, and release higher numbers of infectious virions. This activity of NINJ1 is independent of its role in cell death as key cell-death mutants had no effect on viral replication. Investigating the function of NINJ1, we find that NINJ1 acts as a ‘gate-keeper’ at the stage of virion entry by rendering the cells refractory to viral invasion, thereby reducing the number of cytosolic HSV-1 capsids per cell and the percentage of infected cells. In conclusion, we have identified a novel function for the cell death effector NINJ1 as an anti-viral factor targeting viral entry.

Abstract 6

Double stranded RNA- protein interactome in Sindbis virus infection

Girardi, Erika; Messmer, Mélanie; Lopez, Paula; Fender, Aurélie; Chicher, Johana; Chane-Woon-Ming, Béatrice; Hammann, Philippe; Pfeffer, Sébastien

University of Strasbourg - IBMC CNRS, France

Viruses are obligate intracellular parasites, which depend on the host cellular machineries to replicate their genome and complete their infectious cycle. Long double-stranded (ds)RNA is a common viral by-product originating during RNA virus replication and is universally sensed as a danger signal to trigger the antiviral response. As a result, viruses hide dsRNA intermediates into viral replication factories and have evolved strategies to hijack cellular proteins for their benefit. The characterization of the host factors associated with viral dsRNA and involved in viral replication remains a major challenge to develop new antiviral drugs against RNA viruses. Here, we performed anti-dsRNA immunoprecipitation followed by mass spectrometry analysis to fully characterize the dsRNA interactome in Sindbis virus (SINV) infected human cells. Among the identified proteins, we characterized SFPQ (splicing factor, proline-glutamine rich) as a new dsRNA-associated proviral factor upon SINV infection. We showed that SFPQ depletion reduces SINV infection in human HCT116 and SK-N- BE(2) cells, suggesting that SFPQ enhances viral production. We demonstrated that the cytoplasmic fraction of SFPQ partially colocalizes with dsRNA upon SINV infection. In agreement, we proved by RNA-IP that SFPQ can bind dsRNA and viral RNA. Furthermore, we showed that overexpression of a wild-type, but not an RNA binding mutant SFPQ, increased viral infection, suggesting that RNA binding is essential for its positive effect on the virus. Overall, this study provides the community with a compendium of dsRNA-associated factors during viral infection and identifies SFPQ as a new proviral dsRNA binding protein.

Abstract 7

Canonical and non-canonical contributions of human Dicer helicase domain in antiviral defense

Baldaccini, Morgane; Gaucherand, Léa; Chane-Woon-Ming, Béatrice; Messmer, Mélanie; Pfeffer, Sébastien

CNRS, France

One of the first layer of defense against viral infections is based on the sensing of the invading nucleic acid and/or replication intermediates by the cell. Among the mechanisms that have appeared throughout evolution is RNAi, a very potent antiviral pathway in plants, arthropods or nematods. RNAi is triggered by the type III ribonuclease Dicer, which senses and cleaves viral double-stranded (ds) RNA into small interfering (si) RNAs. Those small RNAs serve as guides for Argonaute proteins, which act in a sequence-specific manner to restrict the infection. In mammals, the importance of RNAi in antiviral innate immunity appears to be restricted to certain cell types and seems to be in conflict with other innate immune pathways that also rely on dsRNA sensing.
We previously showed that the human Dicer protein interacted with a number of dsRNA binding proteins via its helicase domain, and that cells expressing a helicase-truncated version of Dicer displayed a strong antiviral phenotype. Here, I will present our latest results that confirm that the helicase-deleted mutant Dicer protein is indeed antiviral against several (+) RNA viruses. Interestingly, this property does not depend on RNAi, since a catalytically inactive Dicer retains its antiviral phenotype. By RNAseq analysis of cells expressing the helicase-mutant Dicer, we found that they display important changes compared to control cells. Interestingly, it seems that the NF-kB pathway is implicated in these alterations. I will elaborate on these results indicating that Dicer is involved in the regulation of antiviral innate immunity in unexpected ways.

Abstract 8

Identification of the endo-dsRNA proteome in human cells

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The presence of viral double stranded RNA (dsRNA) in the cytoplasm is a danger signal triggering the IFN response in mammalian cells. In addition to dsRNAs of exogenous origin, such as viral ones, endogenous (endo) dsRNAs can also accumulate under certain stress conditions. One of the mechanisms to avoid an unwanted activation of the innate immunity by endo-dsRNAs is RNA editing by ADAR enzymes which convert A to I and destabilize the dsRNA. Whether other regulatory mechanisms play a role in modulating endo-dsRNA sensing upon stress as well as in cellular homeostasis remains to be explored. Our first objective is to identify and characterize new proteins bound to endo-dsRNAs in physiological conditions and upon immunogenic endo-dsRNA induction. In particular, treatment with DNA methyltransferase inhibitors such as 5-azacytidine (5-AZA) was proven to induce accumulation of endo-dsRNAs in the absence of viral infection and ADAR1 depletion was linked to an increase in endo-dsRNA.
We generated CRISPR/Cas9 KO ADAR1 HCT116 cells which were treated or not with 5-AZA to induce endo-dsRNA expression. We first analyzed the transcriptome in treated and untreated wild-type and ADAR1 KO cells. We performed a dsRNA immunoprecipitation coupled to LC/MSMS to identify the endo-dsRNA interactome in human cells under those conditions. This approach allowed us to identify several cellular proteins associated with the expressed endo-dsRNAs. We are assessing their potential role in regulating endo-dsRNA metabolism and immunogenicity. Our study will contribute to the global understanding of the mechanisms of endo-dsRNAs regulation related to autoimmune diseases and other human pathologies.

Abstract 9

Mechanism of Mitochondrial DNA Sensing by the AIM2 Inflammasome

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The AIM2 inflammasome triggers pro-inflammatory pyroptotic cell death upon detection of double-stranded DNA in the cytosol. AIM2 is kept auto-inhibited by an interaction between its two structured domains: the N-terminal pyrin domain (PYD) and the C-terminal HIN200 domain, which are separated by a flexible linker. HIN200 domain binding to dsDNA releases the PYD, which clusters with the PYDs of other AIM2 molecules bound to the dsDNA to initiate inflammasome assembly.
The release of mitochondrial DNA (mtDNA) into the cytosol and its detection by cGAS has been implicated in inflammatory diseases, however the role of AIM2 in this context remains under explored. We first confirmed that mtDNA released upon mitochondrial damage activates the AIM2 inflammasome in murine macrophages, leading to ASC speck formation, cell death and secretion of IL-1b.
To further dissect the structural requirements for the detection of mtDNA, we used cells expressing AIM2-GFP. We found that the full-length protein is recruited to DNA in micronuclei, transfected dsDNA, and mtDNA released upon mitochondrial damage. Deletion of the PYD abrogated mtDNA sensing, whereas detection of micronuclear and transfected dsDNA remained intact. Reintroduction of the PYD from NLRP3, but not PYRIN or ASC, restored mtDNA sensing by AIM2. Microscopy analysis revealed that mtDNA sensed by AIM2 was associated with the mitochondrial marker TOM20, suggesting the DNA may not be released, but remains at least partially contained within a mitochondrial membrane. We are currently exploring the mechanism by which the AIM2 PYD facilitates mtDNA sensing, potentially through a novel protein-protein interaction.

Abstract 10

IFN-β exposure and ARTS deficiency promote the generation of hyper-efferocytic Ly-6C+ macrophages during the resolution of inflammation

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During the resolution of inflammation, Ly-6C+F4/80- monocytes differentiate to Ly-6C-F4/80+ macrophages that exert efferocytic properties and consequently convert to IFN-β-producing macrophages. Here, we report that exposure to IFN-β, or TGF-β, or a deficiency in the pro-apoptotic protein ARTS, results in the conversion of mature macrophages to a rejuvenated Ly-6C+F4/80+CCR2+ phenotype. This phenotype appeared exclusively in peritoneal resolution phase macrophages and not their unchallenged peritoneal, splenic or bone marrow counterparts. Moreover, IFN-β-triggered rejuvenated macrophages were hyper-efferocytic and expressed higher levels of the efferocytic receptor CD36. Inhibition of CD36 ligation resulted in complete abrogation of efferocytosis ex vivo in both mature and rejuvenated macrophages. Altogether, our findings indicate an unprecedented phenomenon in which IFN-β promotes macrophage rejuvenation and efferocytosis that are limited by ARTS-mediated apoptosis during the resolution of inflammation.

Abstract 11

The BiST of Burden: Harnessing biased STING agonists to enhance the resolution of inflammation and limit tissue fibrosis

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Stimulator of IFN Genes (STING) is a cytosolic DNA sensor that plays a central role in host protection against pathogens upon binding of DNA-derived ligands. STING primarily acts by controlling the transcription of type I interferons (IFNs) and pro-inflammatory cytokines. Notably, STING can be inhibited or activated pharmacologically to control STING-associated pathologies. 5, 6-Dimethylxanthenone-4-acetic Acid (DMXAA) is a pharmacological activator of murine STING that induces IFN-β and its affected genes. Here, we report that macrophages from DMXAA-treated mice engulfed significantly higher numbers of apoptotic cells ex vivo, and exhibited enhanced reprogramming reflected by an increased IL-10 and reduced inflammatory cytokine secretion upon LPS exposure. Macrophage reprogramming was significantly hampered in STING- and IFN-β-deficient macrophages. Furthermore, we found, using virtual docking and batch screening, biased STING agonists (BiSTs) that enhanced IL-10 and IFN-β production by splenocytes while inhibiting TNFα. One of these compounds, termed compound 2.1, also induced the murine STING pathway in vivo and in human macrophages. Finally, we found compound 2.1 to enhance the resolution of liver fibrosis induced by CCl4. Thus, our findings indicate that STING can be harnessed to drive IFN-β-mediated IL-10 secretion by resolution phase macrophages and consequently shape their function to enhance the resolution of inflammation and treat fibrotic disorders.

Abstract 12

Crystallography-Guided Characterization of Novel Cytosolic DNase 3' Repair Exonuclease 1 (TREX1) Inhibitors

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Genetic mutation driving profound interferonopathies, virus-targeted inactivation, inflammation and epigenetic silencing affecting a broad spectrum of human diseases validate the STING pathway as a critical node in regulating immunity. Drugging this pathway in cancer has been challenging due to the ubiquitous expression of STING and the need to activate T cell priming broadly among unique antigenic repertoires in advanced metastatic cancer and to reverse immune suppression in the TME. We propose that the cytosolic DNAse 3' repair exonuclease (TREX1), a negative regulator of the cGAS/STING pathway, is an ideal target to selectively induce STING in tumors with systemically administered TREX1 small molecule inhibitors. TREX1 is upregulated in tumor cells in response to genomic instability, DNA replication and therapeutic intervention, providing a therapeutic index for selective activation of the STING pathway. We developed small-molecule inhibitors of TREX1 with nanomolar potency in biochemical and cellular assays. To accelerate structure-activity relationship (SAR) activities, we produced the first high-resolution co-crystal structures of inhibitor-bound human and mouse TREX1. Thermal shift analysis confirmed that magnesium was required for TREX1-inhibitor interactions. Affinity measurements of TREX1-inhibitor-DNA ternary complexes suggested inter-and intra-molecular interactions governed exonuclease activity. These structures identified mouse and human residues driving species specificity and mechanisms underlying inhibitor potency. Systemic administration in mice with lead inhibitors achieved exposures necessary for sustained TREX1 target engagement in tumors and inhibition of outgrowth. Selective inhibition of TREX1 can specifically and locally engage the STING pathway in the TME, enhance tumor-specific immunity, and provide therapeutic benefits.

Abstract 13

Sterile STING activation causes IFN-mediated skin inflammation in keratinocyte-specific Ripk1 knockout mice

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RIPK1 is a key signalling kinase involved in inflammation and regulated cell death. In mouse keratinocytes RIPK1 prevents spontaneous activation of ZBP1/RIPK3/MLKL-mediated necroptosis, which results in skin inflammation. Interestingly, ex vivo cultured RIPK1 deficient keratinocytes show no spontaneous phenotype, suggesting the need for an additional (exogenous) trigger to initiate ZBP1-mediated necroptosis. We show that inflammatory lesion development in keratinocyte-specific knockout Ripk1 (Ripk1E-KO) mice is not rescued by antibiotics treatment or in germ-free conditions. Additionally, we excluded the adaptive immune system as a disease driver by crossing Ripk1E-KO animals into a Rag2/Il2rg-deficient background. Together, these findings demonstrate that Ripk1E-KO mice develop a sterile autoinflammatory skin phenotype.
Interestingly, we found that crossing Ripk1E-KO mice into a Stat1-deficient background significantly rescues the mice from developing inflammatory skin lesions, suggesting an interferon (IFN)-driven pathology. To identify the pathway responsible for pathological IFN production in Ripk1E-KO mice, we genetically deleted crucial adaptor proteins in the three primary pathways upstream of type I IFN production: the RIG-I-like receptor (RLR), Toll-like receptor (TLR) and cGAS-STING pathway. Deletion of Mavs, Trif or MyD88 did not affect skin inflammation thereby excluding a role for RLRs and TLRs in pathogenesis. In contrast, we observed significant rescue by crossing Ripk1E-KO mice into a Sting1 (the gene encoding STING) deficient background. Moreover, STING activation in skin epithelial cells contributed to autoinflammation as keratinocyte-specific deletion of Sting1 improved skin inflammation. We are currently exploring the mechanism(s) of STING activation in Ripk1 deficient keratinocytes.

Abstract 14

SAMHD1 controls innate immunity by regulating condensation of immunogenic self RNA

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The innate immune system plays a crucial role in recognizing and fending pathogen-derived nucleic acids while maintaining tolerance towards self-nucleic acids. Dysregulation of this delicate balance can lead to autoinflammatory and autoimmune disorders such as Aicardi-Goutières syndrome (AGS). The SAM domain and HD domain-containing protein 1 (SAMHD1), one of the AGS-causing genes, has emerged as a key player in the regulation of nucleic acid immunity by modulating the immune response to self-nucleic acids. Here, we show that SAMHD1 functions as a 3´exonuclease specific for single-stranded RNA (ssRNA). Loss of SAMHD1 in AGS patient fibroblasts or in shRNA-transfected HeLa cells leads to the accumulation of cellular ssRNA, which impairs formation of RNA-protein condensates via liquid-liquid phase separation. Using in vitro reconstituted stress granule-like condensates or microinjection of RNA into HeLa cells, we demonstrate that increased ssRNA in cells leads to dissolution of RNA-protein condensates, while immunogenic double-stranded RNA (dsRNA) is sequestered in condensates such as stress granules. Release of sequestered dsRNA from condensates in SAMHD1-deficient cells causes aberrant innate immune activation via retinoic acid-inducible gene I-like receptors, resulting in constitutive type I interferon activation. Our results establish SAMHD1 as a key regulator of cellular RNA homeostasis and demonstrate that buffering of immunogenic self-RNA by condensates regulates innate immune responses.

Abstract 15

Discovery of cyclic nucleotide signaling pathways in humans

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Cyclic GMP-AMP synthase (cGAS) is a nucleotidyltransferase (NTase), which upon recognition of microbial or self dsDNA, synthesizes the second messenger 2´3´ cyclic GMP-AMP (cGAMP). cGAMP binds to and activates the downstream adaptor protein stimulator of interferon genes (STING) leading to induction of an antiviral response, NF-kB signaling, autophagy or various forms of cell death in a cell type specific manner. While in humans only the purine-containing cyclic di-nucleotide 2´3´cGAMP is synthesized, bacteria possess extensive cyclic nucleotide signaling networks including nucleotides with purine and pyrimidine bases and various phosphodiester-linkages. Whether such diverse cyclic nucleotide signaling pathways exist also in human cells is currently unknown.
In order to explore cyclic nucleotide signaling in human cells, we treated various cell types with a nucleotide library containing diverse cyclic nucleotides. Potential cellular responses were analyzed by gene expression employing RNA-seq, virus infection or cell death. Analysis of cellular signaling events revealed responses that are highly specific to selected nucleotides. Furthermore, the data show STING nucleotide preferences in THP1 monocytes and signaling pathways. Overall, our approach will allow us to reveal novel nucleotide signaling pathways in humans. In general, our analysis has the potential to reveal if other cGAS-like NTases found in humans with currently unknown molecular functions are involved in certain cellular processes and nucleotide signaling pathways.

Abstract 16

Unraveling the Role of the Ubiquitin Proteasome System in Innate Immune Signaling in Rare Proteasomopathies

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The ubiquitin proteasome system (UPS) degrades ubiquitin-modified proteins to maintain protein homeostasis (proteostasis) and to control signaling. Proteotoxic stress of various origins can be typically counteracted by the shut-down of global protein translation, or the up-regulation of protein quality control and degradation machineries including stress specific sets of ubiquitin-conjugation and deconjugation factors as well as alternative proteasome isoforms [1]. Loss-of-function mutations in genes encoding proteasome subunits cause two rare and apparently distinct syndromes: (i) proteasome associated autoinflammatory syndromes (PRAAS) or (ii) a neurodevelopmental disorder with rare signs of inflammation (NDD) [2-6]. Although phenotypically diverse, we show that both proteasomopathies share overlapping molecular pathophysiology. Impaired proteasomal degradation capacity is characterized by accumulation of ubiquitin-conjugates suggesting severe proteotoxic stress and activation of recovery pathways such as the NGLY1-DDI2-Nrf1 axis, the integrated stress response (ISR), the unfolded protein response (UPR), as well as type I interferon (IFN) signaling. Moreover, we observed profound changes in the mTOR pathway and induced mitophagy. The role of protein kinase R, IL-24 and other sensors for proteotoxic stress in IFN signalling [2-6], in the proteostasis network and in the maintenance of a balanced immune response as well as novel proteasome subunit mutations will be discussed.

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[2] Brehm et al., J Clin Invest, 126 (2015)
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[4] Isidor et al., Genetics in Medicine, 24 (2022)
[5] Davidson et al., Sci Immunol. 11 (2022)
[6] Ebstein et al., Sci Transl Med 2023 15(698):eabo3189

Abstract 17

Deciphering the driving molecular sensor for the activation of the type I IFN signaling in rare proteasomopathies

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Interferonopathies are caused by dysregulation of type I interferon (IFN) signaling [1]. In rare proteasomopathies, mutations in proteasome subunit genes such as PSMB8 cause a sterile inflammation by the activation of type I IFN signaling [2-8]. However, the driver for the initiation of type I IFN signaling is not fully understood. Recently, Davidson et al. described the induction of protein kinase R (PKR) by IL-24 accumulation, implicating its role as an immune sensor [9]. However, not all cells express IL-24, suggesting other sensors for type I IFN activation. Therefore, two cell lines, a human endothelial cell line (EA.hy926) and a human microglial cell line (C20), were used to establish cell models for proteasomopathies by knocking out PSMB8 using CRISPR/Cas9. Patient samples and these surrogate cell lines exhibit signs of proteotoxic stress, leading to activation of the unfolded protein response (UPR) and the integrated stress response (ISR). Further studies were performed to characterize different molecular pathways in order to decipher a molecular driver of type I IFN signaling. Relevant pathways will be discussed.

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Abstract 18

Myeloperoxidase (MPO) and anti-MPO antibodies synergistically enhance TRL9-mediated immune activation in ANCA-associated vasculitis

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Anti-neutrophil cytoplasmic antibodies (ANCAs) target antigens in neutrophil granules, such as myeloperoxidase (MPO) or proteinase 3 (PR3), and cause small vessel inflammation. The pathogenicity of ANCAs is most often attributed to their ability to directly activate neutrophils, but a comprehensive understanding of their mode of action is lacking. In this study, we elucidate the mechanism for anti-MPO dependent immune activation. We demonstrate that binding of MPO to DNA protects extracellular DNA from enzymatic degradation. Antibodies against MPO promoted its FcγR-mediated uptake into bone marrow-derived dendritic cells (BMDCs). Importantly, we found that complexing DNA with MPO and anti-MPO greatly increased its ability to activate BMDCs and that this activation was critically dependent on Toll-like receptor 9 (TLR9). This effect was observed for both microbial DNA and neutrophil-derived “self” DNA. Our results demonstrate how MPO and anti-MPO cooperate to facilitate DNA sensing and thereby immune activation in ANCA-associated vasculitis.

Abstract 19

Inhibition of ADAR1 RNA editing enzyme in cervical cancer triggers NK cell responses.

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RNA editing consists in the deamination of adenosine to inosine (A-to-I) within double stranded (ds) RNA sequences and it is mediated by ADAR family of enzymes, including ADAR1. The main function of ADAR1, particularly of its p150 isoform, is the editing of endogenous dsRNAs, which strongly resemble viral structures, to prevent aberrant activation of innate immune pathways, such as type-I IFN (IFN-I) production. This study investigates the role of ADAR1 in immune evasion mechanisms in cervical cancer (CC), a disease mostly associated with persistent Human Papilloma Virus (HPV) infection and immune dysfunctions, that is also the fourth most common tumor causing death in women. We characterized ADAR1 expression in CC biopsies, observing higher ADAR1 expression in CC tissues compared to controls, with a negative correlation to overall patient survival.
Silencing of ADAR1 in CC cell lines (SiHa, CaSki) reduced cell proliferation and sensitized cells to exogenous IFN-β treatment. Additionally, ADAR1 inhibition resulted in increased expression of pro-inflammatory cytokines and chemokines, and conditioned supernatants collected from ADAR1-silenced CC cells potentiated NK cell proliferation, migration and cytotoxicity. Our findings suggest that ADAR1 acts as an immune evasion player in CC, restraining IFN production by tumor cells and their sensitivity to IFN-mediated anti-proliferative effects. We also provide a previous unreported role of ADAR1 in opposing NK cell activities, that gives new insights into the immune landscape of CC. Thus, ADAR1 inhibitors able to reduce its expression and/or editing activity may represent a promising therapeutic strategy for CC and potentially other cancers.

Abstract 20

Mechanism and Structure of cGAS nuclear degradation

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Innate immunity is the intrinsic defense system, functioning as the first barrier to sense, react, and remove the pathogenic components. Over the past years, there has been a growing understanding of the molecular complexity that underlies innate immune activation through the cGAS-STING pathway, especially in the aspect of subcellular localization. cGAS was long believed to locate inside the cytoplasm while avoiding the nucleus. Recent studies, however, pointed out that cGAS finds inside nuclei and co-localizes with chromatin. Importantly, cGAS does not produce cGAMP in the chromatin-bound state despite being juxtaposed to genomic DNA, a bone-fide activator of cGAS. In addition to our contribution of the Cryo-EM structure of cGAS/Nucleosome that revealed the mechanism of cGAS inhibition by chromatin, here we further discovered a degradation system with cellular and biochemical assays together with Cryo-EM structures. Here, I will present the mechanism and structure of cGAS nuclear degradation, another negative regulator of the cGAS/STING pathway.

Abstract 21

Sequence specificity of RIG-I/IFN signaling

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Viral RNAs and certain Pol III transcripts exhibit a unique feature called 5′-triphosphate (5′-ppp), which activates the receptor RIG-I, triggering an interferon type I (IFN) response. This response plays a vital role in antiviral defense but can also lead to autoimmune diseases if excessively stimulated. While many RNA virus genomes initiate with 5′-ppp adenosine (5′-pppA), higher eukaryotic Pol III transcripts and genomes of certain pathogenic RNA viruses begin with 5′-ppp guanosine (5′-pppG). The reason for this preference is unknown.
New evidence suggests that structured viral and cellular RNAs containing 5′-pppA induce a stronger RIG-I/IFN response compared to RNAs beginning with 5′-pppG. Altering the initial nucleoside from A to G makes viral RNAs nearly undetectable by the RIG-I machinery in human and mouse cells. Similarly, switching the first G to A in Pol III transcripts enhances their immunogenicity. Structural analysis confirms that the RNA pairs have identical conformations, implying that functional disparities cannot be explained by conformational changes. Significantly, RNA pull-down quantitative mass spectrometry reveals several proteins with a specific affinity for 5′-pppA or 5′-pppG transcripts. These proteins likely regulate RIG-I/IFN signalling triggered by viral and endogenous RNAs.
In summary, we demonstrate the sequence specificity of RIG-I/IFN signalling and proposes that 5′-pppG RNAs may help some viruses and Pol III transcripts evade cellular immune sensors. These findings provide insights into the antiviral response against highly pathogenic RNA viruses and the role of Pol III-derived RNAs in autoimmune disorders.

Abstract 22

cGAS Modulates Chromatin State and Transcription in ATRX-mediated Activation of Interferon Type I

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Mutations in the chromatin remodeller X-linked α-thalassaemia intellectual disability syndrome protein (ATRX) are associated with developmental problems in males and are observed in certain cancer types. Here, we describe the identification of two unrelated patients with the same Y1758C mutation in ATRX, white matter abnormalities and enhanced type I interferon signalling, thereby defining a novel type I interferonopathy.
In cellular models, our findings indicate that ATRX deficiency results in an elevated interferon response. We show that this effect involves the DNA sensor cGAS, but not the production of its second messenger 2'3'cGAMP and its downstream signalling cascade. Rather, we identify an interdependency of ATRX with cGAS at the level of chromatin. Indeed, by interrogating genomic binding sites and protein interactions, we show a preferential genomic co-occupancy of the ATRX/DAXX/H3.3 chaperone complex and cGAS according to ATRX (wild type versus mutant) status. Importantly, such differences are not seen in the absence of cGAS. Further, in the ATRX mutant state, we demonstrate an abnormal enrichment of cGAS at promoters of upregulated genes that include interferon stimulated genes, suggesting a relationship between the redistribution of mutant ATRX and cGAS, nearby genes and transcriptional activation.
Overall, our data reveal a previously unappreciated relationship between ATRX and cGAS in the regulation of chromatin state and transcription, with loss of ATRX function resulting in increased binding of cGAS, DAXX and H3.3 at novel genomic sites, an alteration of transcriptional homeostasis, and a pathogenic inflammatory phenotype.

Abstract 23

SLC15A4 and TASL form a druggable signalling complex required for TLR7-9-induced proinflammatory responses

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Endolysosomal Toll-like receptors (TLRs) play crucial roles in immune responses to pathogens. In contrast, aberrant activation of these pathways is associated with several autoimmune diseases, including systemic lupus erythematosus (SLE). The endolysosomal solute carrier family 15 member 4 (SLC15A4) is required for TLR7, TLR8 and TLR9-induced inflammatory responses and for disease development in different SLE murine models, highlighting its potential as drug target. SLC15A4 has been proposed to affect TLR7-9 activation through its transport activity, as well as by assembling in an IRF5-activating signalling complex with the innate immune adaptor TASL, but the relative contribution of these different functions remains unclear. Here we show that the essential role of SLC15A4 is to recruit TASL to endolysosomes, while its transport activity is dispensable when TASL is tethered to this compartment. Targeting of TASL to the endolysosomal compartment is sufficient to rescue TLR7-9-induced IRF5 activation and proinflammatory responses in SLC15A4-deficient cells, indicating that SLC15A4 acts as signalling scaffold. These findings support interfering with the SLC15A4-TASL complex as a potential therapeutic strategy for SLE. We therefore performed a chemical screen and identified a compound, feeblin, which affects the assembly of the SLC15A4-TASL complex, leading to efficient degradation of TASL and ablation of downstream IRF5 activation. Consequently, feeblin blocks endolysosomal TLR-induced responses in disease-relevant human immune cells. Altogether, our study reveals that SLC15A4-TASL complex assembly is essential for TLR7-9-mediated inflammatory responses and provides the proof-of-concept that chemical interference with this module could represent a novel therapeutics strategy for SLE and related diseases.

Abstract 24

Viral hijacking of hnRNPH1 unveils a G-quadruplex driven mechanism of stress control.

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A common feature of most viruses is their dependence on regulatory RNA elements. Recent research suggested that non-canonical structures called G-quadruplexes (G4s) are overrepresented in viral genomes and have emerged as promising anti-viral targets. Using yellow fever virus (YFV) as a model system, we characterized the formation and the biological consequences of a conserved G4 within the genomes of the Flaviviridae family. We determined that this G4 is essential to promote viral replication and suppress the host cell stress response pathway. In subsequent mechanistic analyses, we pinpoint that this unique G4 function is associated with the nuclear host protein hnRNPH1. Specifically, G4 interaction leads to the retention of hnRNPH1 in the cytoplasm, causing an impaired stress response and alleviation of the anti-viral effects of stress-induced G4-forming tRNA fragments (tiRNAs). In conclusion, our data reveal a unique interplay of hnRNPH1 with host and viral G4 targets, controlling the integrated stress response and viral infection.

Abstract 25

Cell death-optimized 5'-triphosphate RNAs for improved tumor immunotherapy

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Here we describe a molecular mechanism of improved tumor cell death induction upon cytosolic recognition of dsRNA by pattern recognition receptors. Activation of RIG-I by 5'-3p-RNA is leading to an antiviral response, characterized by the induction of type I interferons, proinflammatory cytokines, and apoptosis. Previous findings from our group have demonstrated that 3p-RNA-induced apoptosis is a two-step process consisting of RIG-I–dependent priming and an OAS/RNase L–dependent effector phase.
We hypothesized that modification of the 3p-RNA characteristics influences differential activation of both receptors system leading to an optimal balance between RIG-I mediated cytokine axis and RNase-L mediated cell death axis to enhance priming and antigen availability for an effective antitumoral immune response.
A set of defined in vitro-transcribed 3p-RNA ligands were analyzed in different knockout tumor cell lines activating either RIG-I alone, RIG-I and OAS, or PKR to understand the functional role of each axis and to separate cytokine response from translational inhibition and cell death.
We observed significant length-dependent effects in cytokine and cell death induction upon transfection into tumor cells. 3p-RNA-treatment induced IFN increasingly with length until a maximum was reached, beyond which IFN levels declined rapidly. In contrast, cell viability steadily decreased with increasing 3p-RNA length, coinciding with a more potent activation of OAS/RNase L and PKR-mediated translational arrest thereby increasing cell death induction.
In conclusion, we were able to generate cell death-optimized 3p-RNA which holds great potential to enhance antigen release by carefully balancing cytokine and cell death induction through precise activation of different restriction factors.

Abstract 26

STING- and MAVS-independent sensing of mitochondrial nucleic acids

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Mitochondrial nucleic acid (mtNA) can be sensed by the cGAS-STING and RLR-MAVS machinery when mtNA is released into the cytosol, playing a critical role in cellular homeostasis and the immune response through engagement of type I interferon (IFN-I) signalling. In contrast, mitochondrial perturbation leading to aberrant mtNA leakage into the cytosol has been implicated in a number of disease settings, including cancer, autoimmunity and neurodegeneration. While it has been emphasised that cytosolic mtNAs are detected by cGAS and RIG-I/MDA5, the involvement of other sensors remains unclear.
A large proportion of late-stage colorectal cancer cell lines exhibit altered NA sensing, involving repression of the cGAS-STING signaling pathway, dampening of IFN-I signaling and enhanced tumour growth. These particular characteristics represent a challenge for clinical therapies, and also constitute an interesting model in which to study alternative mtNA innate responses (with potential clinical relevance). Accordingly, we have used the HCT116 colon carcinoma cell line to discover new pathways of mtNA sensing. We find that HCT116 cells respond to mtNA release by inducing an IFN-I response independent of cGAS, STING and MAVS, but dependent on the presence of mtDNA. In addition, preliminary results suggest that DNA-PK is not required for mtNA sensing in our model. Ongoing investigations will explore a potential mtRNA requirement, and alternative mtNA sensors such as ZBP-1, RNA Pol-III, and IFI16. Ultimately, we expect to further our understanding of the mtNA innate sensing apparatus engaged upon mtNA cytosolic leakage.

Abstract 27

Tumor cell-intrinsic STING activation promotes immune checkpoint inhibitor responsiveness

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Immune checkpoint inhibitors (ICIs) have revolutionized cancer therapy. Patients with mismatch repair deficient (dMMR) colorectal cancer (CRC) respond particularly well to ICI therapy compared to patients with mismatch repair proficient (pMMR) CRC. To be effective, ICIs require preexisting immunologically “hot” niches in the tumor microenvironment (TME). How the mutational landscape of cancer cells shapes these niches remains however poorly defined. We found in human and murine CRC models that the superior antitumor immune response of dMMR CRC required tumor cell–intrinsic activation of STING. Mechanistically, dMMR-triggered genomic instability results in aberrant cytosolic DNA, which stimulates cGAS-dependent production of cGAMP to engage STING for type I interferon transcription. Subsequently, we synthetically enforced STING signaling in CRC cells with intact MMR using constitutively active STING variants (STINGN153S). Even in the absence of a genomic instability-driven ligand, the expression of constitutively active STINGN153S in MMR-proficient tumor cells was sufficient to induce tumor cell-intrinsic interferon signaling, enhance antitumor immune responses, and create “hot” TMEs, which sensitized previously “cold” tumors to ICI therapy. Thus, our results introduce a rational strategy for modulating tumor cell–intrinsic programs via engineered STING enforcement to sensitize resistant tumors to ICI therapy.

Abstract 28

Novel dideoxy-cGAMP analogues activate STING and are stable against poxins

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The cGAS-STING innate immunity pathway is activated in response to the presence of abnormal dsDNA of pathogen or host origins[1]. Cytosolic DNA sensor cGAS produces 2'3'-cGAMP, a cyclic dinucleotide, that acts as a second messenger, activating STING and inducing an immune response characterized by the production of type-I interferons and proinflammatory cytokines. The development of STING-binding molecules that can exhibit agonistic activity is currently of tremendous interest considering that those molecules can be suitable for use as vaccine adjuvants or for cancer immunotherapy. 2'3'-cGAMP is specifically cleaved by certain poxvirus nucleases (poxins) in order for them to evade the host immunity[2]. Here, we describe the design and synthesis of 2'3'-dideoxy-CDNs that lack the ribose OH groups and demonstrate remarkable stability against poxins. The presented cGAMP analogues also showed potent activation of STING in in vitro cell based assays, especially 2'3'-dideoxy-cGAMP (1), which showed improved efficiency over the natural 2'3'-cGAMP. Despite their expected reduced affinity to STING, two of our compounds featured a strong anti-tumor response in a hepatocellular carcinoma model.
Acknowledgments: This work was supported by the Deutsche Forchungsgemeinschaft (DFG) via programs SFB1032, SFB749, SPP-1784. Additional funding from EXC117 CiPSM and the European Research Council under the European Union's Horizon 2020 research and innovation program is acknowledged.

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Abstract 29

Immunostimulatory potential of small cellular and viral RNAs may be influenced by RNA modifications and binding partners

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RNA modifications are known to modulate RNA immunogenicity. N-glycosylation has been reported as a novel means of RNA modification, but so far only indirect evidence for "glycoRNA" exists, and little is known about its interaction with the immune system. We used click chemistry and Northern blotting to probe for glycan-modified RNAs in metabolically labelled cells and the murine leukemia virus Moloney strain (MuLV). Glycan-rich fractions were used for stimulation of the pDC cell line CAL-1 and human PBMCs. We found evidence for glycan incorporation in cellular RNA in accordance with previous data, and, interestingly, in MuLV RNA. Signals co-fractionated with small and tRNAs (<200 nts), and evidence was gathered that small MuLV RNAs are host-derived. Treatment of glycan-rich RNA with RNase A/T1 did not abolish the glycosylation signal, which stands in discordance with the original publication. Among all tested enzymes, only nuclease P1 treatment lead to signal reduction. However, using an in-gel visualization workflow without Northern blotting, all nuclease-treated samples showed unaltered signals. These data imply that "glycoRNAs" may be part of a complex, in which the RNA part mediates transfer to Northern blotting membranes, but is not necessarily glycosylated. Interestingly, cells stimulated with fractions of small RNAs of cellular or viral origin, rich in glycans and tRNA, showed inhibited IFN-α response after costimulation with the TLR7 ligand RNA40. Although it is known that the methylation status of tRNA modulates TLR7 activity, further investigations are underway to clarify the role of RNA glycosylation for immunostimulation.

Abstract 30

Inflammation induced by constitutively active STING is mediated by enhanced TNF signaling

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Constitutive activation of STING by gain-of-function mutations triggers manifestation of the systemic autoinflammatory disease STING-associated vasculopathy with onset in infancy (SAVI) in humans and mice. Murine SAVI is characterized by perturbed IFN and TNF signaling resulting in T cell lymphopenia, severe interstitial lung disease, neuroinflammation and neurodegeneration. Here, we show that inhibition of TNF signaling by Infliximab treatment of SAVI mice improved T cell development in the thymus, yet could not prevent the manifestation of severe inflammatory lung disease. However, complete blocking of TNF receptor signaling by genetic knock out of TNFR1 and 2 prevented loss of thymocytes as well as manifestation of interstitial lung disease in STING knock in (STING ki) mice expressing the STING N153S hyperactive variant. The lung disease is characterized by massive inflammatory infiltrates around blood vessels suggesting that endothelial cells might be involved. In primary lung endothelial cells, STING ki mice showed enhanced transcription of cytokines, chemokines and adhesions proteins, which was normalized, in TNFR1/2 deficient background. STING ki lung endothelial cells induced increased transendothelial migration of neutrophils across the endothelial barrier, which was also normalized by TNFR1/2 absence. Finally, we could show that TNFR1 deficiency was sufficient to prevent thymus inflammation and fatal lung disease, thus underlining that SAVI disease in mice is strongly dependent on TNF receptor signaling.

Abstract 31

3-base long 2'-O-Methyl oligonucleotides are potent TLR7 and TLR8 modulators

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RNA therapeutics all rely on chemical modifications, which help to stabilise these molecules against nucleases and are paramount to their drug-like properties. Critically, such modifications are also essential to blunt activation of innate immune nucleic acid sensors by such RNA therapeutics, but a detailed mechanistic understanding of how this operates remains poorly defined.

We have now made the ground-breaking discovery that degradation fragments as short as three bases (3-mers) from 2'-O-methyl-modified phosphorothioate gapmer antisense oligonucleotides can directly bind Toll-Like Receptor (TLR) 7 and TLR8 to impair their RNA sensing activity. Systematic analyses have identified the optimal 3-mer sequences and chemistries impacting TLR7/8 signalling. Functionally, select 3-mer oligonucleotides have divergent effects on TLR8 activity, allowing for both inhibition or potentiation in a sequence-dependent manner, while TLR7 sensing is strictly suppressed by immunoregulatory 3-mers. Mechanistically, molecular dynamics analyses indicate that antagonistic 3-mers do not bind as well as agonistic 3-mers to site 2 of TLR7, potentially underlying their inhibitory function. Critically, TLR7-inhibiting 3-mer oligonucleotides showed significant protection against systemic and topical TLR7-driven inflammation in vivo.

Collectively, our findings add to the understanding of TLR7/8 sensing of RNA, indicating a complex interplay between activating and inhibiting fragments according to base and sugar modification of their nucleotides. Our studies suggest that the distinction between self and non-self RNA by TLR7/8 relies on the competitive activities of 3-base long "immune codons", acting as agonists or antagonists. Finally, our work defines a novel class of ultra-short immunomodulatory oligonucleotides with a broad range of potential therapeutic applications.

Abstract 32

Impact of viral genome methylation on immune sensing in insects

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The RNA genome of flaviviruses like yellow fever virus (YFV) and Dengue virus (DENV) encodes a methyltransferase within the non-structural protein NS5. The latter is involved in 2'-O-methylation of the first nucleotide downstream of the 5' cap resulting in a cap1 structure. A certain mutation of the viral methyltransferase results in a cap0 virus. While the cap1 structure was shown to be a signal for vertebrate cells to distinguish between self and non-self, immune sensing of viral RNA in mosquitoes is poorly understood. Here we like to improve the understanding of how the insect's immune system recognizes viral RNA and how flaviviruses counteract to allow replication and dissemination. We found that YFV cap1 is prevented from recognition by the insect immune system in vitro and in vivo, while YFV cap0 is inhibited in replication. Further experiments will include priming of insect cells or mosquitoes, transcriptome analyses of infected insect cells, and knock-down of potential cap0 binder proteins. Since DENV cap0 replicated comparable to its wild type in mosquito cells, we plan to analyze whether the replication ability is due to a viral antagonist by performing trans-complementation analyses. Also, different levels of internal methylations might contribute to DENV cap0 replication. Hence, the methylation status of different cap1 and cap0 viruses will be compared. Further, the expression of cellular methyltransferases in cap1- or cap0-infected insect and vertebrate cells will be analyzed to estimate the influence of viral infection on their regulation. This study might contribute to improvements in vaccines and vector control.

Abstract 33

RNase H2 mouse model for Aicardi-Goutières Syndrome exhibits a ribogenesis developmental defect

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Human patients with mutations in genes encoding the heterotrimeric enzyme RNase H2 can develop Aicardi-Goutières Syndrome (AGS), a Type I interferonopathy. In AGS, activation of the innate immune system is driven by aberrant accumulation of dsDNA, leading inducing type I interferon signaling. RNase H2 hydrolyzes RNA moieties of RNA/DNA hybrids (present as R-loops in cells) and incises at single ribonucleotides (rNMPs) embedded in DNA. We have generated a mouse model of AGS in which the catalytic A subunit of mouse RNase H2 has a Gly -Ser amino acid at position 37. RNase H2G37S/G37S MEFs have decreased R-loop processing and single rNMP excision, exhibiting increased levels of DNA damage marker yH2AX. Using DRIP-Seq and RNA-Seq, we show that R-loops are differentially present in numerous locations including rDNA. Phenotypes of the RNase H2G37S/G37 mice include microphthalmia, coloboma, small size, hypopigmentation, and perinatal lethality, resembling those of the ribogenesis developmental syndromes - Diamond-Blackfan Anemia, Treacher Collins syndrome, Cartilage Hair Hypoplasia, and Shwachman-Diamond syndrome. Each of these disorders exhibit defective ribogenesis that is closely associated with p53 levels. Crossing Tp53+/- or Tp53-/- with RNase H2G37S/G37 mice results in mice with improved growth and partially rescues many of the abnormal phenotypes. We hypothesize that highly metabolically active tissues have high frequencies of rRNA synthesis which increase the number of R-loops and production of dsDNA breaks. We suggest that frequencies and locations of the R-loops occur in a stochastic manner both in time and space resulting in variability in and degree of observed phenotypes.

Abstract 34

Effect of PKR on IFN induction

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The recognition of non-self nucleic acids by innate pattern recognition receptors (PRR) is a key feature in the defense against invading viruses. Upon recognition of viral RNA Toll-like receptors in the endosome and RIG-I like helicases (RLHs) in the cytosol induce the expression of antiviral genes and thus initiate a type I interferon (IFN) dominated immune response. Additionally, there are cytosolic RNA-sensing receptors with effector function: Activated Protein Kinase R (PKR) phosphorylates eIF2a with subsequent inhibition of global host and viral protein synthesis and oligoadenylate synthetase (OAS) activates ribonuclease L (RNaseL) leading to degradation of host and viral RNA. Here we show, that PKR- and OAS/RNaseL-coactivation through long but not short ppp-dsRNA inhibits the RIG-I induced antiviral type I IFN and chemokine response on the protein expression level. Infection of cells with a PKR, RNaseL or PKR/RNaseL double knockout (KO) by an Influenza mutant with a deletion of the non-structural protein 1 (NS1, known to counteract PKR-mediated replication inhibition) induced an increased IFN activity in PKR as well as PKR/RNaseL KO cells reflecting the situation after stimulation with long ppp-dsRNA. Our results indicate a competition between antiviral signaling and effector pathways if long dsRNA is generated during infection.

Abstract 35

Varicella-Zoster Virus ORF9 Antagonises the DNA Sensor cGAS via Histone Mimicry

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Rationale: Varicella-Zoster virus (VZV) causes chickenpox and shingles. The molecular mechanisms that determine innate immune responses remain poorly defined. Previously, we found that the cGAS protein, a receptor of the DNA sensing pathway, is inhibited by the VZV-ORF9 protein (Hertzog et al., EMBO J 2022). However, the mechanism of cGAS inhibition remained unknown. cGAS is bound and inhibited in the nucleus of cells by binding to the acidic patch of the nucleosomal protein dimer H2A-H2B. An amino acid sequence alignment of VZV ORF9 and H2A revealed sequence similarity to H2A's acidic patch region.
Methodology: HEK293T, THP1, and HFF cells were transiently transfected or transduced to express ORF9 and ORF9 mutants. The introduced mutations revert the negative charge of amino acids in ORF9's putative acidic patch to no longer retain similarity to histones. ORF9 overexpressing cells were stimulated with dsDNA and the type I IFN response was measured via RT-qPCR and ELISA.
Results: ORF9 mutant overexpressing cells stimulated with DNA induced an ISG response similar to that of GFP overexpressing control cells. Compared to ORF9 WT, which supresses ISG induction via cGAS inhibition, the ORF9 mutants lost the ability to supress ISG/IFN induction, potentially via loss of cGAS binding. The loss of function in ORF9 mutants indicates that this viral protein may share the cGAS inhibition mechanism with histones. Consistently, AlphaFold modelling and RMSD values indicate similarities of the secondary structure of ORF9 and H2A.
Conclusions: We propose histone mimicry as a strategy of cGAS inhibition by VZV.

Abstract 36

Innate Immune Sensing of Self-RNA by RIG-I-Like Receptors

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Melanoma Differentiation-Associated Protein 5 (MDA5) is a cytosolic pattern recognition receptor of the RIG-I-like receptor (RLR) family that senses long double-stranded RNA (dsRNA) and, upon activation, induces type I interferon (IFN). Besides its role in antiviral immunity, sensing of insufficiently modified self-RNA by MDA5 has been implicated in autoinflammatory diseases. However, presence and characteristics of endogenous MDA5 agonists under healthy conditions have not been extensively studied so far.
To address this, we utilized a transient overexpression model of MDA5 in 293T cells and showed that MDA5 responds to endogenous dsRNA in a dose-dependent manner. Using mtRNA-free rho0 cells, we found while mtRNA makes up the bulk of MDA5 agonists in total RNA, nuclear-encoded RNA is the major source for accessible MDA5 ligands within cultured cells. In line with this, we found significant levels of dsRNA in HeLa rho0 cells by immunofluorescence staining with an anti-dsRNA antibody. To identify these cellular dsRNAs, we developed a method for targeted, sequence-independent isolation and sequencing of full-length long dsRNAs. This method will be applied to map dsRNA regions in RNA isolated from cells and tissue.
Our findings suggest that cells express MDA5-activating dsRNAs even in non-pathological conditions, and a portion of these is accessible to MDA5. Further experiments will be needed to address the exact identity of these stimulatory RNAs, especially in comparison to ligand sources under pathological conditions, their regulation to prevent autoimmunity or a possible physiological role in priming of innate immunity.

Abstract 37

Defects in mitochondrial RNA processing induce IFN signalling upregulation

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Type I interferonopathies are genetic diseases characterized by chronic activation of type I interferon (IFN) signalling and antiviral responses, often due to the abnormal sensing of self-nucleic acids. It has been recently shown that nucleic acid of mitochondrial origin can also trigger an IFN response, and thereby possibly contribute to pathology. As an exemplar, we previously showed that mutations in ATAD3A are associated with the release of mitochondrial DNA into the cytosol, which is then sensed by the cGAS-STING pathway to trigger the induction of IFN stimulated genes (ISGs) (PMID: 34387651). Here, we take advantage of another primary mitochondrial cytopathy to investigate further the relationship between mitochondrial dysfunction and IFN signalling. Specifically, a homozygous missense mutation in nuclear encoded MTPAP has been connected with a complex spastic ataxia phenotype, and an impairment of poly-A polymerase activity targeting mitochondrial messenger RNAs (mt-mRNAs) (PMID: 25008111). The shortened poly-A tail of mt-mRNAs was correlated with their destabilisation, resulting in the downregulation of mitochondrial respiratory chain protein levels. We are now studying cells from patients carrying compound heterozygous mutations in MTPAP, exhibiting a perinatal encephalopathy reminiscent of the type I interferonopathy Aicardi-Goutières syndrome (PMID: 31779033). We have observed baseline upregulation of ISGs in patient cells, suggesting a novel pathway linking mitochondrial nucleic acid maintenance and IFN signalling. We are currently exploring the mechanism(s) by which nucleic acids are sensed in the context of MTPAP dysfunction.

Abstract 38

The pUL36 deubiquitinase and pUL37 deamidase activities of herpes simplex virus dampen the innate immune response during skin infection

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Herpes simplex viruses (HSV) infect the skin, oral and genital mucosa, and the nervous system. While pattern recognition receptors (PRRs) detect HSV vRNAs and vDNAs, many HSV proteins intercept the PRR signaling. The tegument HSV1-pUL36 and HSV1-pUL37 proteins harbor deubiquitinase (DUB) or deamidase (DAM) activities, respectively, which both restrict cGAS/STING signaling and IFN expression, but target different host proteins.
We have generated HSV-1 mutants lacking the DUB or the DAM activity by replacing their respective catalytic cysteine (pUL36-C65A; pUL37-C819S). HSV1-pUL36ΔDUB replicates less efficiently in the murine brain and induces higher expression of IFN-β and ISGs (Bodda et al. 2020, JEM). Here, we investigated whether these DUB or DAM activities contribute to HSV-1 spread in the skin.
In human keratinocytes, HSV1-pUL36ΔDUB and HSV1-pUL37ΔDAM formed slightly smaller plaques and were slightly delayed in virus production. HSV1-pUL36ΔDUB was less able to restrict the transcription of several host genes related to immune response such as ISG15, MxB, and RSAD2, while HSV1-pUL37ΔDAM revealed only few changes in the transcriptome. In a novel ex vivo murine skin explant model, both mutants spread as efficiently as their parental HSV-1 strain and formed almost as many infectious centers of similar size. HSV1-pUL36ΔDUB and HSV1-pUL37ΔDAM induced stronger IFN-b promotor activation around the infectious centers. HSV1-pUL36ΔDUB also showed a stronger induction of ISG transcripts such as Cxcl10 and ISG15 than the parental virus. Our data indicate that the tegument DUB and DAM activities of HSV1 dampen the innate immune response in human keratinocytes and in murine skin infection ex vivo.

Abstract 39

Nucleic acid sensing is regulated by CDK4/6

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Cells are equipped to defend themselves from invading pathogens through sensors such as cGAS, which upon binding DNA induces type I interferon (IFN) expression. Whilst IFNs are crucial for limiting viral infection and activating adaptive immunity, uncontrolled production causes excessive inflammation and autoimmunity. cGAS binds DNA of both pathogenic and cellular origin and its activity is therefore tightly regulated. This is particularly apparent during mitosis, where cGAS association with chromatin following nuclear membrane dissolution and phosphorylation by mitotic kinases negatively regulate enzymatic activity. Here we describe a novel mechanism by which DNA sensing and other innate immune pathways are regulated during cell division, dependent on cyclin dependent kinases (CDK) 4 and 6. Inhibition of CDK4/6 using chemical inhibitors, shRNA-mediated depletion, or overexpression of cellular CDK4/6 inhibitor p16INK4a, greatly enhanced DNA- or cGAMP-induced expression of cytokines and IFN-stimulated genes (ISG). Mechanistically, CDK4/6-dependent inhibition mapped downstream of cytoplasmic signalling events including STING and IRF3 phosphorylation, limiting innate immune induction at the level of IFNβ mRNA expression. This regulation was universal, occurring in primary and transformed cells of human and murine origin, and broad, as IFNβ expression was inhibited in a CDK4/6-dependent manner downstream of multiple pattern recognition receptors. Together these findings demonstrate that host innate responses are limited by multiple mechanisms during cell division, thus defining cellular replication as an innate immune privileged process that may be necessary to avoid aberrant self-recognition and autoimmunity.

Abstract 40

RNaseT2 restricts TLR13-mediated autoinflammation in vivo

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RNA-sensing Toll-like receptors reside in the endolysosomal compartment, shielded from endogenous nucleic acids. Inside the endolysosome, the endoribonuclease RNase T2 processes long-structured RNA into short oligoribonucleotides, a step critically required for human TLR8 activation. In contrast to its pro-inflammatory role in TLR8 signalling, RNaseT2-deficient patients display a neuroinflammatory phenotype, indicative of aberrant activation of nucleic acid sensing pathways. In the murine system, RNaseT2 deficiency has been reported to result in an elevated interferon signature in various tissues, as well as dysregulated haematopoiesis and autoinflammation. Of note, TLR8 is not functional in mice, but TLR13 acts as its functional orthologue, recognising single-stranded RNA in a sequence-specific manner.
By studying Rnaset2-/- mice, we observed a predominantly inflammatory phenotype characterised by peripheral leukocytosis, as well as by overt splenomegaly dominated by an increase in myeloid cells. Interestingly, these phenotypes were completely attenuated by ablation of the TLR adapter protein MyD88. Further genetic studies revealed that a TLR13-MyD88 dependent signalling axis induces autoinflammation in Rnaset2-/- mice. Considering normal myelopoiesis in Rnaset2-/- mice, we suspect that a TLR13 ligand engages peripheral myeloid cell activation to instigate inflammation. In this regard we hypothesize that RNaseT2 deficiency leads to the accumulation of RNA molecules from either endogenous or bacterial sources. Altogether, these results suggest that RNase T2 - in addition to its proinflammatory role related to TLR8 signalling - also plays an important role in limiting erroneous TLR13 activation.

Abstract 41

Structural mechanism of OAS1 activation by RNA modifications

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The 2´-5´ oligoadenylate synthases (OAS) are interferon-induced dsRNA sensors of the innate immune system and play a critical role in antiviral activity. Upon binding to dsRNA, OAS polymerizes ATP into 2´-5´ linked oligoadenylates, which activate latent endoribonuclease L (RNase L) leading to subsequent cell death. The OAS family consists of three catalytically active members, OAS1, OAS2 and OAS3 that differ in their antiviral activity through currently unknown recognition mechanisms. The activity of OAS1 can be enhanced by specific RNA sequences, structural motifs, and lengths. Our previous work revealed an increased activation of OAS1 by 5'-triphosphate RNA, which is a well-known ligand of RIG-I, an immune receptor leading to interferon production and inducing cell death. However, current structures do not reveal the recognition mechanism of 5´ppp-RNA and why it leads to increased activation of OAS1. Here, we aim to reveal the structural mechanism of phosphorylated RNA recognition by OAS1 and compare binding affinities and mechanism of OAS1 with RIG-I activation by phosphorylated RNA. In order to understand the impact of different RNA characteristics on OAS1, OAS2 and OAS3, we test RNAs with defined lengths, sequences, structural motifs and modifications. Our results reveal novel dsRNA sequences that, when combined with 5´tri-phosphorylated RNA, can increase OAS1 activity far beyond the current preferred known agonistic consensus sequence.

Abstract 42

CK1a, a rheostat kinase modulating the CGAS-STING pathway

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Aberrant cytosolic double-stranded DNA (dsDNA) originating from pathogen infection (eg: virus, bacteria) or cellular stress (eg: chromosomal instability (CIN)-induced micronuclei, mitochondrial DNA leakage) triggers an evolutionarily conserved antimicrobial defence system orchestrated by Cyclic GMP-AMP synthase (cGAS) and Stimulator of Interferon Genes (STING), leading to inflammation and apoptosis. Dysregulation of the cGAS-STING pathway results in defective innate immune response and leads to autoinflammatory and autoimmune disorders. The fine tuning of the cGAS-STING pathway is therefore critical.
Herein, we report that Casein Kinase 1 alpha (CK1a), a kinase previously linked to antigen receptor and Wnt-b-catenin pathways, exerts a dual “gating” function in the cGAS-STING signaling pathway. On one hand, we unveil that CK1a and its partner Glycogen Synthase Kinase 3 beta (GSK3b) allow optimal DNA sensing by maintaining the abundance of cGAS, thereby positively regulating the downstream phosphorylation cascade and type I IFN production. Whilst on the other, we find that CK1a puts a break on type I IFN signalling downstream of STING stimulation.
Overall, our study highlights that CK1a has important immunomodulating activity in the cGAS-STING pathway that could be of therapeutical interest in the context of inflammatory diseases or autoimmununity.

Abstract 43

Analysis of yellow fever vaccination on DC subsets at transcriptomic and epigenomic level

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The life-attenuated yellow fever vaccine (YF17D) is one of the most efficient vaccines leading to a life-long immunity after one injection in humans. This efficiency makes the vaccine interesting for research to understand the underlying mechanism and adapt this knowledge to further vaccine research. In a cohort of yellow fever vaccinees, differentially expressed genes could be identified in all blood DC and monocyte subtypes after vaccination by using bulk RNA-sequencing and single-cell RNA-sequencing with a peak on day 7. In addition, multidimensional flow cytometric analysis showed upregulation of activation and maturation markers, and chemokine receptors, e.g., CD86, PD-L1, Axl, and Siglec-1, after YF17D vaccination in DC and monocyte subtypes. Longer-lasting changes in gene expression up to 28 days post vaccination were also detected in monocytes and DCs which may be due to ongoing adaptive responses or epigenetic remodeling of these cell types or their precursor cells. These long-lasting effects in the monocyte/DC compartment may be relevant for the response to subsequent vaccinations or infections. To further investigate chromatin accessibility and transcriptomic changes simultaneously on the single-cell level, we are currently doing 10x Genomic single-cell multiome ATAC and RNA sequencing with PBMC samples enriched for DCs, monocytes, and NK cells from YF17D vaccinees at early and late time points after vaccination. This approach will allow us to connect epigenomic changes with gene expression levels in individual cells and populations to unravel gene regulatory networks and potential epigenetic reprogramming of innate immune cells after application of a live viral vaccine.

Abstract 44

Deciphering the interactions between RNA viruses and retrotransposons in Drosophila melanogaster

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Insects are vectors for viruses causing disease in humans, plants, and animals. During viral infection, RNA genomes of non-retroviral RNA viruses are reverse transcribed into viral DNA (vDNA) by reverse transcriptases (RTs) encoded by endogenous retrotransposons. vDNA enhances the RNA interference-based antiviral response by giving rise to transcripts serving as sources of small interfering RNAs. This process keeps viral titers below a lethal threshold and is required for establishment of persistent viral infections in Drosophila melanogaster and mosquitoes. Therefore, virus-retrotransposon interactions are key to the host response to infection and a greater understanding of these interactions may facilitate new strategies to disrupt virus transmission.
To better characterize the impact of virus-retrotransposon interactions on viral infection cycles and host fitness, we developed isogenic D. melanogaster stocks harboring persistent infections with four naturally occurring viruses. Infections with any of the four viruses reduced fly lifespan, indicating that persistent viral infection imposes a previously unreported fitness cost. We next focused on a positive sense, single stranded RNA virus called Drosophila A virus (DAV) to determine how virus-retrotransposon interactions may influence host fitness during persistent viral infection. We found that vDNA forms of DAV are produced during persistent DAV infection and we measured RT activity throughout development in tissues from non-infected and DAV-persistently infected flies to correlate the appearance of vDNA with RT activity. Our results reveal novel spatiotemporal information regarding the formation of vDNA and provide new insights to further explore the physiological role of transposons in maintaining homeostasis during virus infections.

Abstract 45

A conserved isoleucine in the binding pocket of RIG-I controls immune tolerance to mitochondrial RNA

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RIG-I is a cytosolic receptor of viral RNA essential for the immune response to numerous RNA viruses. Accordingly, RIG-I must sensitively detect viral RNA yet tolerate abundant self-RNA species. The basic binding cleft and an aromatic amino acid of the RIG-I C-terminal domain(CTD) mediate high-affinity recognition of 5'triphosphorylated and 5'base-paired RNA(dsRNA). Here, we found that, while 5'unmodified hydroxyl(OH)-dsRNA demonstrated residual activation potential, 5'-monophosphate(5'p)-termini, present on most cellular RNAs, prevented RIG-I activation. Determination of CTD/dsRNA co-crystal structures and mutant activation studies revealed that an evolutionarily conserved Isoleucine within the CTD sterically inhibits 5'p-dsRNA binding. By contrast a RIG-I mutant missing this isoleucine was activated by both synthetic 5'p-dsRNA and endogenous long dsRNA within the polyA-rich fraction of total cellular RNA. The RIG-I mutant specifically interacted with a long, highly structured, polyA-bearing, non-coding mitochondrial(mt) RNA, and depletion of mtRNA from total RNA abolished RIG-I mutant activation. Altogether, our study demonstrates that avoidance of 5'p-RNA recognition is crucial to preventing mtRNA-triggered RIG-I-mediated autoinflammation.

Abstract 46

Insights into the pathophysiology of Aicardi-Goutières syndrome implementing CRISPR/Cas9-engineered iPSCs as an experimental model.

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Aicardi-Goutières syndrome (AGS), a rare monogenetic type I interferonopathy, is caused by mutations in genes participating in nucleic acid metabolism. AGS pathophysiology is hypothesized to result from accumulation of aberrant self-derived nucleic acids, resulting in activation of a type I interferon (IFN)-mediated response. However, the precise molecular mechanisms causing AGS remain unclear. Among AGS-associated genes, sterile alpha motif and HD domain-containing protein 1 (SAMHD1) is reported to be frequently mutated in the context of multiple malignancies. The investigation of SAMHD1-associated AGS provides an excellent experimental model for autoinflammation and cancer.

To study the role of specific mutations in SAMHD1 and to unravel the underlying disease-causing mechanisms, we generated induced pluripotent stem cells (iPSCs) from an AGS patient harbouring a mutation in SAMHD1 (1). Furthermore, to gain inside into the consequences of specific SAMHD1 point mutations associated with both cancer and AGS we generated CRISPR/Cas9 isogenic knock-in/out cell lines in apparently healthy iPSCs (2). We analyzed the impact of the mutations on inflammation after directed in vitro-differentiation into dendritic cells and neuronal progenitor cells. Patient derived as well as knock-out cells display higher interferon-stimulated gene expression (ISG) compared to controls.

SAMHD1 plays a role in replication fork processing. In absence of functional SAMHD1, aberrant DNA fragments from stalled forks could induce an IFN response. Indeed, we demonstrate that mutations in SAMHD1 alter replication dynamics and impair DNA end-resection in iPS-derived knock-in cells supporting the idea that aberrant DNA replication structures underlie the interferonopathy pathomechanism.

1) DOI: 10.1016/j.scr.2019.101697
2) DOI: 10.1016/j.scr.2019.101679

Abstract 47

RIG-I activation induces osteoblastic differentiation and calcification of human aortic smooth muscle cells

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Retinoic acid-inducible gene-I (RIG-I) is a cytosolic pattern recognition receptor that senses viral RNA. While its function is essential to the antiviral innate immune response through type-I interferon induction, gain-of-function mutations are associated with Singleton-Merten Syndrome (SMS), an innate immune disorder characterized by dental and skeletal abnormalities, as well as early and severe aortic calcification.
As this genetic disorder provides evidence that enhanced RIG-I signaling can cause aortic calcification, we are aiming to understand the underlying molecular mechanisms in more detail. Using primary human aortic smooth muscle cells (HAoSMC), we examined the effect of RIG-I activation on the expression of osteogenic factors and calcification. We found that activation of HAoSMC with a specific RIG-I ligand did not only trigger a type I IFN response, but also increased mRNA levels of the osteogenic factors bone morphogenic protein 2 (BMP-2), bone sialoprotein 2 (BSP2) and the structural bone component osteopontin. Moreover, HAoSMC cultivated in a pro-calcifying medium (PCM) showed calcium depositions that could be visualized by staining with Alizarin Red. Additional RIG-I stimulation in PCM-cultivated cells showed an earlier and stronger process of calcification. Lack of RIG-I or its downstream signaling molecule mitochondrial antiviral-signaling adaptor protein (MAVS) decreased the expression of interferon-stimulated genes and osteogenic factors. Calcification was also reduced in either RIG-I- and MAVS-deficient cells. Altogether, our results demonstrate that RIG-I and the downstream signaling molecule MAVS are involved in the inflammatory process that drives aortic pathogenesis. Further studies are required to identify the underlying molecular mechanisms in greater detail.

Abstract 48

Dissecting the crosstalk between type I interferon responses and DNA repair pathways

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Upon genome instability, replication or genotoxic stress, pathological self-DNAs accumulate in the cytosol of the cell and are recognized by receptors to promote pro-inflammatory cytokines and type I interferon (IFN) production. In the past years numerous DNA sensing machineries have been described to elicit inflammation, including the cyclic GMP-AMP synthase–stimulator of interferon genes (cGAS-STING) pathway and the DNA repair DNA-dependent protein kinase (DNA-PK) complex.
We will discuss a newly identified cross-talk between cGAS and the DNA-PK complex. Indeed, upon DNA recognition, DNA-PK promotes cGAS activation to enable higher type I IFN, cytokine and chemokine production, that ultimately shapes tumor immunogenicity. Furthermore, intriguingly, we observed that DNA-PK can also impair STING signaling, thus revealing that DNA-PK acts as a rheostat to control inflammation level. Finally, we will discuss how components of the cGAS-STING pathway are able to affect DNA-PK repair functions. Therefore, our findings suggest that dissecting the tight inter-connection between the DNA repair and type I IFN responses may reveal novel therapeutic options for pathologies, such as autoimmune disorders and cancer.

Abstract 49

The interferon-inducible antiviral GTPase MxB promotes herpesvirus capsid disassembly and premature genome release in cells

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The interferon-inducible dynamin-like GTPase MxB restricts retroviruses as well as herpesviruses. In cell-free capsid-host protein interaction assays, MxB binds to capsids of human alphaherpesviruses and induces their disassembly (Serrero et al. 2022, eLife). Here, we investigated MxB-induced capsid disassembly in cells during infection with HSV-1.

We generated retinal pigment epithelial cell lines (RPE) expressing MxB(1-715)-FLAG, the short isoform MxB(26-715)-FLAG, the GTPase deficient MxB(1-715/T151A)-FLAG, the monomeric MxB(1-715/M547D)-FLAG, or MxB(1-715/R11-13A)-FLAG in which a triple-arginine-motif, required for HIV restriction, has been replaced, or FLAG-MxA. In these cell lines we measured the impact of different Mx proteins on virus production, plaque morphology, intracellular fate of incoming capsids, incoming genomes, capsid assembly, and induction of interferon and ISGs. HSV-1 gene expression and capsid assembly were restricted in cells expressing MxB(1-715)-FLAG but not with FLAG-MxA. Using click chemistry, we labeled incoming HSV-1 genomes and detected more disintegrated capsids and cytoplasmic genomes in cells expressing MxB(1-715)-FLAG than in the control cells.

Our data indicate that MxB also disassembles capsids in cells and thereby reduces the efficiency of nuclear targeting of incoming and/or the assembly of progeny capsids. The reduced capsid protection of viral genomes may enhance the activation of DNA sensors, which are key players in the amplification and regulation of host innate immune defenses to infection.

Abstract 50

MAVS activation kinetics is determined by its subcellular localization

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Peroxisomes are highly dynamic intracellular organelles that fulfill important metabolic functions, being essential for lipid metabolism and a balanced redox species-environment. On top of that, peroxisomes are also important platforms for antiviral signal transduction. Detection of cytosolic viral RNA by the members of the RIG-I-like receptor (RLR) family, induces the activation of the mitochondrial antiviral signaling adaptor (MAVS) at peroxisomes and mitochondria, which culminates in the production of interferons (IFNs) and IFN-stimulated genes (ISGs).
The initial studies concerning peroxisomal and mitochondrial MAVS signaling were carried out in different stable cell lines expressing MAVS transgenes that were localized to each of the organelles and have reported conflicting results related to the antiviral response kinetics and the type of IFNs produced. To finally disclose the specific differences between the two signaling pathways and their activation features, we developed a novel genetic approach to evaluate MAVS activation at each subcellular compartment. By employing a doxycycline-inducible system, we implemented a strict regulation of our transgene expression to reduce possible confounding factors. We can now demonstrate that the peroxisomal MAVS strongly induces an early type I and III IFN-dependent response, in contrast to a late mitochondrial response. We further observe that peroxisomal MAVS generates a transcriptional antiviral response of similar magnitude when compared to the mitochondrial counterpart.
These data emphasize the versatility of the peroxisome-dependent antiviral response, in terms of IFN signaling, and reaffirm the distinctiveness of peroxisomes in generating a prompt cellular defense state against viral infections.

Abstract 51

Understanding the molecular basis of cGAS activation during HIV-1 infection

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Cyclic guanosine monophosphate–adenosine monophosphate (cGAMP) synthase (cGAS) is a sensor of cytosolic DNA and an innate immune sensor of retroviruses, including the human immunodeficiency virus type-I (HIV-1). The retrovirus-specific innate co-sensor PQBP1 is required for cGAS recruitment to incoming virus particles once capsid disassembly and DNA synthesis are initiated [1]. This recruitment mechanism suggests a two-step authentication strategy where PQBP1 binding to the capsid is a prerequisite for cGAS recruitment. However, cGAS recruitment additionally requires loss of capsid integrity associated with reverse transcription1. Initiation of reverse transcription starts in the cytosol [2], nevertheless, completion of cDNA reverse transcription and uncoating of HIV-1 capsid occur within the nucleus of the host cell [3,4]. These observations raise the possibility that cytoplasmic and nuclear sensing of HIV may be distinct processes, regulated by different reverse transcription events and capsid configurations. Our aim is to study the progression of cGAS binding to the molecular products of early, intermediate and late stage of HIV-1 reverse transcription in the cytoplasm and nucleus of infected THP-1 macrophages by performing pulldown of endogenous protein and high-throughput sequencing of cGAS-bound nucleic acids at different time point of infection. With this purpose, we generated and characterized CRISPR/Cas9 THP1-1 clones expressing cGAS tagged with 3xFLAG in the gene locus.

1. Yoh et al., 2022: doi: 10.1016/j.molcel.2022.06.010
2. Cosnefroy et al., 2016: doi: 10.1186/s12977-016-0292-7
3. Zila et al., 2021: doi: 10.1016/j.cell.2021.01.025
4. Dharan et al., 2020: doi: 10.1038/s41564-020-0735-8.

Abstract 52

Leishmania mexicana dysregulates the cytosolic DNA sensing pathway in infected THP-1 macrophages

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Leishmania parasites are the causative agents of Leishmaniasis, a neglected tropical disease. To replicate, Leishmania evades the host's innate immune response to infect macrophages and persist within them. Leishmania express virulence factors to aid infectivity by inhibiting host signalling, some downstream of pathogen recognition receptors (PRRs). Leishmania parasites have been shown to inhibit Toll-like receptor signalling, but there has been little investigation into Leishmania interaction with cytosolic nucleic acid sensors.
Human monocytic THP-1 cells were infected in vitro with L. mexicana parasites, and the activation of the cytosolic DNA and RNA sensing pathways were investigated using quantitative real-time PCR (qRT-PCR) and western blot. We find that THP-1 cells infected with L. mexicana parasites have an enhanced response to subsequent DNA, but not RNA, stimulation. This can be seen by an increased activation of a key DNA sensing adaptor protein, stimulator of interferon genes (STING) and its downstream targets, in addition to an increased interferon response to DNA. Through protein analysis we also observed that STING and other key components of the DNA sensing pathway are modified during infection. We identified the modification to STING as a cleavage event resulting in partial loss of its C-terminal tail. While cleavage to this form is highly upregulated during infection, we find that this alternative form of STING is also present at lower levels in uninfected cells, and may have a regulatory role that Leishmania parasites could exploit for their own benefit.

Abstract 53

RIG-I like Receptor Activation by mRNA Incorporating Modified Nucleosides

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The remarkable success of nucleoside-modified, in vitro-transcribed (IVT) mRNA vaccines against SARS-CoV-2 has brought mRNA technology to the medical mainstream, with more than 12 billion vaccine doses administered worldwide to date. Effective IVT mRNA translation requires the inhibition of innate immune RNA receptors. The co-transcriptional incorporation of modified nucleosides such as N1-methylpseudouridine (m1Ψ) and 5-methylcytosine (m5C) has been utilized to prevent activation of endosomal Toll-like receptors by transfected mRNA. While inhibitory effects on cytosolic RIG-I like receptor (RLR) activation through the reduction of double-stranded IVT byproducts have been suggested, a systematic investigation and direct comparison of nucleoside-modified mRNA sensing by the RLRs RIG-I and MDA5 has not been performed.
Here, we investigate the effects of commonly used modified nucleosides on the activation of MDA5 and RIG-I by IVT mRNA preparations and structurally defined RLR-agonistic RNAs. We quantify mRNA translation as well as activation of the type I interferon response in primary immune cells and gene-edited monocytic cell lines and analyze the differential contribution of MDA5 and RIG-I to the innate immune response against IVT mRNA.
Using this approach, we find that most nucleoside modifications have a less pronounced effect on RLR activation than previously reported, in particular on MDA5 activation. In addition, the effect of modified nucleosides on innate immune activation varies with both length and secondary RNA structure. Our results highlight the importance of rigorous assessment of innate immune stimulation for individual mRNA sequences and nucleoside modifications, with implications for the clinical effectivity of mRNA formulations and their potential inflammatory side effects.

Abstract 54

The Role of Mitochondrial Stress in the Pathogenesis of Dermatomyositis

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Dermatomyositis (DM) is a rare autoimmune disease characterized by skin and muscle inflammation significantly impairing quality of life. The disease is characterized by activation of the type I interferon (IFN) pathway. Beside the well-known IFN signature in blood we also observed a chronic upregulation of type I IFN stimulated genes in fibroblasts isolated from DM skin.
While the molecular pathways inducing type I IFN in DM remain to be elucidated, we were able to show that ISG expression partially decreases after STING knockdown in DM fibroblasts. Due to the central role of STING in intracellular DNA sensing via cGAS, we propose that self-nucleic acids could trigger type I IFN induction in DM fibroblasts.
Following this hypothesis, we performed RNA sequencing in fibroblasts from DM-patients and healthy controls. Gene Set Enrichment Analysis revealed upregulation of ISG and significant downregulation of genes involved in respiratory chain and mitochondrial protein import.
Therefore, we assessed mitochondrial function in patient fibroblasts via Seahorse Assay. We detected that the basal oxygen consumption rate as well as the ATP-production were significantly reduced compared to healthy controls. Further analysis showed a significant upregulation of mitochondrial reactive oxygen species in DM fibroblast as a sign of mitochondrial stress.
These findings suggest that IFN induction in DM fibroblasts may be driven by mitochondrial DNA leakage into cytosol inducing cGAS-STING-sensing and upregulation of ISGs which perpetuate inflammation. Deciphering these pathogenic pathways will allow to develop novel therapeutic strategies for this chronic and often devastating disease.

Abstract 55

Antagonistic nanobodies reveal mechanism of GSDMD pore formation and unexpected therapeutic potential

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Activation of various inflammasomes by different triggers, including aberrant nucleic acids, converges on the cleavage of gasdermin D (GSDMD) by pro-inflammatory caspases, followed by oligomerization of the N-terminal domain (GSDMDNT) and the assembly of pores penetrating target membranes. Yet, it remained unclear what triggers the conformational changes that allow membrane insertion, as methods to study pore formation in living cells were limited. We raised nanobodies specific for human GSDMD and found two nanobodies that prevent pyroptosis and IL-1β release when expressed in the cytosol of human macrophages. Nanobody binding to GSDMDNT blocked its oligomerization, while inflammasome assembly and GSDMD processing itself were not affected. The nanobody-stabilized monomers of GSDMDNT partitioned into the plasma membrane, suggesting that pore formation is initiated by insertion of monomers, followed by oligomerization in the target membrane. When GSDMD pore formation was inhibited, cells still underwent caspase-1-dependent apoptosis, likely due to the substantially augmented caspase-1 activity. This hints at a novel layer of regulation of caspase-1 activity by GSDMD pores. Moreover, we revealed the unexpected therapeutic potential of antagonistic GSDMD nanobodies, as recombinant nanobodies added to the medium prevented cell death by pyroptosis, likely by entering through GSDMD pores and curtailing the assembly of additional pores. GSDMD nanobodies may thus be suitable to treat the ever-growing list of diseases caused by activation of the (non-) canonical inflammasomes.

Abstract 56

A CRISPR/Cas9 based in vitro model to investigate the molecular basis of the PQBP1 dependent HIV-1 sensing complex

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Polyglutamine-binding protein 1 (PQBP1) is an innate sensor specifically required for cGAS-mediated innate response to human immunodeficiency virus 1 (HIV-1) and other lentiviruses (1). PQBP1 participates in a unique two-step authentication process. PQBP1 recognizes incoming HIV-1 particles. Capsid disassembly and DNA synthesis trigger PQBP1-dependent recruitment of cGAS followed by enzymatic activation (2). Monocyte-derived dendritic cells from enpenning-syndrome patients harboring mutations in PQBP1 possess a severely attenuated innate immune response to HIV-1 (1). Control experiments suggest that both, RNA and general DNA sensing pathways are intact in patient cells underscoring the specific requirement of PQBP1 for HIV sensing.

To understand the involvement of the PQBP1 c-terminus, we assessed the levels of enzymatic cGAS product, cGAMP, upon HIV-1 challenge. THP1 cells resembling patient PQBP1 revealed cGAMP staining comparable to non-infected cells in contrast to robust induction of cGAMP in WT-reconstituted cells. C-terminal deleted PQBP1 does not recruit cGAS to capsid as shown by proximity ligation assay in THP1 cells.

To further understand the molecular basis of the PQBP1 regulated sensing complex, we generated induced pluripotent stem cells (iPSCs) from the patient mentioned above (3). Moreover, Isogenic knock-in cell lines resembling specific mutations in the pqbp1 locus were enginereed by CRISPR/Cas9.

We differentiated iPSCs into sensing competent i-dendritic cells (iDC).). HIV-infection experiments demonstrated that patient derived iDCs possess an altered cGAS recruitment pattern and an attenuated response to HIV-1 compared to controls. These findings will decipher the molecular mechanisms of the sensing complex comprised of cGAS, PQBP1 and capsid.

1) PMID:26046437 2) PMID:35809572 3) PMID:31698189

Abstract 57

cGAS guards centromeric DNA to detect the virulence protein ICP0

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cGAS senses viral DNA in the cytosol, leading to activation of antiviral defenses. However, most DNA viruses enter and replicate in the nucleus, where cGAS is present.

How viruses may be detected by nuclear cGAS in the nucleus is ill-defined. Nuclear cGAS is maintained inactive by tethering to chromatin and is enriched on centromeric DNA. We hypothesized that centromere alterations induced by viruses might activate nuclear cGAS. The transcription activator and SUMO-targeted ubiquitin ligase ICP0 (HSV-1), disorganizes the centromere by targeting centromeric proteins. We find that ectopic expression of ICP0 in human dendritic cells induces innate immune activation, nuclear cGAMP accumulation and type III IFN production in a cGAS-dependent manner. We show that ICP0 activates cGAS independently from the MORC3 pathway. ICP0 is nuclear in DCs and leads to centromeric protein B (CENP-B) degradation. Nuclear localization and ubiquitin ligase activity of ICP0 are required for innate immune activation. ATAC-seq profiling and DNA quantification reveals that ICP0 increases respectively the accessibility and the amount of centromeric DNA (cenDNA). ICP0-induced cenDNA expansion requires DNA polymerase activity and the ICP0 target USP7 (Ubiquitin Specific Peptidase 7).

Altogether, these results indicate that cGAS senses the activity of ICP0 by detecting changes in centromere DNA abundance, consistent with the guard model of pathogen detection. We propose that cGAS guards centromeric DNA in interphase to detect virulence factors.

Abstract 58

Dynamic Evolution of Avian RNA Virus Sensors: Repeated Loss of RIG-I and MDA5

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Two key cytosolic receptors belonging to the retinoic acid-inducible gene I (RIG-I)-like receptor (RLR) family sense the viral RNA-derived danger signals: RIG-I and melanoma differentiation-associated protein 5 (MDA5). The evolutionary dynamics of RLRs has been studied mainly in mammals, where rare cases of RLR gene losses were described. By in silico screening of avian genomes, we described two independent disruptions of MDA5 in two bird orders. Further, we extendedthis analysis to approximately 150 avian genomes and report 16 independent evolutionary events of RIG-I inactivation. Interestingly, in almost all cases, these inactivations were coupled with genetic disruptions of RIPLET/RNF135, an ubiquitin ligase RIG-I regulator. Complete absence of any detectable RIG-I sequences is unique to several galliform species, including the domestic chicken (Gallus gallus). We further aimed to determine compensatory evolution of MDA5 in RIG-I-deficient species. While we were unable to show any specific global pattern of adaptive evolution in RIG-I-deficient species, in galliforms, the analyses of positive selection and surface charge distribution support the hypothesis of some compensatory evolution in MDA5 after RIG-I loss. This work highlights the dynamic nature of evolution in bird RNA virus sensors.

Abstract 59

Diadenosine tetraphosphate (Ap4A) serves as a 5’ RNA cap in mammalian cells

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RNA modifications have long been connected to foreign nucleic acid detection and the innate immune response. In particular, the canonical eukaryotic N7-methylguanosine (m7G) RNA cap and its modifications are a key component of this recognition process. While the m7G cap was long thought to be the only eukaryotic RNA cap, the recent discovery of non-canonical RNA caps such as nicotinamide adenine dinucleotide (NAD), flavin adenine dinucleotide (FAD), and others has changed our understanding of post-transcriptional gene regulation. Here, we present the discovery of a new type of RNA cap in eukaryotes –diadenosine tetraphosphate (Ap4A). Free Ap4A is the most abundant dinucleoside polyphosphate in eukaryotic cells and can be incorporated into RNA by RNA polymerases as a non-canonical initiating nucleotide (NCIN). We use liquid chromatography-mass spectrometry (LC-MS) to show that Ap4A-RNA capping is not dependent on the intracellular concentration of free Ap4A. We further identify two enzymes that are capable of cleaving the Ap4A-RNA cap in vitro, NUDT2 and DXO. Interestingly, we show that even though free Ap4A has been linked to the innate immune response and its dysregulation, Ap4A-RNA does not induce the expression of genes responsible for nucleic acid sensing such as RIG-I, MDA-5, IFNB1 or members of the IFIT family. As such, Ap4A-RNA is recognized as self by the cell and exists as a natural part of the cellular transcriptome. This discovery of a new cap opens a previously unexplored area of eukaryotic RNA regulation.

Abstract 60

ADAR editing and RNA interference prevent the accumulation of toxic palindromic repeat RNAs in C. elegans.

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We have previously reported that the molecular targets of ADAR editing in Caenorhabditis elegans are mostly palindromic repeat elements present in genes that can form dsRNA hairpins. In the absence of ADAR editing in C. elegans mutants with increased activation of the RNA interference (RNAi) pathway, these palindromic repeat RNAs cause toxicity to the host. We have characterized the responses to palindromic repeat RNA that are activated in this context, and we have defined a pivotal role for DRH-1, the sole C. elegans homolog of RIG-I and MDA5 that is required for anti-viral RNAi. These data show that the ADARs (together with negative regulation of RNAi) prevent an autoimmune anti-viral response to endogenous dsRNA similar to the function of ADAR in human.
Using small RNA sequencing, we showed that small interfering RNAs are generated from palindromic dsRNA. Using a partial genome RNAi screen for gene depletions that suppress the toxic phenotypes, we found that this autoimmune response requires RNAi pathway factors, transcription factors, splicing factors and RNA transport proteins.
Our data also indicates that other classes of cellular RNAs may be targeted by the anti-viral RNAi machinery. As an example, we identified mis-regulation of several classes of cellular RNAs in ADAR and enhanced RNAi mutants including histone RNAs, rRNA, and mitochondrial RNA. We will report on our current research focusing on defining the mechanisms that prevent mis-guided silencing responses to cellular RNA.

Abstract 61

Influence of mRNA secondary structure on innate immune activation

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Viruses have evolutionary acquired diverse molecular features that allow them to replicate in different hosts. One crucial feature is adaptation of the viral RNA sequence and structure, allowing high translation efficiency and immune evasion. One example is suppressed CpG dinucleotides found in various human-infecting viruses.
This project aims to investigate their potentially disadvantageous role in virus replication. There might be sensing mechanisms of the host's defense system for a certain CpG frequency that trigger an antiviral immune response, leading to impaired translation or increased RNA degradation.
To identify the receptors and effectors of this system, RNA sequences of viral proteins have been modified to exhibit different CpG frequencies while maintaining the amino acid sequence. These mRNAs were transfected into monocytes to analyze the translational efficiency and immune reactions. How supposed codon optimization of the sequence benefits translation or whether the resulting increase of CpG is interfering with the efficiency is yet to be verified.
In the following, cells lacking certain RIG-I like receptors (RLRs) and pull down assays can hopefully shed light on the sensing mechanism. Further, changes in the RNA sequence inevitably lead to changes in the secondary structures. Advanced bioinformatic analysis of correlation between certain secondary substructures and effects in the cellular system will complement the understanding of interactions of RNA with the innate immune system.
The final goal is finding mRNA sequences with balanced immune escape and RLR-mediated adjuvant activity while maintaining high translation activity using bioinformatic and molecular biologic approaches in order to improve vaccine design.

Abstract 62

Hsc70-4 as a Cell Surface Receptor for Extracellular dsRNA Uptake in Drosophila melanogaster S2 Cells

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The small interfering RNA (siRNA) pathway constitutes a pivotal antiviral defense against RNA viruses in insects, functioning through RNA interference mediated by Ago2-guided cleavage of viral genomes. Despite the known cellular uptake of double-stranded RNA (dsRNA) through endocytosis, the specific protein(s) responsible for this internalization from the extracellular environment remain elusive. Here, we investigate the role of Hsc70-4, a member of the heat shock protein family, as a potential cell surface receptor or co-receptor for dsRNA internalization in Drosophila melanogaster S2 cells. Immunofluorescence assays were conducted on permeabilized and non-permeabilized S2 cells using a specific anti-Hsc70-4 antibody to determine its subcellular localization. Permeabilized cells exhibited cytoplasmic and plasma membrane staining, whereas non-permeabilized cells showed punctate staining on the outer surface of the plasma membrane, indicating Hsc70-4's presence on the cell surface. To assess Hsc70-4's role as a receptor/co-receptor for dsRNA uptake, we employed immunofluorescence and luciferase-based silencing assays. Pretreatment of S2 cells with anti-Hsc70-4 antibody resulted in a significant reduction of internalized Cy3-labeled dsRNA, suggesting Hsc70-4's involvement in the uptake process. Furthermore, luciferase assays revealed a direct correlation between antibody concentration during pretreatment and decreased silencing efficiency, further supporting Hsc70-4's role as a dsRNA receptor. In conclusion, our experiments provide evidence that Hsc70-4 is expressed on the cell surface of S2 cells where it may act as a receptor for extracellular dsRNA. These findings shed light on the molecular mechanisms underlying insect antiviral defense and offer potential targets for enhancing antiviral responses in insects or developing novel antiviral strategies.

Abstract 63

ZBP1 forms amyloidal signalling complexes upon recognition of Z-RNA/DNA

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Activation of the nucleic acid sensor ZBP1 induces an innate immune response with relevance in viral restriction, autoinflammation and anticancer immunity. The two N-terminal Zα domains of ZBP1 bind specifically to double-stranded DNA or RNA in the left-handed Z-conformation, structures that are referred to as Z-DNA or Z-RNA, respectively. Recognition of Z-DNA/RNA by ZBP1's Zα domains induces NF-κB activation, IFN-I production and induces regulated cell death under the forms of apoptosis and necroptosis. How the detection of Z-RNA/DNA by ZBP1 activates downstream signalling is incompletely understood.
Using herpes simplex virus 1 (HSV-1) as an activator of ZBP1, live cell microscopy, in vitro reconstitution assays and structure-function analysis, we here propose a model whereby Zα domain-mediated recognition of Z-DNA/RNA induces the assembly of multiple ZBP1 molecules along the axis of Z-DNA/RNA. This is followed by proximity-induced oligomerisation of three centrally positioned RIP homotypic interaction motifs (RHIMs), resulting in the formation of a stable amyloidal signalling complex. We show that this enables the recruitment of the RIPK3 and RIPK1 kinases to induce necroptosis of the infected host cell.
In the future, we wish to employ this model to understand how ZBP1 activates signalling pathways other than necroptosis, characterise the physiological agonist of ZBP1 in the context of infection and autoinflammation and design therapeutic nucleic acids that specifically target ZBP1.

Abstract 64

The role of innate immune stimulation for BAFF induction in cutaneous lupus erythematosus

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Lupus erythematosus (LE) is a chronic autoimmune disease with diverse clinical manifestations, whose pathogenesis remains elusive. Research has shown that monogenic lupus forms are driven by heightened type 1 interferon (Type 1 IFN) levels activated via the cell-intrinsic cyclic GMP-AMP synthase (cGAS) and stimulator of interferon genes (STING) pathway. The B-cell activating factor (BAFF), plays a pivotal role in activating B-cells and is expressed predominantly in innate immune cells, but also in tissue-resident keratinocytes. Notably, BAFF levels are elevated in cutaneous lupus lesions. Cultured LE human fibroblasts (hFBs) have demonstrated elevated levels of BAFF compared to healthy controls. Environmental factors, such as photosensitivity, cold weather, and viral infections, can trigger lupus patients. To simulate these factors in vitro, cultured hFBs were exposed to UV-irradiation, cold temperatures, and nucleic acid (NA) stimulation. BAFF expression in irradiated LE hFBs was significantly higher ($p=0.006$) than in unexposed hFBs. Furthermore, NA stimulation with poly dA:dT significantly upregulated ($p=0.03$) BAFF in LE fibroblasts, whereas poly I:C did not produce the same effect. Transcriptome sequencing of 5 multifactorial lupus patients and 5 healthy control skin samples showed upregulated IFN-associated gene expression in LE fibroblasts and keratinocytes compared to controls. Interferon stimulated genes (ISGs) such as IFI27, IFI44, ISG15 Mx1 and OAS2 were upregulated in the sequencing analysis. IFN-κ was upregulated in LE keratinocytes versus control cells. The functional dependency of BAFF and type 1 IFN in monogenic and multifactorial lupus forms remains to be understood, with the cGAS-STING pathway potentially serving as the connecting bridge.

Abstract 65

How is the innate immune system alerted to DNA damage in skin cells?

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Ultraviolet (UV) radiation is known to induce DNA damage and generate an inflammatory response in the skin. However, the role of innate immune response in responding to this exposure is unclear. The pattern recognition receptor cGAS (cyclic GMP-AMP synthase) is capable of detecting double-stranded DNA in the cytosol to activate the adaptor STING (Stimulator of Interferon Genes). cGAS can detect cytosolic DNA that becomes exposed due to micronuclei rupture occurring in the days following DNA damage to generate an immune response. In human keratinocytes, IFI16 contributes to cGAS-induced STING activation. However, IFI16 can also independently generate a more rapid response to DNA damage involving an altered STING signalling complex that occurs in a matter of hours following DNA damage detection. The aim of the project is to characterise these different innate immune signalling pathways in response to different types of DNA damage, including UV-B, in human skin cells.
In HaCaT cells we find that chemotherapy agents, replication stress inducers and UV irradiation induce a DNA damage response including rapid histone γH2A.X phosphorylation. Using ELISA and qRT-PCR analysis we show an innate immune response, which involves the secretion of pro-inflammatory cytokines such as IL-6 occurs in response to these forms of DNA damage. IL-6 secretion was dependent on the DNA sensing factors IFI16 and STING after DNA damage induction.
Overall, we find that different types of DNA damage can induce a cell-intrinsic innate immune response in human skin cells. This may play a role in UV-induced inflammation and skin cancer progression.

Abstract 66

IKK-epsilon prevents RIPK1-dependent inflammation in the absence of TBK1

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TBK1 plays a crucial role in regulating various cellular processes, such as anti-viral type-I interferon responses, metabolism, and TNF receptor signaling. TBK1 homolog IKKε is also involved in anti-viral and TNF responses. However, the specific contributions and potential overlaps between IKKε and TBK1 in cell death, inflammation, and tissue balance are not well understood. In this study, we demonstrate that IKKε serves as a protective factor against RIPK1-mediated transient alopecia, splenomegaly, myeloid cell expansion, and systemic inflammation in the absence of TBK1. When both IKKε and TBK1 kinase activities were inhibited in myeloid cells, it led to RIPK1-dependent cell death and systemic inflammation, mediated by IL-1 family cytokines. Our tissue-specific investigations further revealed that IKKε and TBK1 are crucial in preventing cell death and inflammation in the intestine, while their functions are less critical for liver and skin homeostasis. In summary, our study showed the tissue-specific roles of IKKε and TBK1 and demonstrated their cooperation with canonical IKKs to safeguard against RIPK1-mediated cell death and inflammation.

Abstract 67

DNA damage-induced spontaneous tumorigenesis in the hematopoietic system is unaffected by the cGAS/STING pathway

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Genome damage is a main driver of malignant transformation and it activates the cGAS/STING DNA sensing pathway. cGAS/STING signaling triggers inflammation, cell death and senescence, which altogether can limit expansion of genome-damaged cells and thereby potentially prevent malignant transformation. Indeed, targeted activation of cGAS/STING represents a promising strategy to boost anti-tumor immunity and to induce tumor cell death in established tumors. In contrast to this acute scenario, the contribution of chronic activation of cGAS/STING in response to endogenous DNA damage in the control of spontaneous malignant transformation is currently controversially discussed. We investigated how cGAS/STING signaling affects the development of hematopoietic tumors in mice with defective ribonucleotide excision repair (RER) and with defective p53. Loss of RER caused genome instability with concomitant activation of the cGAS/STING axis whereas loss of p53 did not lead to a prominent activation of cGAS/STING. Both mouse models succumbed to leukemia and lymphoma, respectively, with unaltered kinetics even when cGAS or STING signaling were abrogated. In addition to that, fitness of hematopoietic cells was unaltered by the loss of cGAS upon induction of chronic and acute DNA damage, challenging the concept cGAS-mediated regulation of DNA repair in mice. Our study suggested that in contrast to their targeted activation in established tumors, cGAS or STING do not alter the course of spontaneous malignant transformation of RER- or p53-deficient hematopoietic cells.

Abstract 68

An autoinflammatory syndrome with high interferon signature due to compound heterozygous truncating IFNLR1 mutations

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We describe a child with recurrent fever, panniculitis, and pancytopenia, due to compound heterozygosity for two mutations in IFNLR1, which together with IL10R2 forms the IFN-λ receptor. The first mutation (M1) truncates at the extracellular portion of IFNLR1, the second mutation (M2) truncates distal of the JAK1 binding site. Patient PBMCs were unresponsive to IFN-λ stimulation, while responsiveness to IFN-α did not differ from wildtype controls, indicating complete loss of IFN-λ signaling. IFN-λ acts antiviral similar to type I IFN at mucosal interfaces. However, the patient did not show recurrent infections, but instead exhibited an IFN signature in blood and spontaneous STAT1 phosphorylation in LCLs. Overexpression of M1, but not M2, in IFNLR1-deficient HaCat cells led to phosphorylation of STAT1, which persisted in cells with additional knockout of either the IFN-α or IFN-γ receptors, or both, suggesting that STAT1 activation induced by M1 occurs independently of type I, type II, or type III IFN signaling. Whole blood assays using LPS revealed a deficiency in IL-10-mediated regulation of IL-12 in the patient, suggesting that IFNLR1 mutants may interfere with anti-inflammatory IL-10 signaling. This may reflect impaired formation of the IL-10 receptor, which shares the IL10R2 subunit with the IFN-λ receptor. Moreover, basal and stimulatory levels of IL-18 and IFN-γ were elevated in the patient, suggesting the presence of enduring pro-inflammatory cytokines, contributing to autoinflammation. These findings identify INF-λ receptor deficiency as a novel immune dysregulation disorder and suggest yet unknown functions of IFN-λ in innate immunity.

Abstract 69

Understanding how mitochondrial DNA signals danger

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Mitochondria are central players in cell death and inflammatory signalling. Permeabilization of the outer mitochondrial membrane (MOMP) during intrinsic apoptosis is essential for cytochrome C release and caspase activation ultimately driving cell death. Importantly, upon caspase inhibition MOMP potently induces inflammation and caspase-independent cell death (CICD). In the context of cancer, the inflammation elicited by CICD can turn a “cold” tumor “hot”, restoring immune infiltration and driving tumor regression. As such CICD harbors a high potential as an anti-tumorigenic treatment. The origin of the inflammatory response lays in danger signals such mitochondrial DNA (mtDNA) released during CICD. While mtDNA is inert when residing in the matrix, upon its cytoplasmic release, mtDNA can activate various pro-inflammatory pathways. Most notably the induction of cGAS-STING signalling, leading to a type I interferon response. Crucially, how mtDNA gains access to the cytosol driving inflammation remains ambiguous. I will discuss my research investigating mtDNA release during CICD. A deeper understanding mtDNA release is vital for future development of drug candidates and the use of CICD as a cancer therapy.

Abstract 70

Phlebovirus infection drives NLRP1 inflammasome activation and pro-inflammatory cytokine release

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Recently, we and others found that arthropod-borne alphaviruses activate the inflammasome sensor NLRP1 in human keratinocytes and thus an inflammatory response in the skin (Bauernfried et al. 2020, Jenster et al. 2023). Phleboviruses (order Bunyavirales), a distinct group of RNA viruses, are globally emerging mosquito- or sandfly-transmitted arboviruses that cause a wide spectrum of disease. Rift Valley fever virus (RVFV), for example, causes fever, hepatitis, encephalitis, or hemorrhagic fever, i.e. pathologies associated with a strong inflammatory response. However, only little is known about the local anti-phleboviral immune response in the skin.
We thus infected human keratinocytes with RVFV and examined inflammasome assembly, the release of pro-inflammatory IL-1β, and the interferon response. Indeed, inflammasome assembly and IL-1β release of RVFV-infected human keratinocytes were comparable to alphavirus-infected cells and depended on NLRP1 and its downstream adapter protein ASC. Similarly, active viral replication was required, whereas interferon signaling was dispensable. Furthermore, RVFV-infected cells displayed robust p38 MAP kinase activation and characteristics of the ribotoxic stress response (RSR), suggesting that NLRP1 activation might be driven by the RSR kinase ZAKα and downstream p38. While p38 signaling was essential, inhibitors or depletion of ZAKα, however, only partially reduced inflammasome assembly and IL-1β release, indicating the presence of additional signaling events that feed into NLRP1 activation upon RVFV infection.
In summary, RVFV infection drives NLRP1 inflammasome activation in human keratinocytes. The subsequent release of mediators like IL-1β might thus play a role in shaping the inflammatory environment in the skin - and thereby the immune response at the initial site of RVFV transmission.

Abstract 71

Unveiling the cGAS-STING Pathway in Hepatitis C Virus Infected human hepatoma cell line

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Hepatitis C virus (HCV) is a significant human pathogen known to infect hepatocytes, leading to potentially severe outcomes such as cirrhosis and hepatocellular carcinoma. The HCV genome, approximately 9.6 kb in length, encodes a single open reading frame flanked by 5′ and 3′ untranslated regions (UTRs). This genetic material employs an internal ribosome entry site (IRES) to recruit ribosomes for translation, ultimately generating a polyprotein. This polypeprotein is then co- and post-translationally processed into structural (core, E1, E2) and nonstructural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B) proteins. Importantly, beyond their role in viral RNA replication, HCV proteins have been shown to modulate cellular responses to viral infection. HCV infection triggers an innate immune response aimed at protecting the host against its effects. Within the host a pathogen recognition receptor (PRRs), recognize pathogen-associated molecular patterns (PAMPs). Among these PRRs, cGMP-AMP synthase (cGAS) and Stimulator of Interferon Genes (STING) stand out. While well-understood in the context of DNA viral infections, their role in the context of RNA viruses like HCV is less clear, though some RNA viruses have been reported to be restricted in a cGAS- and/or STING-dependent manner.
Our preliminary data has shown an increase in cGAS expression during HCV infection. However, the mechanism of cGAS activation and its specific role in HCV infection remain elusive. Additionally, it is crucial to ascertain whether the cGAS-STING pathway operates concurrently with the well-established RIG-I-MAVS-IRF3 pathway during HCV infection. This study aims to elucidate the mechanism of cGAS-STING pathway activation during HCV infection as it is typically associated with DNA viruses. We are investigating whether STING can initiate an innate immune response independently or if cGAMP, produced by cGAS, is necessary for its activation in HCV-infected cells. This research will enhance our understanding of how the cGAS-STING pathway deploys immune defenses against HCV and complements existing RIG-I-MAVS pathways.

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International Conference on Nucleic Acid Immunity

October 26-28th 2023
Dresden, Germany

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