Nucleic Acid Immunity Meeting 2022

Venue: Royal College of Physicians of Edinburgh (RCPE)

Abstract Book

KEYNOTE SPEAKERS

Andrea Ablasser

EPFL, Lausanne, Switzerland

STING: insight into negative regulation and implications for neurodegeneration

Raphaela Goldbach-Mansky

*NIH, Bethesda, US***A**

Lessons taught by patients with presumed interferonopathies

James Chen

University of Texas Southwestern Medical Centre, Dallas, USA

cGAS in immune defence - from human to bacteria

INVITED SPEAKERS *(in session order)*

Session 1: Chemistry and structural biology of nucleic acid sensing
Chair: Min Ae Lee-Kirsch

Pingwei Li

Texas A&M University, USA

The structural basis of nuclear tethering and inactivation of cGAS

Carina de Oliveira Mann

Institute of Virology, Technical University Munich, Germany

Nucleotidyltransferases in antiviral immunity

Hana Cahova

Institute of Organic Chemistry and Biochemistry of the CAS, Prague, Czech Republic

HIV-1 infection affects NAD capping of host cell snRNA and snoRNA

Session 2: Cell stress and mitochondria-derived nucleic acid sensing
Chair: Axel Roers and Veit Hornung

Stephen Tait
Cancer Research UK Beatson Institute, Glasgow, UK
Targeting mitochondria as age-old drivers of inflammation

Agnel Sfeir
Memorial Sloan Kettering Cancer Center, New York, USA
Nuclear sensing of breaks in mitochondrial DNA enhances immune surveillance

A Phillip West
Texas A&M University School of Medicine, USA
Mitochondrial DNA sensing in immunity and disease

Session 3: Genome stability links to nucleic acid immunity
Chair: Andrew Jackson

Nicolas Manel
Institut Curie, Paris, France
Activation of innate immune sensors

John Maciejowski
Memorial Sloan Kettering Cancer Center, New York, USA
Immune control and genomic instability at micronuclei

Roger Greenberg
University of Pennsylvania, Philadelphia, USA
Pattern recognition receptor responses to DNA damage

Session 4: Mechanisms and regulation of nucleic acid sensing
Chair: Gunther Hartmann

Nan Yan
University of Texas Southwestern Medical Center, Dallas, USA
STING trafficking and signalling activation

Veit Hornung
LMU Gene Center Munich, Germany
Molecular mechanisms of detection of non-self nucleic acids by the innate immune system

Dan Stetson
University of Washington, Seattle, USA
ABCC1/MRP1 exports cGAMP and controls cGAS-dependent immunity

Kensuke Miyake
University of Tokyo, Japan
Diseases due to lysosomal nucleic acid stress

Philip Kranzusch
Dana-Farber Cancer Institute, Harvard Medical School, USA
Evolution of antiviral immunity from bacteria to animal cells

Session 5: Inborn errors of immunity and disease
Chair: Yanick Crow

Elke Krüger
University of Greifswald, Germany
Recognition of proteotoxic stress by innate immune signalling: Lessons from rare proteasomopathies

Hiroki Kato
Institute of Cardiovascular Immunology, University Hospital Bonn, Germany
Type I interferonopathy and regulatory T cells

Min Ae Lee-Kirsch
Department of Paediatrics, TU Dresden, Germany
SAMHD1 controls innate immunity by regulating condensation of immunogenic self RNA

Dusan Bogunovic
Icahn School of Medicine at Mount Sinai, New York, USA
Erroneous self-sensing in inborn errors of immunity in humans

SHORT TALKS *(in session order)*

TLR8 is activated by 5'-methylthioinosine, a Plasmodium falciparum-derived intermediate of the purine salvage pathway

Stefan Bauer
Institute of Immunology, Philipps University Marburg, Germany

The innate immune recognition of the malaria-causing pathogen Plasmodium falciparum (P. falciparum) is not fully explored. Here, we identify the nucleoside 5'-methylthioinosine (MTI), a Plasmodium-specific intermediate of the purine salvage pathway, as a pathogen-derived Toll-like receptor 8 (TLR8) agonist. Co-incubation of MTI with the TLR8 enhancer poly(dT) as well as synthetic or P. falciparum-derived RNA strongly increase its stimulatory activity. Of note, MTI generated from methylthioadenosine (MTA) by P. falciparum lysates activates TLR8 when MTI metabolism is inhibited by immucillin targeting the purine nucleoside phosphorylase (PfPNP). Importantly, P. falciparum-infected red blood cells incubated with MTI or cultivated with MTA and immucillin lead to TLR8-dependent interleukin-6 (IL-6) production in human monocytes. Our data demonstrate that the nucleoside MTI is a natural human TLR8 ligand with possible in vivo relevance for innate sensing of P. falciparum.

ADAR1 prevents autoinflammation by suppressing spontaneous ZBP1 activation

Richard de Reuver, Simon Verdonck, Evelien Dierick, Josephine Nemegeer, Eline Hessmann, Sadeem Ahmad, Maude Jans, Filip Van Nieuwerburgh, Alexander Botzki, Lars Vereecke, Geert van Loo, Wim Declercq, Sun Hur, Peter Vandenabeele and Jonathan Maelfait.

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The RNA editing enzyme Adenosine Deaminase Acting on dsRNA 1 (ADAR1) limits accumulation of endogenous immunostimulatory dsRNA. In humans, reduced ADAR1 activity causes the severe inflammatory disease Aicardi-Goutières Syndrome (AGS). In mice, complete loss of ADAR1 activity is embryonically lethal while mutations similar to those found in patients with AGS cause lethal autoinflammation. Mechanistically, adenosine-to-inosine (A-to-I) base modification of endogenous dsRNA by ADAR1 prevents chronic overactivation of the dsRNA sensors MDA5 and PKR, which drive pathology in mice. Here we show that ADAR1 additionally inhibits spontaneous activation of the left-handed Z-nucleic acid sensor ZBP1. Activation of ZBP1 elicits caspase-8-dependent apoptosis and MLKL-mediated necroptosis of ADAR1 deficient cells. ZBP1 contributes to the embryonic lethality of Adar knockout mice and drives early mortality and intestinal cell death in Adar/Mavs doubly deficient animals. The Z-nucleic acid binding Zα domain of ADAR1 is critically required to prevent ZBP1-mediated intestinal cell death and skin inflammation. Finally, the Zα domain of ADAR1 promotes A-to-I editing of Alu elements to prevent dsRNA formation through pairing of inverted Alu repeats, which can otherwise induce ZBP1-mediated cell death. This shows that recognition of Alu duplex RNA by ZBP1 may contribute to the pathological features of AGS resulting from loss of ADAR1 function.

Lysosomal dysfunction due to BORC-deficiency exacerbates TLR7 responses

Olivia Majer
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Nucleic acid-sensing Toll-like receptors reside within intracellular membranes and signal from endolysosomes. Their endosomal location reduces the risk of encountering self-nucleic acids and inducing auto-reactive responses. However, endosomes display a remarkable heterogeneity and there is little known about how TLR function is integrated and controlled within that complex endosomal network. The hetero-octameric protein complex BORC (BLOC-1 related complex) assembles on a subset of endolysosomes and together with the small GTPase Arl8b controls lysosome positioning and maturation. In epithelial cells, BORC deletion induces lysosomal clustering in the perinuclear region accompanied by an increase in lysosomal acidity and proteolytic activity. Since optimal lysosomal conditions are essential for TLR processing and activation, we decided to study the role of BORC in endosomal TLR signaling. Deletion of individual BORC subunits or Arl8b in primary macrophages strongly sensitizes TLR7 to stimulation, without any effect on other TLRs. The observed specificity can be explained by the exclusive interaction of Arl8b/BORC with TLR7, but none of the other TLRs; indicating that individual nucleic acid-sensing TLRs might populate functionally distinct endosome subsets. Contrary to epithelial cells, BORC depletion does not result in visible lysosome clustering in macrophages, presumably due to their unique endosomal organization as professional phagocytes. However, we detected an altered endosomal distribution of TLR7, indicative of dysregulated receptor trafficking. We are currently investigating how BORC controls the localization of TLR7 and whether a shift in the receptor's endosome occupancy can predispose to self-recognition and autoimmunity.

Interactions between multidomain dsRNA-binding proteins, including ADAR RNA editing enzymes, suppress antiviral responses to self RNA

Liam P Keegan

Ketty Sinigaglia[1], Dragana Vukic[1], Anna Cherian[1], Janka Melicherova[1], Pavla Linhartova[1], Standa Stejskal[1], Radek Malik[2], Radislav Sedlacek[3], Mary A. O'Connell[1] and **Liam P Keegan**[1].

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ADAR RNA editing enzymes deaminate adenosine to inosine in dsRNA and prevent aberrant immune induction in vertebrates and also in our Drosophila Adar mutant model. Human ADAR mutation combinations reducing ADAR1 RNA editing cause Aicardi Goutieres Syndrome (AGS) and Adar null mutant mouse embryos die by day E12.5, both with aberrant interferon induction. Drosophila Adar mutants affecting the single fly also show aberrant innate immune induction. Adar, Mavs double mutants in which signaling downstream of the Mda5 cytoplasmic antiviral dsRNA sensor is prevented, survive till birth, although these mice still die within a few days or weeks. We show that normal lifespan is restored in Adar, Mavs, Eif2ak2 triple mutant mice also lacking the dsRNA-activated protein kinase R (PKR), identifying this as the other key aberrantly activated immune sensor. Early death of Adar, Mavs mice may be due to loss of enterocyte progenitor cells leading to loss of villi in intestines, which is entirely prevented in the Adar, Mavs, Eif2ak2 triple mutant animals. Aberrant PKR activation occurs in Adar null mutant embryos; surprisingly however, this is mainly due to the absence of Adar1 protein itself; PKR activation is prevented in AdarE912A mutant embryos expressing deamination-inactive Adar1 protein. Human ADAR1-DICER interactions were also reported and aberrant innate immune induction in the Drosophila Adar mutant, mediated through Dicer 2 acting as a dsRNA sensor, is blocked in the presence of even catalytically-inactive Adar protein. Control of innate immune responses by interactions of dsRNA-binding protein domains is ancient and may follow conserved patterns.

Illuminating intracellular STING trafficking and degradation

Katherine R Balka[1,2], Tahnee L Saunders[1], Rachael M Lane[1,2], Rajan Venkatraman[1,2], Harrison M York[1], Senthil Arumugam[1], Benjamin T Kile[1,3], Meredith O'Keeffe[2] and Dominic De Nardo[1,2].

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The cGAS-STING pathway is important for mediating an innate immune response during viral and bacterial infection. Microbial double-stranded DNA within the host cytosol is recognised by the DNA sensor cyclic GMP-AMP synthase (cGAS). Once DNA-bound, cGAS generates a second messenger cyclic dinucleotide (CDN) molecule that binds and activates Stimulator of Interferon Genes (STING). In resting conditions, STING localises to the endoplasmic reticulum (ER) membrane. Upon binding to CDNs, STING traffics to the Golgi and associates with signalling molecules, including the kinases TBK1 and IKK-epsilon. These kinases facilitate activation of transcription factors including IRF3 and NF-kappaB, thus promoting the expression of antiviral type I interferons and pro-inflammatory cytokines. STING further traffics from the Golgi to endolysosomal regions which triggers its degradation, preventing sustained inflammation.

The kinetics and mechanisms controlling post-Golgi STING trafficking events and degradation remain poorly defined. I have utilised live-cell and super resolution imaging approaches to track the dynamic spatiotemporal movement of STING within macrophages. Through an unbiased global phosphoproteomic screen performed in primary mouse macrophages, I have identified novel mechanisms that control STING trafficking and degradative events. My data indicates that the ESCRT pathway internalises ubiquitinated STING into multivesicular bodies (MVBs) facilitating degradation. I have further found that the lysosomal degradation of STING appears independent of TBK1/IKK-epsilon-mediated signalling events. In recent years it has emerged that dysregulation of STING localisation and degradation can promote aberrant STING activation and propagate autoimmune and autoinflammatory diseases. Therefore, there is considerable need to understand how STING trafficking and degradation is controlled.

Genetic screen identifies novel determinants of anti-tumor immunity

Alberto Ciccia

Giuseppe Leuzzi, Alessandro Vasciaveo, Angelo Taglialatela, Xiao Chen, Wendy Mao, Raquel Cuella-Martin, Jen-Wei Huang, Samuel B. Hayward, Tarun S. Nambiar, Ali Ghasemzadeh, Charles G. Drake, Chao Lu, Andrea Califano, **Alberto Ciccia**.

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Genome instability can trigger cancer-intrinsic innate immunity by facilitating the recruitment and activation of cytotoxic T cells that mediate tumor rejection. However, cancer cells overexpress the immune checkpoint regulator PD-L1 in response to immunostimulatory signals, dampening the T-cell-mediated response against tumors. In our studies, we conducted flow cytometry-based CRISPR-Cas9 screens to identify DNA repair and chromatin regulators that control innate immune signaling and PD-L1-dependent immune checkpoint responses. Among the hits of our screen, we identified SMARCAL1, a SNF2-family DNA translocase that promotes genome stability in response to replication stress, as a novel factor that favors cancer immune evasion in a two-pronged manner. We show that SMARCAL1 deficiency in tumor cells activates the cGAS-STING pathway, resulting in immune signaling induction. Concomitantly to the activation of the cGAS-STING pathway, SMARCAL1 loss leads to a downregulation of PD-L1 expression in cancer cells despite the activation of cancer-intrinsic innate immunity. Collectively, our studies demonstrate that SMARCAL1 promotes tumor immune evasion through a dual mechanism involving both the suppression of innate immune signaling and the induction of PD-L1-mediated immune checkpoint responses.

cGAS/STING-dependent sensing of endogenous RNA

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Innate immune detection of endogenous nucleic acids through the cGAS/STING and the RIG-like receptor (RLR) pathways has been shown to underlie several hereditary inflammatory diseases that are characterised by high levels of type I interferon (IFN). Research over more than the last decade revealed that genetic defects in nucleic acid metabolising enzymes lead to spontaneous but selective activation of either cGAS/STING or RLR signalling, resulting in the secretion of IFN. Another striking outcome of these studies was the connection of DNA damage to the activation of cGas. The latter connection was also established in human cells lacking the AGS gene SAMHD1, which has dNTPase and DNA repair functions. However, mice lacking SAMHD1 activate the IFN response in the absence of apparent DNA damage.

We used SAMHD1 knockout mice to study the relationship between DNA damage, cancer development and IFN production in vivo. We found low-level chronic DNA damage in Samhd1 knockout mice, which reduced the tumor free survival on a p53-knockout background, while loss of DNA mismatch repair had no such effect. Surprisingly, we could not establish a link of increased DNA damage to higher IFN production. In contrast, we observed that the IFN response in SAMHD1-deficient cells is driven by the MDA5/MAVS pathway only when the cGAS/STING pathway is functional. Our results implicate endogenous dsRNA as the primary pathogenic nucleic acid ligand in SAMHD1-deficient cells. Furthermore, we propose an important role of the cGAS/STING pathway in physiological and pathophysiological innate immune priming.

Cross-species analysis of viral nucleic acid interacting proteins identifies TAOKs as innate immune regulators

Andreas Pichlmair

Friederike Pennemann, Assel Mussabekova, Christian Urban, Alexey Stukalov, Line Lykke Andersen, Vincent Grass, Teresa Maria Lavacca, Cathleen Holze, Lila Oubraham, Yasmine Benamrouche, Enrico Girardi, Rasha E Boulos, Rune Hartmann, Giulio Superti-Furga, Matthias Habjan, Jean-Luc Imler, Carine Meignin, **Andreas Pichlmair**.

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The cell intrinsic antiviral response of multicellular organisms developed over millions of years and critically relies on the ability to sense and eliminate viral nucleic acids. We used an affinity proteomics approach in evolutionary distant species (human, mouse and fly) to identify proteins that are conserved in their ability to associate with diverse viral nucleic acids. This approach shows a core of orthologous proteins targeting viral genetic material and species-specific interactions. Functional characterization of the influence of 181 candidates on replication of 6 distinct viruses in human cells and flies identifies 128 nucleic acid binding proteins with an impact on virus growth. We identify the family of TAO kinases (TAOK1, -2 and -3) as dsRNA-interacting antiviral proteins and show their requirement for type-I interferon induction. Depletion of TAO kinases in mammals or flies leads to an impaired response to virus infection characterized by a reduced induction of interferon stimulated genes in mammals and impaired expression of srg1 and diedel in flies. Overall, our study shows a larger set of proteins able to mediate the interaction between viral genetic material and host factors than anticipated so far, attesting to the ancestral roots of innate immunity and to the lineage-specific pressures exerted by viruses.

cGAS activation in TREX1-deficient cells triggered by DNA waste from genome replication

Axel Roers

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Innate DNA sensors detect foreign and endogenous DNA to induce responses to infection and cellular stress or damage. Inappropriate activation by self-DNA triggers severe autoinflammatory conditions, including Aicardi Goutières syndrome (AGS) that can be caused by defects of the cytosolic DNase 3'repair exonuclease 1 (TREX1). TREX1 loss-of-function alleles are also associated with systemic lupus erythematosus (SLE). Chronic activation of innate antiviral immunity in TREX1-deficient cells depends on cGAS, implying that accumulating TREX1 DNA substrates cause the inflammatory pathology. Retrotransposon-derived cDNAs were shown to activate cGAS in TREX1-deficient neuronal cells. We addressed other endogenous sources of cGAS ligands in cells lacking TREX1. We find that induced loss of TREX1 in primary cells induces a rapid IFN response that requires ongoing proliferation. The inflammatory phenotype of Trex1-/- mice was partially rescued by additional knock out of exonuclease 1, a multifunctional enzyme providing 5' flap endonuclease activity for Okazaki fragment processing and postreplicative ribonucleotide excision repair. Our data imply genome replication as a source of DNA waste with pathogenic potential that is efficiently degraded by TREX1.

Differential RLR activation between BNT162b2 and mRNA-1273, humans and mice, determines anti-SARS-CoV-2 immunity to mRNA vaccination

Eva Bartok

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Vaccination with in-vitro-transcribed (IVT), nucleoside-modified mRNA has proven to robustly induce anti-SARS-CoV-2 immunity, yet the mechanisms of the initial innate immune response remain unclear. Here, we demonstrate that direct sensing of the RNA within the BNT162b2 and mRNA-1273 vaccines by RIG-I like receptors (RLR) critically contributes to their CD8+ T cell and antibody-dependent immune response. BNT162b2 activates RLRs more potently than mRNA-1273 due to purification differences, which we could also recapitulate with other IVT-generated RNA. Furthermore, while mRNA-1273 critically requires the type-I interferon receptor (IFNAR) for antiviral immunity, the efficacy of BNT162b2 is RLR-dependent but IFNAR-independent. Despite a clear requirement for MDA5 for effective vaccination of mice, we observed that RIG-I is predominantly activated by mRNA vaccines and IVT-RNA in human cells due to incomplete 5'triphosphate-capping. Altogether, our study demonstrates the essential importance of RNA sensing for mRNA-vaccine activity and a critical difference in receptor requirements between humans and murine models.

Life-threatening viral disease in a novel form of autosomal recessive IFNAR2 deficiency in the Arctic

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Type I interferons (IFN-I) play a critical role in human antiviral immunity, as demonstrated by exceptionally rare deleterious variants of IFNAR1 or IFNAR2. We investigated five children from Greenland, Canada and Alaska presenting with viral disease, including life-threatening COVID-19 or influenza, in addition to meningoencephalitis and/or haemophagocytic lymphohistiocytosis following live-attenuated viral vaccination. Affected individuals bore the same homozygous IFNAR2 c.157T>C, p.Ser53Pro missense variant. Although absent from reference databases, p.Ser53Pro occurred with a minor allele frequency of 0.034 in their Inuit ancestry. The serine to proline substitution prevented cell surface expression of IFNAR2 protein, small amounts of which persisted intracellularly in an aberrantly glycosylated state. Cells exclusively expressing the p.Ser53Pro variant lacked responses to recombinant IFN-I and displayed heightened vulnerability to multiple viruses in vitro – a phenotype rescued by wild-type IFNAR2 complementation. This novel form of autosomal recessive IFNAR2 deficiency reinforces the essential role of IFN-I in viral immunity. Our findings may have public health implications, including development of strategies for population screening, prophylaxis, and management of viral infections and live attenuated vaccines in these populations.

The SLC15A4-TASL complex controls IRF5 activation by TLR7/9

Manuele Rebsamen

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Endolysosomal nucleic acid sensing Toll-like receptors (TLRs) play a crucial role in recognition of pathogens and initiation of protective immune responses. Conversely, dysregulation of these processes is associated with auto-inflammatory and autoimmune conditions, including systemic lupus erythematosus (SLE).

We recently identified a novel signaling complex required for TLR7/9-induced responses composed by the endolysosomal solute carrier SLC15A4 and a previously uncharacterized protein encoded by CXorf21, which we named "TLR adaptor interacting with SLC15A4 on the lysosome" (TASL) (Heinz, …, Rebsamen* and Superti-Furga*, Nature 2020). Interestingly, both SLC15A4 and CXorf21 are genetically associated with SLE. Deletion of SLC15A4 or TASL abrogated responses to TLR7/9 agonists in multiple human immune cells by specifically impairing the activation of the transcription factor IRF5, without affecting NF-κB and MAPK pathways. TASL contains a conserved pLxIS motif that mediates the recruitment and activation of IRF5 and acts therefore as a novel innate immune adaptor, displaying a clear mechanistic analogy with the IRF3 adaptors STING, MAVS and TRIF.

Recent investigations further demonstrated the general requirement of SLC15A4-TASL complex for TLR7/9-induced IRF5 activation and suggest that the crucial role of SLC15A4 in this pathway is to mediate the recruitment of TASL to the endolysosomal compartment.

Altogether, these data identify TASL-SLC15A4 as a critical signaling complex linking nucleic acids sensing by TLR7/9 to IRF5 activation and downstream responses, provide a mechanistic explanation for the involvement of these proteins in SLE and suggest a novel, combined target for pharmacologic intervention.

Effects of Human STING Polymorphisms on Pharmacological Activation and Post-translational Regulation of Pathway Sensitivity

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**Equal contribution.*

The intracellular receptor STING (Stimulator of Interferon Genes) is an essential sensor of the endogenous second messenger 2'3'-cyclic GMP-AMP (2'3'-cGAMP) produced by cGAMP-Synthase (cGAS) in response to cytosolic DNA. However, STING can also be activated by bacterial cyclic dinucleotides (CDN), such as cyclic di-AMP, which is produced by Listeria monocytogenes and other gram-positive bacteria. Several STING agonists are currently in clinical trials for cancer therapy but might also be harnessed for treatment of infectious diseases, while on the other hand, modulation and inhibition of STING is a promising therapeutic approach for DNA-driven interferonopathies, such as a subset of systemic lupus erythematosus and Aicardì-Goutières Syndrome cases. In humans, five common STING alleles exist that vary in their sensitivity and ligand preferences, which may result in physiologically relevant differences in pathogen recognition between individuals and hold implications for therapeutic STING activation.

To be able to faithfully assess the immune response and protein regulation under physiological expression and without additional donor variation, we used CRISPR-Cas9 mediated HDR to generate consecutive point mutants of the endogenous STING locus in THP-1 cells resulting in homozygous cell lines for each of the five major human STING alleles. We assessed the immune response to natural and artificial CDN and investigated posttranslational modification as well as regulation of endogenous protein levels. Findings were validated in genotyped human primary cells and further mechanistic studies on posttranslational modifications and mouse-human differences were performed by transient reconstitution of STING-deficient 293FT cells.

We found considerable genotype-dependent differences in the response to CDN dependent on STING genotype. Importantly, STING polymorphisms affected the response to 2'3'-cGAMP, bacterial CDN and ligand-independent auto-activation to different extent. We also found that both STING genotype and CDN ligand identity strongly influenced the requirement of posttranslational modifications for efficient signalling. Moreover, our results indicate that the R293Q mutation affects general protein stability while R71H reduces STING dimer interactions at the protein N-terminus.

Our findings suggest that human STING genotype variation elicits stronger influence and sensing of natural CDN as well as on protein regulation and pharmacological modulation than previously known. Thus, results on experimental activation and modulation as well as possible STING-based treatments can be only correctly interpreted in conjunction with knowledge of STING genotype in primary cells as well as cell lines. In extension, we expect that STING-based therapies will greatly profit from personalized medicine approaches, adjusting compound identity and concentration based on individual genotype.

POSTERS *(in alphabetical order)*

Alkylating antineoplastic agents induce DNA modifications which potentiate innate immune activation

Katarzyna Andryka-Cegielski[1,4], Thomas Zillinger[1,2], Maike Adamson[1,4], Sofía Beatriz Soler[1,4], Maximilian Appel[1,4], Saskia Schmitz[1,4], Christina Mertens, Patrick Müller[1], Eliana D Giammarino[1], Winfried Barchet[1,3], Gunther Hartmann[1], Eva Bartok[1,4].

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The detection of DNA by the innate immune system is essential for protection of the host against pathogens yet can also trigger and maintain autoinflammation. Several recent studies have demonstrated that chemotherapeutic drugs can lead to detection of self DNA by the cGAMP synthase (cGAS) / Stimulator of interferon genes (STING) pathway, which has been associated with both beneficial and deleterious effects during tumor therapy. Here, we demonstrate that chemotherapeutic agents cause DNA modifications which increase their resistance to degradation by the potent cytosolic DNase three-prime repair exonuclease 1 (TREX1) thus licensing DNA sensing. This effect can be observed at concentrations at and below the peak plasma level of these drugs during tumour chemotherapy. In the study we also identify micronuclei raised from chemotherapy treatment like a potential site for interaction between modified DNA and TREX1 and link this to DNA receptor activation.

Influence of RNA modifications on RNA processing and TLR8 activation

Marleen Bérouti

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**Equal contribution.*

Toll-like receptors (TLRs) are key pattern recognition receptors of the innate immune system. Among those, TLR7 and TLR8 are ssRNA sensors located in the endosome. Fragments needed for binding and activation of those TLRs are processed by specific endonucleases. Further, endogenous RNA modifications play an important role in TLR7 and TLR8 activation to discriminate between self and viral or bacterial RNA. The influence of structural determinants and RNA modifications on RNase cleavage specificity and whether the resulting fragments still activate the immune receptors requires further investigation.

Here, we report how structural features and common RNA modifications influence TLR8-mediated immunogenicity. Using high-resolution mass spectrometry, we analyze the exact cleavage products of modified ssRNA digested by RNase T2 and a representative member of the RNase A family. Further, we investigate whether the produced fragments still activate TLR8 in cellulo. Our results suggest that structural determinants as well as base- or ribose-modifications alter the processing capacity of endonucleases and that this leads to a change in TLR8 activation.

Mechanisms of genetic immunity in *Saccharomyces cerevisiae*

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How eukaryotic organisms outside of Animalia protect themselves against exogenous DNA is currently not well understood. To address this, in our lab, we use the transformation of plasmid DNA into Saccharomyces cerevisiae to understand whether mechanisms of genetic immunity could be at play inside these cells. We have tracked the fate of pre-labelled plasmid DNA in live yeast cells using fluorescence microscopy. From this, we observe that while ~50% of cells uptake plasmid DNA, only a small fraction of these cells become stably transformed. By combining assessments of the level of plasmid DNA entry into cells and the proportion of cells that become stably transformed, we observe that the effects of several factors known to improve plasmid transformation efficiency in yeast, such as lithium acetate and salmon sperm DNA, are not at the level of plasmid entry into cells alone. From transformations of pre-labelled TetO plasmids into TetR-expressing yeast cells, we also show that many entering plasmids are not accessible to cytoplasmic TetR binding. We hypothesize that this may be due to plasmid DNA being “packaged”. Related to this, we have recently identified a previously uncharacterised positively charged peptide, which intriguingly localises at the nuclear periphery in cells only after transformation. Together, these results from our ongoing work provide evidence for there being cell intrinsic mechanisms in S. cerevisiae to help to restrict the propagation of plasmid DNA.

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 by a translational shutdown after stimulation with long dsRNA in a PKR/RNaseL dependent manner. 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) induces 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.

Regulation and dynamics of nuclear cGAS signaling during infection with HSV-1

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Cyclic GMP-AMP synthase (cGAS) is an innate immune sensor that detects the presence of foreign DNA. Upon binding to DNA, cGAS catalyzes the formation of the dinucleotide cyclic GMP-AMP (cGAMP), which activates Stimulator of Interferon Genes (STING) to induce an antiviral state through the production of type I interferons (IFNs) and inflammatory cytokines. Recent work by our lab and others has demonstrated that cGAS is predominantly nuclear and tethered tightly to the nucleosome. Tethering inactivates cGAS, and point mutations that impair tethering render cGAS constitutively active against host DNA. However, the fate of tethered cGAS remains unclear – is chromatin tethering merely a sequestration mechanism for cGAS, or can nuclear cGAS be activated to respond to viral infection?

Preliminary data from our lab demonstrate that during infection with HSV-1, a fraction of nuclear cGAS becomes untethered from chromatin, suggesting that nuclear cGAS can be activated during viral infection. To reveal the mechanism of nuclear cGAS activation, we will use cells expressing a cGAS-APEX2 fusion protein, allowing for precisely timed in situ biotinylation of proteins interacting with cGAS during viral infection. Additional work will use confocal microscopy to determine the dynamics and localization of cGAS after untethering, combined with biochemical assays to assess cGAMP production. These findings will elucidate the host and viral factors involved in initiating nuclear cGAS signaling, as well as the dynamics of nuclear cGAS during infection. This will reveal novel upstream activators and regulatory mechanisms of the cGAS pathway, generating new insights into fundamental principles of self/non-self discrimination.

Photosensitivity and cGAS-dependent type I IFN activation in lupus patients with TREX1 deficiency

Claudia Günther

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The exonuclease three prime repair exonuclease 1 (TREX1) safeguards the cell against DNA accumulation in the cytosol and thereby prevents innate immune activation and autoimmunity. TREX1 mutations lead to chronic DNA damage and cell-intrinsic type I interferon (IFN) response. Associated disease phenotypes include Aicardi-Goutières syndrome, familial chilblain lupus and systemic lupus erythematosus.

Given the role of ultraviolet (UV) light in lupus pathogenesis, we assessed sensitivity to UV light in lupus patients with TREX1 mutation by phototesting which revealed an enhanced photosensitivity. TREX1-deficient fibroblasts and keratinocytes generated increased levels of reactive oxygen species in response to UV irradiation as well as increased levels of 8-oxo-guanine lesions after oxidative stress. Likewise, the primary UV-induced DNA lesions cyclobutane pyrimidine dimers (CPD) were induced more strongly in TREX1-deficient cells. Further analysis revealed that single-stranded DNA regions, frequently formed during DNA replication and repair, promote CPD formation. Together, this resulted in a strong UV-induced DNA damage response that was associated with a cyclic GMP-AMP synthase (cGAS)-dependent type I IFN activation.

In conclusion, these findings link chronic DNA damage to photosensitivity and type I IFN production in TREX1 deficiency and explain the induction of disease flares upon UV exposure in lupus patients with TREX1 mutation.

Functions of the DEAH-box helicase DHX36 in type I interferon signaling

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Pattern recognition receptors (PRRs) of the innate immune system recognize specific pathogen associated molecular pattern (PAMPs) such as unique structures of nucleic acids in the case of RNA viruses. The DEAH-box helicase DHX36 (RHAU) plays a pivotal role during the detection of viral RNA and the subsequent signaling transduction.

We could show that the deletion of DHX36 in HEK293T cells leads to an increased phosphorylation of the viral nucleic acid sensor PKR in untreated as well as stimulated conditions. Additionally, this causes an enhanced expression of interferon stimulated genes (ISGs) with and without a viral trigger, which completely depends on PKR. Furthermore, DHX36 protein levels decrease during poly (I:C) treatment and this downregulation of DHX36 is accompanied by an increase of global mRNAs harboring specific secondary nucleic acid structures called G quadruplexes, which are specifically bound by DHX36.

We speculate that the RNA binding ability of DHX36 and its involvement in the interferon signaling pathways are closely connected to facilitate a controlled interferon response.

5'-Triphosphate RNA triggers cell death through an RIG-I-dependent priming and an OAS1/RNase L-mediated effector phase

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Viral double-stranded RNA (dsRNA) with a 5'-Triphosphate-moiety may be sensed by RIG-I leading to the induction of type I interferons (IFN), proinflammatory cytokines and cell death. While IFN and cytokine induction by RIG-I signaling is well described, there have been contradictory models on the mechanisms leading to cell death. Using human and murine CRISPR/Cas9-mediated knockout (KO) melanoma cell lines we analyzed the distinct signaling mechanisms downstream of RIG-I activation by 5'-triphosphosphate RNA (3p-RNA) leading to IFN and cell death induction, respectively. IFN production and cell death were, as shown earlier by others, strongly dependent on intact RLR signaling. Surprisingly, co-culturing KO and wildtype cells or priming cells with IFN, however, rescued the ability of RIG-I-, MAVS- and IRF3-deficient cell lines to undergo apoptosis in response to 3p-RNA suggesting that RIG-I signaling is merely required to prime other 3p-RNA sensors that will ultimately execute cell death. Affinity purification followed by mass spectrometry revealed 3p-RNA-specific binding of oligoadenylate synthetase 1 (OAS1). Overexpression of OAS1 was able to rescue the ability of RIG-I-deficient cells to undergo apoptosis in response to 3p-RNA. The execution of apoptosis was dependent on the concerted action of translational arrest triggered by OAS1/RNase L, and upregulation of the pro-apoptotic BH3-protein NOXA by RIG-I to rapidly deplete the anti-apoptotic BCL-2 family member MCL-1 and thereby induce intrinsic apoptosis in melanoma cells. This mechanism also translated to different innate RNA receptor systems if NOXA induction and translational inhibition co-occurred showing a general mechanism of cell fate decision upon dsRNA sensing.

Visualisation of ASC signaling assemblies in cells by cryo-electron tomography

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Nod-like receptors (NLRs) form oligomeric inflammasomes that sense pathogen- or danger-associated molecular patterns. Viral infection can activate the NLRP1 and NLRP3 inflammasomes. NLRP1 is activated by double-stranded RNA, a viral signature. Inflammasomes recruit the adaptor protein ASC, forming micron-sized oligomeric signalling platforms known as ASC specks. Caspase-1 is then recruited to ASC specks, where it is activated by autoproteolysis. Caspase-1 activation in turn induces activation of proinflammatory cytokine and pyroptotic executor protein GSDMD. The size, shape and growth kinetics of ASC signaling assemblies can differ substantially depending on whether the constituents are biochemically reconstituted, overexpressed in cells, or endogenously expressed in cells.

We used cryo-correlative light and electron microscopy to image ASC signalling assemblies in the cellular context. Our image reconstructions provide the most detailed view of ASC signaling assemblies to date, under more physiological conditions. We identified ribosomes and small vesicles within the ASC signalling sites. We used live-cell imaging to confirm the presence of Golgi-derived vesicles in the ASC signalling sites. Consistent with previous findings, fragmentation of the mitochondrial network and loss of mitochondrial membrane potential were observed after inflammasome activation. Notably, our tomographic reconstructions show that mitochondrial cristae remodelling occurs at more advanced stages of inflammasome activation.

Examining the status of T cells in AGS patients carrying mutations in MDA5 or ADAR1

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Systemic expression of gain-of-function MDA5 G821S in mice leads to autoinflammation, with contribution from immune cell types, such as activated dendritic cells and macrophages. In this model we also observed changes in T cell populations, a shift of naive to effector CD4 and CD8 T cells, an increase in activation markers and alterations in cytokine production in presence of stimuli. This finding prompted us to further investigate a possible role for T cells in disease progression in our mouse model. In addition, there is little information about the state of T cells in human patients affected by type I interferonopathies such as AGS, which we would like to examine.

With help from clinicians, we received samples from AGS patients carrying a gain-of-function mutation in MDA5, and from patients carrying mutations in the deaminase or Z-DNA binding domains of ADAR1. We analysed T cell ratios and activation status by markers such as CD25 from the freshly isolated PBMCs, and the production of T cell specific cytokines including IL-4, IL-10 and IFNg, after culturing them with stimuli such as a-CD3 and a-CD28 antibodies. In this work we hope to better understand if and how T cells and their functions are altered in the context of type I interferonopathies.

TNFα receptor signaling is a driver of SAVI disease in mice

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Activating mutations in STING, a key adaptor in cGAS dependent cyctosolic DNA sensing, were shown to cause STING-associated vasculopathy with onset in infancy (SAVI), a severe pediatric disease with poor prognosis. We established a mouse model with the mutation most commonly found in patients (Sting1 p.N153S/WT). All mice developed a systemic autoinflammatory disease with various pathologic characteristics, e.g. massive reduction of T cell count and manifestation of fatal inflammatory lung disease. Expression of STING N153S/WT resulted in activation of type I IFN and NFκB dependent signaling pathways in many cell types followed by systemic inflammation. STING gain-of-function mutation led to elevated TNFα serum level and to increased of Tnfα gene expression in various tissues.

Currently, we observed that the production and secretion of TNFα is necessary for manifestation and progression of SAVI disease in mice. Both TNFα receptors have contrary physiological properties and have very different effects on the severity of SAVI disease. Genetic knock out of Tnfr1 was able to ameliorate this disease, whereas separate knock out of Tnfr2 did not show any curative effects in these mice. A double knock out of Tnfr1 and Tnfr2 resulted in normalization of Tnfα gene expression and improvement of SAVI associated lung disease and T cell loss.

RIG-I like receptor activation by mRNA incorporating modified nucleotides

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Delivery of in vitro transcribed mRNA into target cells enables the introduction of any protein of interest and serves as a promising new tool for various clinical applications including vaccination strategies and cancer therapy. Only recently, the development of COVID-19 mRNA vaccines has gained public attention. The therapeutic performance is largely influenced by successful mRNA delivery, stability and translation efficiency.

Here, the incorporation of modified nucleotides has proven beneficial. Besides, the mRNA is able to activate innate RNA-sensing pattern recognition receptors, such as the cytosolic receptors RIG-I and MDA5. Typically, these are involved in the detection of viral infections and induce a type I interferon response upon activation that may reduce protein translation and thus be disadvantageous for the therapeutic outcome. Therefore, it is critical to regulate innate receptor activation.

So far, there is only limited knowledge of whether nucleotide modifications affect RIG-I and MDA5 activation. For this, we investigate the type I interferon response and protein expression upon in vitro transfection of mRNA incorporating commonly used modified nucleotides. With this study, we hope to elucidate the correlation between immune activation and translation efficiency in order to optimize the mRNA design for future therapeutic applications.

Elucidating the molecular sensor for the activation of the type I interferon response in rare proteasomopathies

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Type I interferon (IFN) signaling is typically activated through the binding of nucleic acids of foreign or self-origin to pattern recognition receptors [1]. In rare proteasomopathies caused by loss-of-function mutations in proteasome genes such as PSMB8, a sterile inflammation induced by dysregulation of type I IFN signaling has been observed in response to proteotoxic stress [2,3,4]. The molecular characteristics of type I IFN activation in proteasomopathies involved the integrated stress (ISR) and the unfolded protein response (UPR) [4,5,6,7]. Recently, Davidson et al. showed that the activation of the ISR protein kinase R (PKR), driven by accumulated cytoplasmic IL-24, acts as an innate immune sensor in cells with proteasome deficiency [8]. As not all cell types express IL-24, the mechanism of type I IFN activation in cells other than immune cells remained unclear. In this regard, several human cell types were analyzed for IL-24 expression. In particular, a human endothelial cell line (EA.hy926) and a human microglia cell line (C20) are used as cell models by knocking out PSMB8 via CRISPR/Cas9 to investigate IL-24 expression. Besides PKR, additional kinases of the ISR are further analyzed to decipher the molecular pathway for the activation of the type I interferon response in response to proteasome deficiency.

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Genetic immunity in yeast

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The interaction between exogenous DNA and prokaryotic cells is well characterized, from the innate restriction and modification system to the acquired CRISPR/Cas. Such systems are not described as thoroughly in eukaryotes. We study the interaction between budding yeast and exogenous DNA to better understand such possible immunity barriers in eukaryotes. We first asked the question of whether yeast cells restrict the propagation of uptaken exogenous DNA. We transformed yeast cells with a covalently labelled plasmid and tracked its fate by microscopy. The results showed that around 50% of the cells in a population show at least one plasmid focus after transformation, yet only around 1% keep this plasmid and express it stably.

To look into possible genes involved in this restriction, we looked for genes that restrict transformation in yeast using SATAY transposition screening. The screen indicated the presence of multiple barriers in the cells for the exogenous plasmid: the cell wall, endocytosis, the vacuole, nuclear import, chromatin remodelling, and the DNA repair machinery. The screen also revealed a group of unannotated positively charged peptides whose function is unknown as restrictors of transformation. We currently are using in vitro and in vivo tools to characterize the involvement of those peptides in a potential immunity against exogenous DNA in yeast.

ADAR1 as a novel target for combating infectious diseases

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Adenosine-to-Inosine deamination of dsRNA by ADAR1 is essential to prevent activation of innate immune pathways by endogenous cellular dsRNAs. Several publications have demonstrated that targeting ADAR1 can be utilized to reactivate immune responses in cancer, generating interest in developing inhibitors of ADAR1 for use in cancer immunotherapy. It is unclear if ADAR1 can also be targeted to provide protection from infectious disease.

In malaria studies of the Fulani ethnic group, who are protected from P. falciparum malaria, we observed reduced A-to-I RNA editing in CD14+ monocyte cells following infection in the Fulani compared to a control unprotected ethnic group. Therefore, we have investigated whether this reduction in A-to-I RNA editing during infection with the parasite can confer protection against malaria. Analysis of publicly available RNA-sequencing data from different malaria models show transient but significant changes in levels of RNA editing in individuals following Plasmodium infection. Reduced A-to-I editing levels are associated with protection from malaria. In addition, and most strikingly, Adar1+/- heterozygous mutant mice are protected from malaria, with significantly reduced parasitemia during blood stage infection with rodent malaria parasite P.yoelii.

Our data support a model where ADAR1 activity and levels of A-to-I RNA editing are transiently reduced in innate immune cells in response to Plasmodium infection and reduced levels of ADAR1 activity contribute to protection from parasitemia during malaria. This implies that ADAR1 inhibitors may have future utility in the treatment or prevention of some infectious diseases.

Identification of novel proteasome variants in three unrelated cases of proteasome-associated autoinflammatory syndrome (PRAAS)

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The ubiquitin-proteasome system (UPS) plays a central role in a myriad of cellular processes including protein homeostasis, cell cycle progression, signal transduction as well as cell death and survival. Mutations in genes coding for proteasome subunits and/or proteasome assembly helpers typically cause recurring autoinflammation referred to as proteasome-associated autoinflammatory syndrome (PRAAS) [1-3]. Inflammation in PRAAS is mainly dominated by a type I interferon (IFN) response that emerges a consequence of increased proteotoxic stress by mechanisms involving the unfolded protein response and the integrated stress response [4-7]. Here, we report on three unrelated PRAAS patients carrying novel inherited proteasome missense and/or nonsense variants. Two cases were associated with two compound heterozygous pathogenic alterations in the PSMB8 and PSMB10 genes, while one patient showed trigenic inheritance pattern implicating the PSMB8, PSMA5 and PSMC5 genes. Our in vitro investigations provide evidence on the pathogenicity of these variants with all identified mutations substantially impacting the steady-state expression of the affected proteasome subunits and/or their incorporation into mature 26S proteasomes. Altogether, our findings widen the spectrum of PRAAS known variants and may help improving genetic counseling as well as the molecular diagnosis of patients with clinical manifestations suggestive of sterile autoinflammation.

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DNA-PKcs regulates macrophage transcriptional responses during bacterial infection

Abigail J Morales

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The maintenance of genome integrity is critical for cell and organismal survival; however, eukaryotic cells perpetually sustain various types of DNA damage. DNA double-strand breaks (DSBs) are among the most insidious of these lesions, as they can lead to chromosomal translocations if they persist unrepaired. A substantial body of work has established that various cell types utilize DNA DSBs as signaling intermediates that impact tissue-specific processes. This is executed, in part, through the regulation of transcriptional networks. Recently published work established that macrophages, an important leukocyte subset, sustain genomic DNA damage after producing reactive nitrogen intermediates (RNI) that are intended to disable invading microbes. Notably, the resulting DNA damage response (DDR) impacts macrophage transcriptional networks during bacterial infection.

Among the transcription factors activated downstream of microbial sensing is NF-κB. Previous work has established that NF-κB is also activated downstream of DNA damage. In contrast to NF-κB activation following pathogen sensing, this DNA damage-elicited signaling cascade originates in the nucleus and depends, in part, on phosphorylation of the IKK regulatory subunit NEMO at Serine 85. We find that in activated macrophages, NEMO is phosphorylated at Serine 85, suggesting DNA damage-elicited NF-κB activation. Though previous reports have established that ATM is the kinase responsible for this signaling event, our findings suggest that in macrophages, optimal NEMO S85 phosphorylation depends on the related DDR kinase DNA-PKcs. Additionally, in the absence of functional DNA-PKcs, NF-κB-driven pro-inflammatory cytokine production is markedly reduced, underscoring a key role for DNA-PKcs in modulating transcriptional responses during bacterial infection.

P38 kinases mediate NLRP1 inflammasome activation after ribotoxic stress response and virus infection

Florian I Schmidt

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Inflammasomes integrate cytosolic evidence of infection or damage to mount inflammatory responses. The inflammasome sensor NLRP1 is expressed in human keratinocytes and coordinates inflammation in the skin. We found that diverse stress signals induce human NLRP1 inflammasome assembly by activating the MAP kinase p38 through different upstream kinases: UV irradiation and different microbial molecules that initiate the ribotoxic stress response activate p38 through the MAP3 kinase ZAKα. RNA delivery by infection with arthropod-borne alphaviruses, including Semliki Forest, Ross River, and Chikungunya virus, also activated NLRP1 in a p38-dependent manner, but likely involve other upstream kinases. P38-mediated NLRP1 activation relies on critical serine/threonine residues in the NLRP1 linker region as well as lysines in the NLRP1 pyrin domain (PYD). Stimulation of p38 by overexpression of MAP2 kinases MKK3 or MKK6 is sufficient for NLRP1 activation, and NLRP1 is directly phosphorylated by p38.

We thus propose that phosphorylation of the NLRP1 linker region by p38 initiates PYD ubiquitination, proteasome-dependent N-terminal degradation, and release of UPA-CARD fragments that nucleate inflammasomes. In line with this, NLRP1 activation by direct nanobody-mediated ubiquitination was independent of p38 activity. Taken together, we define p38 activation as a unifying signaling hub that controls NLRP1 inflammasome activation by integrating a variety of cellular stress signals relevant to the skin.

Analysis of Mice with MDA5-G821S Conditional Knock-In in Astrocytes and Neural Stem Cells

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Gain of function mutations in IFIH1 gene encoding MDA5 have been identified in Aicardi-Goutières syndrome (AGS) patients. We previously reported that mice expressing constitutively active MDA5 G821S (MDA5 GS) develop lupus-like autoimmune symptoms and AGS-like encephalopathy with high lethality. Analysis of brain showed upregulation of type I IFNs, astrogliosis and microgliosis.

Based on these recent findings, we newly established mouse lines with cell-type specific expression of MDA5 GS under GFAP and Nestin promoters, in order to understand the contribution of astrocytes and neural stem cells to the brain pathogenesis. Neither of the two conditional MDA5 GS lines are lethal and only Nestin-MDA5 GS mice had a weight loss of less than 20% compared to control mice. Analysis of brains by flow cytometry and immunofluorescence staining showed an increase in the number of microglia and astrocytes with increased expression of activation markers, especially in cortex, hippocampus and the cerebellum of both GFAP- and Nestin-MDA5 GS mice. The expression of type I IFNs, ISGs and inflammatory cytokine genes was also upregulated in the brain, and the upregulation was much higher in Nestin-MDA5 GS mice. Our findings suggest the involvement of both cell types in the brain pathogenesis, however, Nestin-expressing cells have a stronger contribution and they could be a potential target for therapeutic approaches in AGS patients.

Enhancing the anti-tumor activity of NK cells via CRISPR/Cas genome editing and innate immune activation

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NK cells utilize a repertoire of activating and inhibitory receptors to mediate cytotoxic responses against virally infected and malignantly transformed cells in an antigen-independent manner. In turn, many tumors have developed diverse strategies to evade NK cell recognition and activation, including the upregulation of the non-classical HLA molecule HLA-E, which is recognized by the inhibitory receptor NKG2A as well as the activating receptor NKG2C.

In this study, we demonstrate that HLA-E is broadly expressed on tumor cell lines and, in analogy to other immune checkpoints, upregulated by tumor-cell intrinsic RIG-I activation. Moreover, while RIG-I stimulated tumor cells are more efficiently killed by primary NK cells, this effect is further increased by deletion of tumor-cell HLA-E expression.

In order to limit tumor-cell protection through the HLA-E/NKG2A axis, we have established protocols for the CRISPR/Cas9-mediated genome editing of primary NK cells, including the generation of NKG2A-/- NK cells. Primary NKG2A-/- NK cells showed a general increase in cytotoxicity in vitro when compared to wildtype controls. Altogether, this study provides a rationale for approaches combining NK-cell editing and other tumor immunotherapies in vivo. Moreover, by establishing a system for genome editing in primary NK cells, we also open avenues for targeting other promising candidate genes for antitumoral therapy.

The phagosome as a source of immunostimulatory molecules in TRAP-deficient cells

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Type I interferonopathies are a collection of inherited autoinflammatory disorders characterised by a type I interferon response, most commonly the result of aberrant innate immune system activation by endogenous nucleic acids. Biallelic pathogenic mutations in ACP5, encoding tartrate-resistant acid phosphatase (TRAP), result in a type I interferonopathy, and in many cases a monogenic form of systemic lupus erythematosus (SLE). Additionally, patients display impaired skeletal development with spondyloenchondrodysplasia. TRAP is a lysosomal phosphatase that is expressed in macrophages and osteoclasts, and which is able to dephosphorylate a wide range of substrates under acidic pH conditions. High levels of type I interferon in TRAP-deficient patients suggest accumulation of danger associated molecular patterns (DAMPs) that activate innate immune signalling. However, the source of immunostimulatory molecules in TRAP-deficient cells is currently unknown.

We hypothesise that TRAP normally processes one or more DAMPs in (phago-)lysosomes to prevent aberrant innate immune signalling and interferon overproduction. To address this, we are using a combination of TRAP-deficient mouse models and human cell lines. We are currently investigating whether TRAP-deficient cells display defects in phagocytosis or impaired lysosomal degradation of apoptotic cells, resulting in accumulation of immunostimulatory nucleic acids. Overall, our research will contribute to understanding the pathogenesis of type I interferonopathies and common autoimmune disease, potentially linking phagocytosis, lysosomal function and immune system activation.

Intercellular cGAMP transmission induces innate immune activation and tissue inflammation in Trex1 deficiency

Lino L Teichmann
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Intercellular transmission of the second messenger 2',3'-cGAMP, synthesized by the viral DNA sensor cGAMP synthase (cGAS), is a potent mode of bystander activation during host defense. However, whether this mechanism also contributes to cGAS-dependent autoimmunity remains unknown. Here, using a murine bone marrow transplantation strategy, we demonstrate that, in Trex1-/- associated autoimmunity, cGAMP shuttling from radioresistant to immune cells induces NF-κB activation, interferon regulatory protein 3 (IRF3) phosphorylation and subsequent interferon signaling. cGAMP travel prevented myeloid cell and lymphocyte death, promoting their accumulation in secondary lymphoid tissue. Nonetheless, it did not stimulate B cell differentiation into autoantibody-producing plasmablasts or aberrant T cell priming. Although cGAMP-mediated bystander activation did not induce spontaneous organ disease, it did trigger interface dermatitis after UV light exposure, similar to cutaneous lupus erythematosus. These findings reveal that, in Trex1-deficiency, intercellular cGAMP transfer propagates cGAS signaling and, under conducive conditions, causes tissue inflammation.

Vaccinia virus-mediated delivery of bivalent AIM2 nanobodies defines cell-type-specific inflammasome activation

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Absent in melanoma-2 (AIM2) is a pattern recognition receptor that is activated by cytosolic DNA and nucleates an inflammasome complex comprised of the adaptor protein ASC and the effector protein pro-caspase-1. AIM2 is activated by both host- and pathogen-derived dsDNA, resulting in a highly inflammatory form of cell death called pyroptosis. Yet, molecular mechanisms that precede its activation remain to be elucidated. It is particularly unclear, why dsDNA in human monocytes activate NLRP3 instead of AIM2 inflammasomes, which necessitates further investigation into the mechanism of AIM2 activation. Therefore, it is of great interest to develop tools that perturb AIM2 functions at the endogenous levels and in primary cells to address these questions.

In this study, we immunized an alpaca with purified AIM2PYD protein and cloned the resulting nanobodies into a phagemid library. We successfully identified a collection of AIM2-specific nanobodies by phage display and found that three of the identified nanobodies moderately inhibit activation of AIM2 after poly dA:dT transfection and vaccinia virus (VACV) infection, but did not interfere with NLRP3 inflammasome assembly. To improve inhibition, we generated bivalent nanobodies that exhibited 10-fold improved potency and completely abrogated AIM2 inflammasome activation. We constructed recombinant VACV strains that express the bivalent nanobodies from a synthetic early/late promoter to deliver nanobodies into infected primary cells. The nanobodies encoding recombinant VACV substantially diminished inflammasome activation in PMA differentiated THP-1 cells. Furthermore, using these viruses as well as the NLRP3 inhibitor drug Crid3, we find that primary GM-CSF differentiated macrophages assemble AIM2 inflammasomes in response to VACV infection, while infection of primary CD14+ monocytes triggers NLRP3 inflammasome activation.

Hence by exploiting the newly generated AIM2 inhibitory nanobodies, we are able to show that VACV induces the activation of either NLRP3 or AIM2 inflammasome in primary human monocytes and macrophages in a cell-type-specific manner.

Characterization of endogenous RNAs triggering MDA5 activation

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Pathogen-associated molecular patterns (PAMPs) such as nucleic acids are recognized by pattern recognition receptors (PRRs) with high specificity. Unique features such as cap structure, double-strandedness, base content, or nucleotide modifications help to distinguish self from non-self RNAs. Adenosine to inosine deamination by ADARs is the most abundant type of RNA editing in metazoans. A-to-I RNA editing has been shown to play an essential role in mammalian embryonic development and tissue homeostasis, and is implicated in the pathogenesis of many diseases including autoimmune and inflammatory tissue injury [1]. In mammals, ADAR1 and ADAR2 are responsible for A-to-I editing, while ADAR3 is seemingly inactive. ADAR1 is constitutively expressed but also has an IFN-inducible isoform. Mice lacking the interferon inducible version of ADAR1 show embryonic lethality at day 12.5, accompanied by a strong IFN signature. Interestingly, ADAR1 deficiency is rescued by ablation of MDA-5, which senses viral RNAs. Interestingly, only ADAR1 editing prevents the production of type I interferons, inflammatory cytokines, and embryonic lethality [2].

This suggests that inosines in endogenous RNAs edited by ADAR1 prevent immune signaling through MDA5. It is assumed that cellular double-stranded RNAs can activate MDA-5 in the absence of inosines. ADAR1 might target specific regions of RNAs that are crucially required to prevent activation of MDA5. Here we aim to identify RNAs that are critically edited by ADAR1 to prevent the activation of MDA-5 signaling.

We utilized an RNase protection assay to identify cellular RNAs that stimulate MDA5. We also test how inosine in dsRNA can prevent multimerization of MDA5 on dsRNA substrates. Further, we find that A to I edited RNA also suppresses in vivo consequences of transfection with long dsRNAs, such as interferon stimulated gene expression (ISG) and stress granule formation (SG).

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The RIG-I/MAVS Signaling Pathway Contributes to the Pathogenesis of Aortic Valve Stenosis in the Mouse Model of Wire Injury

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Aortic valve stenosis is characterized by an active calcification process driven by high mechanical stress-induced inflammation in the aortic valve leaflets. Interestingly, Singleton-Merten-Syndrome (SMS), a rare monogenetic disease, which can be caused by gain-of-function mutations in the retinoic acid-inducible gene I (RIG-I), is also characterized by diverse calcification abnormalities including calcification of aortic valve and proximal aorta. Therefore, we aim to investigate the role of the RNA receptor RIG-I in the development and progression of aortic valve stenosis. By using the mouse model of wire injury, aortic valve stenosis was induced in wildtype (WT), RIG-I and MAVS (mitochondrial antiviral signaling protein) knockout (KO) mice. The development of the disease was monitored by ultrasound, while tissue samples were collected 6 weeks after the induction of aortic valve stenosis and analyzed using immunohistochemistry and fluorescence microscopy.

The results showed a significant reduction of aortic peak blood velocity, an indicator of aortic stenosis, in mice deficient for RIG-I as well as for MAVS, the central adaptor molecule of RIG-I. Immunofluorescence staining revealed less CD45 and CD68 positive immune cells infiltrating the aortic valve in MAVS KO mice compared to WT mice. In addition, MAVS deficiency led to a reduced myofibroblast activation in response to injury, indicated by the decreased expression of the mesenchymal cell marker aSMA in MAVS KO mice. The reduced severity of the aortic valve stenosis in RIG-I and MAVS KO mice suggests a possible role of the RIG-I like receptor pathway in the pathophysiology of this disease.

Immune recognition of Cap0 RNA in mosquitoes

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Various infectious diseases are caused by mosquito-borne flaviviruses affecting people worldwide. Efficient transmission of these viruses highly depends on the pathogen's ability to overcome the host immune system. Previous, including our own studies, showed that flaviviruses evade the vertebrate immune system by capping their viral genome via a cap-N1-2'O-methyltransferase encoded in the non-structural protein NS5.

Using the yellow fever virus (YFV) vaccine strain (YFV-17D cap1) and a methyltransferase deficient mutant (YFV-17D cap0) we showed that the replication of YFV-17D cap0 was also impaired in mosquito cells. Concordantly, expression of a luciferase cap0-mRNA was suppressed, indicating a cap0 sensing translation regulation mechanism. Since YFV-17D is unable to replicate in mosquitoes, we established a YFV-Asibi cDNA clone for analyses in vivo. Similar to YFV-17D cap0, YFV-Asibi cap0 was suppressed in mosquito cells, however to a slightly lower extent. This indicates that YFV-Asibi expresses antagonist(s) against the insect antiviral discrimination mechanism of cap0-RNA.

Current studies comparing chimeras between YFV-Asibi and YFV-17D aim to identify potential antagonist(s). After oral infection of Aedes aegypti mosquitoes, YFV-Asibi cap1 replicated in the mosquito midgut and in secondary tissues like legs and wings. Conversely, replication of YFV-Asibi cap0 was suppressed in the midgut and nearly blocked in secondary tissues. Intriguingly, efficient replication of YFV-Asibi cap0 occurred after intrathoracic infection, indicating a potential receptor discriminating between cap1- and cap0-RNAs expressed in the midgut or the midgut barrier. This is the first hint to an innate 5'RNA-modification recognizing effector protein in insect cells beyond the well characterized adaptive RNAi mechanisms.

Tumor cell-intrinsic STING signaling controls immunogenicity of mismatch repair-deficient colorectal cancers

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Colorectal cancer (CRC) is one of the most prevalent malignancies in the world with nearly one million deaths in 2020. Roughly 15% of CRCs harbor inherited or sporadic loss of function mutations in mismatch repair (MMR) genes. Enrichment of neoantigens in these hypermutated MMR deficient (dMMR) colorectal cancers is proposed to be a major contributor to anti-PD1 immune checkpoint inhibitor (ICI) therapy responsiveness. However, about 50% of dMMR tumors are non-responsive to ICI therapy suggesting that additional mechanisms govern the ICI sensitivity of dMMR tumors. Using murine CRC models, we demonstrate that the deletion of MMR proteins results in an accumulation of cytosolic DNA which is recognized by cGAS to activate the STING-TBK1-Interferon signaling pathway within the tumor cells. Upon subcutaneous injection in vivo, dMMR compared to MMR proficient (pMMR) tumor cells grow smaller tumors which is strictly dependent on the presence of STING. Moreover, STING signaling in these dMMR tumors shapes an immunologically active tumor microenvironment (TME) which is characterized by high expression of TIL attracting chemokines (Cxcl5, Cxcl10) and cytotoxic effector molecules (Gzmb, Ifng) and by increased CD8+ T cell and NK cell infiltrates which collectively promote a strong antitumor immune response in the TME of dMMR tumors. Together, these results provide new insights into how tumor cell-intrinsic STING signaling controls antitumor immunity.

Early development silences the interferon response to avoid self-recognition and developmental problems

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In mammals, the type I interferon (IFN) response constitutes the major innate immune pathway against viruses. Although essential for fighting viral infections, this pathway is inactive during the early stages of mammalian development.

To better understand the mechanisms and rationale behind this suppression, we interrogated the expression of major innate immune genes during in vitro differentiation of both mouse and human embryonic stem cells (ESC). This revealed that genes involved in dsRNA recognition and signalling are developmentally regulated and may be involved in silencing the IFN response in ESCs.

By manipulating the expression of specific dsRNA sensors and signalling genes, we have now engineered ESCs capable of an IFN response. Interestingly, this shows to have two major consequences: 1) activation of the IFN response in the absence of infection and 2) deregulation of the pluripotency and differentiation programme. Interestingly, ESCs are characterized by a large-scale DNA (CpG) de-methylated state, which leads to increased expression of endogenous transposable elements, some of which are known to induce the IFN-response. Our current hypothesis is that early development requires silencing the IFN response to avoid self-recognition and prevent catastrophic consequences during development.

Janus kinase inhibition in complement component 1 deficiency

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We investigated 4 children from 2 consanguineous families affected with early-onset SLE accompanied by strong type I interferon (IFN) activation, and tested the therapeutic effects of JAK inhibition. Patients presented with acral vasculitis, oral ulcera and ANA and were largely unresponsive to hydroxychloroquine, prednisone, methotrexate and rituximab. Exome sequencing revealed a homozygous truncating mutation in C1R in family 1 and C1Q in family 2. Western blot analysis demonstrated the presence of truncated C1r and C1q in patient fibroblasts, which failed to be secreted. Activation of the classical complement pathway was absent in patient sera and fully reconstituted by C1 substitution. In SLE, type I IFN can be induced by immune complexes (IC), which activate nucleic acid–sensing Toll-like receptors (TLRs). We determined that the innate immune activation in the patients is TLR7 and TLR9-dependent. Patients exhibited a strong IFN signature in blood. In response to poly(I:C), type I IFN was strongly induced in whole blood of a healthy individual, while it could not be further stimulated in patient cells and markedly decreased upon incubation with the JAK inhibitor baricitinib. Treatment of 2 patients with baricitinib led to sustained clinical improvement.

In summary, we delineate deficiencies in C1r and C1q as bona fide type I IFN interferonopathies and demonstrate the therapeutic efficacy of baricitinib.

Design of next-generation RIG-I ligands

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One of the key innate pattern-recognition receptors that senses viral RNA is the RNA helicase RIG-I (retinoic acid-inducible gene I). The structure of RIG-I consists of N-terminal tandem caspase activation and recruitment domains (CARDs), a central helicase domain, and a C-terminal regulatory domain (CTD). In its inactivated form, the CARDs are bound to the helicase domain. However, binding of a minimal RNA ligand (8-10 nt) by the CTD causes RIG-I to undergo a conformational change that activates its downstream antiviral signaling pathway. Our group has identified the ligand of RIG-I to be a short, blunt-ended, double-stranded RNA with a 5'-triphosphate (3pRNA).

Here we use chemical synthesis of different 3pRNAs to analyse the immunostimulatory properties of minimal RNA ligand structures with different sequences, chemical modifications, and guanosine overhangs. We use primary human immune cells, primary mouse bone marrow dendritic cells and THP-1 cells to examine pre-assembly of RIG-I ligands and their functional impact.

We found that the type I IFN-response of different RNA ligands is concentration dependent, with non-optimal ligand structures requiring higher concentrations. In this setting we developed a new generation of 3pRNA ligands defined by sequence, chemical modifications and tertiary structure formation. This new class of highly potent 3pRNA ligands have the potential to substantially improve clinical applications of RIG-I activation in mRNA vaccine development and for the treatment of viral infection and cancer.

Development and functional analysis of photocaged RNA agonists for spatiotemporal control of RIG-I activation

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RIG-I (Retinoic Acid Inducible Gene I) is an intracellular innate immune receptor which detects 5'triphosphorylated blunt end double-stranded viral RNA and upon activation triggers a type I interferon response. Based on its activity to induce an immunogenic cell death in tumor cells, RIG-I agonists are currently developed for immunotherapy of cancer. Tumor-targeted delivery of RIG-I agonists is not yet available. Here we aim at light-induced targeted activation of systemically administered photocaged RIG-I agonists in tumor tissue.

Synthetic photocaged RNA agonists of RIG-I were developed to investigate the spatiotemporal control of RIG-I activation in different experimental setups, including primary human immune cells, primary mouse bone marrow dendritic cells and THP-1 cells. We generated a HEK293FT reporter cell line expressing EGFP under control of the interferon beta promoter to non- invasively track RIG-I stimulation on the single-cell level. Readouts include microscopy, ELISA and Luciferase Assays.

We found that appropriate irradiation of cells previously incubated with photocaged RIG-I agonists stimulated the production of type I interferon production, providing the proof of concept for light-mediated control of RIG-I activation. In the absence of light irradiation, photocaged ligands were inactive.

Our newly developed photocaged ligands now allow to delineate the precise kinetics of RIG-I signalling and of paracrine cell to cell effects of RIG-I stimulation while bypassing the influence of transfection. Spatiotemporal control of RIG-I activation in vivo is expected to improve the therapeutic efficacy of RIG-I agonists while reducing systemic side effects, thereby opening new avenues for RIG-I-mediated cancer immunotherapy.

Severe type I interferonopathy associated with an inborn homozygous STAT2 mutation

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Type I interferonopathy is a group of monogenic Mendelian rare diseases where constitutive up-regulation of type I interferon (IFN) signalling leads to systemic auto-inflammation. Patients with such diseases often display intracranial calcification, developmental delay and higher expression of interferon-stimulated genes (ISGs). In this study, our laboratory ascertained a patient with signs of type I interferonopathy from a consanguineous family. Whole-exosome sequencing revealed a germline mutation c.656C>T in STAT2 resulting in p.(A219V) transition. STAT2 is a well-known effector in transducing IFN signalling downstream of the IFNAR1/2 receptor and associated kinases JAK1 and TYK2 to initiate transcriptional activation of ISGs. STAT2 also functions in later stage of IFN signalling where it binds to a classic IFN negative regulator USP18 to form a dimer, which displaces JAK1 from IFNAR2 and inhibits chronic activation of IFN receptor.

This study investigated the pathogenicity of the newly discovered STAT2 mutant p.(A219V). To achieve this goal, 3 aims were set forth: 1) to assess if the variant displays defect in transducing IFN signalling; as well as 2) defect in restricting IFN receptor activity; and 3) to predict other potential disease-causing STAT2 variants through mutagenesis screen.

Through stable lentiviral reconstitution of STAT2 wildtype or p.(A219V) into STAT2 null human fibrosarcoma cell line U6A, we were able to show that cells bearing p.(A219V) mutation retained the ability to transduce IFN signalling. However, STAT2 p.(A219V) failed to support receptor desensitization upon second IFN stimulation, resulting in sustained STAT2 phosphorylation and ISGs up-regulation. Furthermore, STAT2 p.(A219V) showed defective binding to USP18, providing an explanation for the prolonged IFN pathway activation in patient. Future work entails structural biology and modelling to map the STAT2-USP18 interaction region and to predict other potential disease-causing variants.