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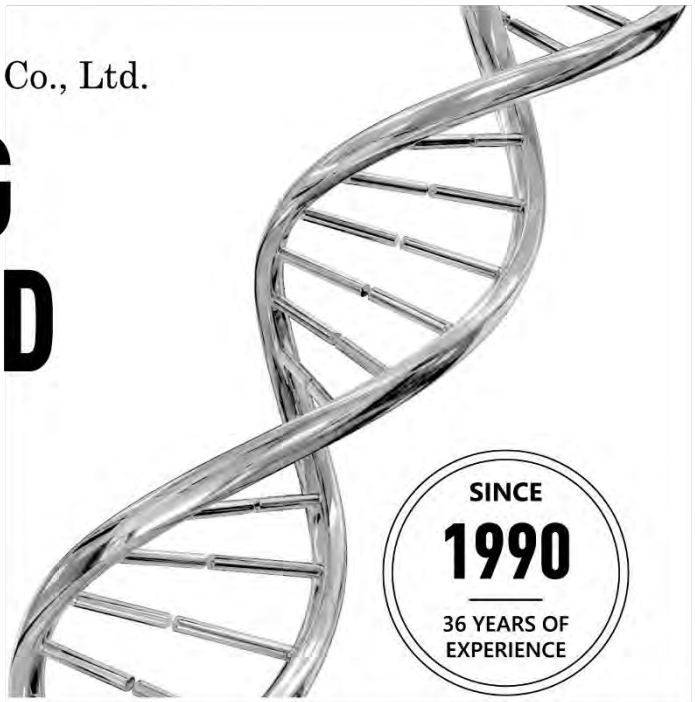

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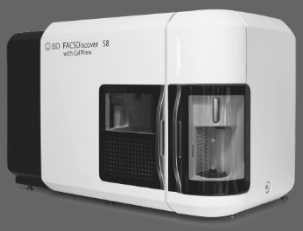

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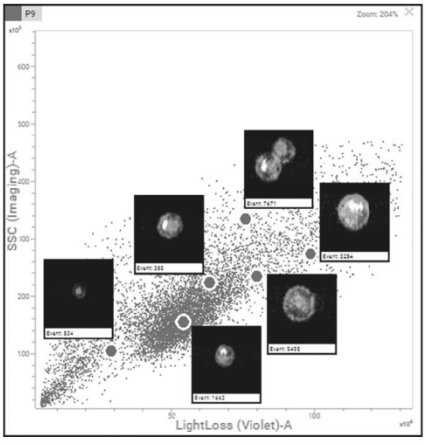

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MTNA Becomes an Official Journal of NAIS

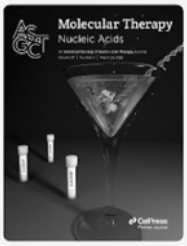
A NEW STRATEGIC PARTNERSHIP

Paloma Giangrande, PhD, Editor-in-Chief of *Molecular Therapy Nucleic Acids* (MTNA), and **James Chen**, President of the *International Society of Nucleic Acid Immunity* (NAIS), are pleased to announce a new strategic partnership dedicated to advancing excellence and innovation across the global nucleic acids research community.

EXPANDING SCOPE, DEEPENING IMPACT

> Through this partnership, **MTNA will officially expand its scope to include nucleic acid immunity**. Together, MTNA and NAIS will deepen scientific engagement, broaden opportunities for knowledge exchange, and strengthen the global foundation of nucleic acids research.

This collaboration aims to accelerate the translation of groundbreaking discoveries into impactful nucleic acid-based therapeutics to treat and correct genetic and acquired diseases worldwide.



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The 3rd Annual Meeting of the International Society of Nucleic Acid Immunity (NAIS26)

16-18 September 2026

Nara Kasugano International Forum, Nara, Japan

Organized by

International Society of Nucleic Acid Immunity (NAIS)
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NAIS26 Office

C/O WPI - Immunology Frontier Research Center (IFReC), The University of Osaka
3-1 Yamadaoka, Suita, Osaka 565-0871, Japan
https://www.ifrec.osaka-u.ac.jp/nais26/

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C/O WPI - Immunology Frontier Research Center (IFReC), The University of Osaka
3-1 Yamadaoka, Suita, Osaka 565-0871, Japan
https://www.ifrec.osaka-u.ac.jp/nais26/
E-mail: ifrec-sympo@ifrec.osaka-u.ac.jp
Date of Publication: 4 September 2026

Welcome to
the 3rd International School on Advanced Immunology

It is our great pleasure to welcome you to the **3rd Annual Meeting of the International Society of Nucleic Acid Immunity (NAIS26)**, to be held in the historic city of **Nara, Japan, from September 16 to 18, 2026**.

Since its establishment, NAIS has provided a unique forum for researchers from around the world to exchange ideas and advance our understanding of the complex interactions between nucleic acids and the immune system. The first two annual meetings were held in Europe, bringing together researchers from different countries and disciplines. We are particularly pleased that NAIS26 will be the first annual meeting to be held outside Europe, marking an important step in expanding the international reach of the NAIS community. We hope that holding the meeting in Japan will further strengthen connections between researchers in Europe, Asia, and other regions, and promote broader and more diverse scientific exchange.

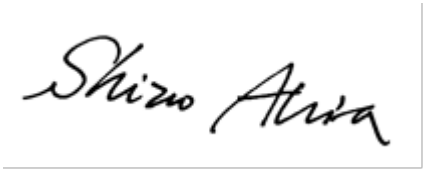
NAIS26 will feature a rich scientific program including keynote lectures, invited talks, oral presentations, poster presentations, and opportunities for informal scientific exchange. We hope that the meeting will provide a stimulating environment for presenting new discoveries, discussing emerging concepts, and exchanging ideas across disciplines. We also hope that these interactions will foster new collaborations and inspire innovative research directions in the field of nucleic acid immunity.

An important feature of this meeting is the opportunity to meet researchers at different stages of their careers and from different parts of the world. We particularly encourage young scientists and students to actively participate in discussions and poster sessions, and to take advantage of the meeting as an opportunity to establish new scientific connections.

We are especially pleased to welcome you to Nara, one of Japan’s oldest cities and a place where history, culture, and nature coexist. Nara is home to numerous historic temples and shrines and is also famous for the deer that freely roam the city. We hope that your stay in Nara will provide not only a scientifically rewarding experience but also an opportunity to enjoy and experience a unique aspect of Japanese culture.

We sincerely thank all invited speakers, presenters, participants, committee members, and sponsors for their valuable contributions and generous support. We hope that NAIS26 will be a memorable occasion for scientific discussion, new ideas, lasting collaborations, and friendship.

Welcome to NAIS26, and we hope you enjoy the meeting and your stay in Nara!



Shizuo Akira
Director of the NAIS26 Organizing Committee
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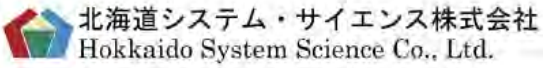

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Timetable

2026	16 September Wednesday	17 September Thursday	18 September Friday
08:00			
09:00	Registration (30)	Excursion	L12 Ken J. Ishii (30)
	Remarks (10)	Free Time Kasuga-Taisha Shrine Todai-ji Temple	L13 Asako Yamayoshi (30)
	Keynote Takashi Fujita (30)		L14 Jean-Charles Guéry(30)
10:00	L01 Hiroki Kato (30)		S07 Dong Ki Lee (15)
	Poster Pitch Session (60)		P53 Remzi Onur Eren (15)
11:00		Lunch (80)	Poster Pitch Session (60)
12:00	Lunch Poster Session	Meet the Editor (30)	Lunch Poster Session
		L06 Shizuo Akira (30)	
13:00		L07 Zhijian “James” Chen (30)	
	L02 Carl Wakley (30)	Break (15)	
14:00	L03 Enzo Poirier (30)	L08 Andrea Ablasser(30)	L15 Art Krieg (30)
	S01 Michael T. Lotze (15)	L09 Jean-Luc Imler(30)	L16 Jan Rehwinkel (30)
	S02 Hisashi Arase (15)		
15:00	P57 Daxing Gao (15)	S04 Nicolas Manel (15)	S08 Gunther Hartmann (15)
	Break (15)	S05 Conggang Zhang (15)	S09 Michael Gantier(15)
	L04 Kensuke Miyake (30)	Break (15)	P13 Jose Luis Guzmán Martín (15)
		L10 Susan Carpenter (30)	Break (15)
16:00	L05 Osamu Takeuchi (30)	L11 Hao Wu(30)	L17 Rayk Behrendt (30)
	S03 Kazuki Kato (15)	S06 Taro Kawai (15)	L18 Judy Lieberman (30)
	P03 Marleen Berouti (15)	P62 Lingyin Li (15)	Remarks/Awards (15)
17:00	Group Photo		
18:00	Gagaku Performance	Meet the Experts	
	Welcome Reception		
19:00			
20:00			

Wednesday, 16 September 2026

9:00 **Registration**
Please register at the Registration Desk in the Entrance Hall of the Nara Kasugano International Conference Center.

Posters should be mounted on the assigned poster boards in **Meeting Room 1 on the first floor and Meeting Rooms 3 & 4 on the second floor** and should remain mounted throughout the meeting.

9:30 **Opening Remarks**
Zhijian 'James' Chen
President of International Society of Nucleic Acid Immunity
Howard Hughes Medical Institute, USA
University of Texas Southwestern Medical Center, USA

Chair: Taro Kawai (Nara Institute of Science and Technology (NAIST), Japan)

9:40 **Keynote Talk**
Takashi Fujita
Center for advanced Modalities and DDS (CAMaD), The University of Osaka, Japan
Institute for Life and Medical Sciences, Kyoto University, Japan
Physiological functions of RIG-I-like receptors

10:10 **L01** **Hiroki Kato**
University Hospital Bonn, University of Bonn, Germany
Targeting the Poxvirus RNA Cap Maturation Enzyme as an Antiviral Strategy Against Mpox

10:40 **Poster Pitch Session**
Presenters with **odd-numbered Poster IDs** will briefly introduce their posters.

11:40 **Lunch and Poster Session**
Lunch will be provided in the Reception Hall on the second floor.
After lunch, presenters with **odd-numbered Poster IDs** will present their posters at their assigned poster boards.

Chair: Nicolas Manel (Institut Curie, France)

13:30 **L02** **Carl Walkley**
Centre for Innate Immunity and Infection Diseases, Hudson Institute of Medical Research, Australia
Department of Molecular and Translational Sciences, Monash University, Australia
Editing-Dependent and Editing-Independent Functions of ADAR1 In Regulation of "Self" vs "Non-Self" Discrimination of Cellular dsRNA

14:00 **L03** **Enzo Poirier**
Institut Curie, PSL Research University, INSERM U932, France
Ancestral Immunity: the Immune Modules Conserved Across Domains of Life

14:30 **S01** **Michael T. Lotze**
University of Pittsburgh Medical Center, Hillman Cancer Center, USA
Beyond DAMPs: HMGB1 as the Molecular Courier of Nucleic Acid Immunity

14:45 S02 **Hisashi Arase**
Immunology Frontier Research Center, The University of Osaka, Japan.
Research Institute for Microbial Diseases, The University of Osaka, Japan.
Bridging Innate and Adaptive Immunity through Neoself Antigens

15:00 P57 **Daxing Gao**
State Key Laboratory of Immune Response and Immunotherapy, University of Science and Technology of China, China
C9ORF72 safeguards against ageing by facilitating cytosolic DNA clearance via STING-mediated autophagy

15:15 **Break**

Chair: Jean-Luc Imler
(Guangzhou Medical University, China / University of Strasbourg, France)

15:30 L04 **Kensuke Miyake**
Synergy Institute for Futuristic Mucosal Vaccine Research and Development, Chiba University, Chiba, Japan
Medical Mycology Research Center, Chiba University, Chiba, Japan
Disease-driving TLR7 responses to lysosomal RNA/nucleoside stress

16:00 L05 **Osamu Takeuchi**
Graduate School of Medicine, Kyoto University, Japan
mRNA Decay in the Regulation of Immune Cells

16:30 S03 **Kazuki Kato**
Institute of Science Tokyo, Japan
Self vs Non-self RNA discrimination by RIG-I like receptors

16:45 P03 **Marleen Bérouti**
Gene Center and Department of Biochemistry, Ludwig-Maximilians-Universität, Germany
2‘,3‘-cUMP Is the Endogenous Ligand of TLR8

17:00 **Group Photo**

18:00 **Gagaku Performance**
Gagaku is the traditional music of the Japanese imperial court and is one of the oldest forms of orchestral music in the world. It has a history of more than 1,000 years and has been performed at court ceremonies and other important occasions in Japan.
Gagaku is characterized by its elegant and distinctive melodies and the unique sounds of traditional Japanese instrument. The music offers a glimpse into Japan’s rich cultural heritage and its long musical tradition.

18:30 **Welcome Reception**
A reception will be held for all participants at the Reception Hall on the 2nd floor.

Thursday, 17 November 2026

9:00 **Excursion: Deer-Calling Experience**
Meeting Point: Tobihino, Nara
(Please refer to page 92 for directions and a map.)

Nara is a unique city in Japan where wild deer freely roam the streets and parks as part of everyday life.
The “Shikayose” (Deer Calling) is a traditional Nara experience in which deer that have spent the night resting in the mountains are called down by the sound of a horn. This will be a truly special experience during your stay in Japan.
Participation is free of charge for those who wish to join.

After the Deer Calling Experience (around 9:30), participants will have free time until the start of the afternoon sessions. During this time, they may visit nearby sites that represent Japan’s rich cultural heritage, such as Todai-ji Temple and Kasuga Taisha Shrine, both of which are part of the UNESCO World Heritage Site “Historic Monuments of Ancient Nara.

11:00 **Lunch**
Lunch will be provided in the Reception Hall on the second floor.

Chair: Gunther Hartmann (University of Bonn, Germany)

12:20 **Meet the Editor of JITC at NAIS26**
Michael T. Lotze
Editor-in-Chief of Journal for ImmunoTherapy of Cancer (JITC)
How to Write a Great Paper and Get it Published

12:50 **L06** **Shizuo Akira**
WPI Immunology Frontier Research Center, The University of Osaka, Japan
Center for Advanced Modalities and Drug Delivery System Center, The University of Osaka, Japan
Regnase-1: An Endoribonuclease Linking Immunity and Metabolism

13:20 **L07** **Zhijian ‘James’ Chen**
Howard Hughes Medical Institute, USA
University of Texas Southwestern Medical Center, USA
A DNA-activated cGAS-like Enzyme in Bacterial Anti-Phage Immune Defense

13:50 **Break**

Chair: Rayk Behrendt
(University of Bonn & University Hospital Bonn, Germany)

14:05 **L08** **Andrea Ablasser**
Swiss Federal Institute of Technology Lausanne (EPFL), Switzerland
Immune sensing of DNA – mechanisms of (in)activation

14:35 **L09** **Jean-Luc Imler**
Sino-French Hoffmann Institute, Guangzhou Medical University, China
University of Strasbourg, Institut de Biologie Moleculaire et Cellulaire, France
Double-stranded RNA triggered antiviral immunity in insects

15:05 S04 **Nicolas Manel**
Institut Curie, INSERM U932, PSL University, France
Activation of the cGAS-STING pathway

15:20 S05 **Conggang Zhang**
School of Pharmaceutical Sciences, Tsinghua University, China
Distinct transmembrane mechanisms of STING activation

15:35 Break

Chair: Hui Yang (Fudan University, China)

15:50 L10 **Susan Carpenter**
Department of Molecular and Cellular Biology, University of California, Santa Cruz, USA
Integrative multiomics analysis and CRISPR screening identify functional noncanonical translation loci in the mouse immune system

16:20 L11 **Hao Wu**
Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, USA
Program in Cellular and Molecular Medicine, Boston Children's Hospital, USA
MyD88 is a pattern recognition receptor for cytosolic dsDNA

16:50 S06 **Taro Kawai**
Graduate School of Science and Technology, Nara Institute of Science and Technology (NAIST), Japan
Identification of ZAIP as a negative regulator of inflammatory enhancer activation

17:05 P62 **Lingyin Li**
Stanford University, USA
Arc Institute; USA
Extracellular cGAMP transmission drives neurodegeneration

18:00 Meet the Experts (Traditional Japanese Dinner with Guest Speakers)
A seated dinner will be held in an intimate setting with guest speakers who are experts in their respective fields. Participants will have the opportunity to enjoy an authentic traditional Japanese dinner while interacting with the invited speakers and other participants. Participation is limited to those who have registered for the dinner in advance.
Please refer to page 93 for directions to the venue.

Friday, 18 September 2026

Chair: Michael P. Gantier (Hudson Institute of Medical Research, Australia)

9:00 **L12** **Ken J. Ishii**
The Institute of Medical Science, The University of Tokyo, Japan
The University of Tokyo Pandemic Preparedness, Infection and Advanced Research Center (UTOPIA), the University of Tokyo, Japan
From B- and Z-Form Nucleic Acids to Immune Control

9:30 **L13** **Asako Yamayoshi**
Department of Life Science and Technology, Institute of Science Tokyo, Japan
Novel Exosome-Hijacking Drug Delivery System using Antibody-Oligonucleotide Conjugates

10:00 **L14** **Jean-Charles Guéry**
University of Toulouse, INSERM, CNRS, Infinity – Toulouse Institute for Infectious and Inflammatory Diseases, France.
Tlr7-biallelism defines a hyperfunctional state of female B lymphocytes

10:30 **S07** **Dong Ki Lee**
OliX Pharmaceuticals Inc, South Korea
OliX US, USA
University of Bonn, Germany
Sungkyunkwan University, Korea
Identification of an unconventional and potent small double-strand RNA MDA5 agonist

10:45 **P53** **Remzi Onur Eren**
CECAD, University of Cologne, Germany
Modulating Innate Immune Signaling Pathways in STING-Driven Inflammatory Diseases

11:00 **Poster Pitch Session**
Presenters with **even-numbered Poster IDs** will briefly introduce their posters.

12:00 **Lunch and Poster Session**
Lunch will be provided in the Reception Hall on the second floor.
After lunch, presenters with **even-numbered Poster IDs** will present their posters at their assigned poster boards.

Chair: Susan Carpenter (University of California, Santa Cruz, USA)

14:00 **L15** **Arthur M Krieg**
Zola Therapeutics, USA
Therapeutic Applications of Nucleic Acid Self-nonself Discrimination Through TLR7/8/9

14:30 **L16** **Jan Rehwinkel**
MRC Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, UK
A secreted decoy receptor buffers type I interferon signalling

15:00 **S08** **Gunther Hartmann**
University Hospital Bonn, University of Bonn, Grermany
Natural human STING variation determines cysteine-dependent regulation

15:15 **S09** **Michael P. Gantier**
Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Australia

Mouse TLR8 is a Functional Sensor of Nucleic Acid Fragments

15:30 P13 José Luis Guzmán Martín
University of Bonn, Germany
Disrupting MAVS Filament Extension Abrogates Antiviral Immunity

15:45 Break

Chair: Hao Wu (Harvard Medical School, USA)

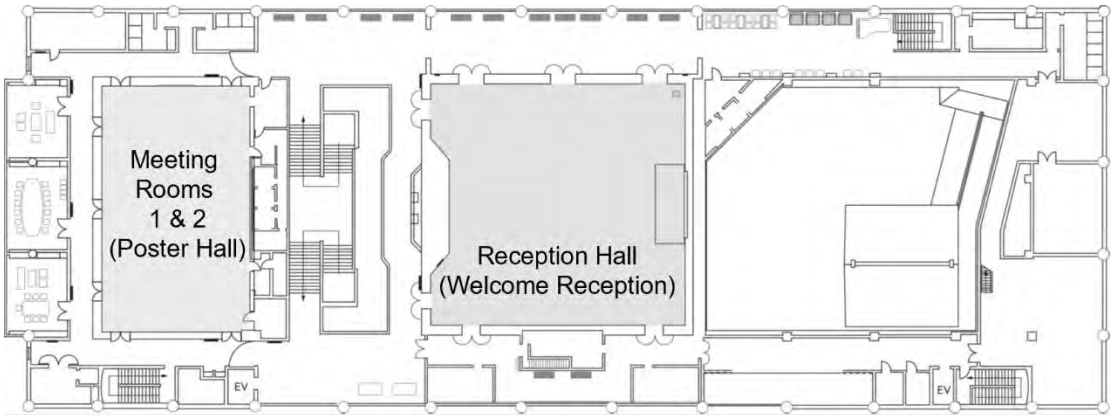
16:00 L17 Rayk Behrendt
University Bonn, University Hospital Bonn, Nucleic Acid Immunity & Genome Defense, Institute of Clinical Chemistry and Clinical Pharmacology, Germany.
The ART Of Defense: Endogenous Reverse Transcription In Antiviral Immunity

16:30 L18 Judy Lieberman
Program in Cellular and Molecular Medicine, Boston Children's Hospital, USA
Department of Pediatrics, Harvard Medical School, USA
Mismatch repair deficiency activates CD8 T cell tumor immunity without tumor antigens

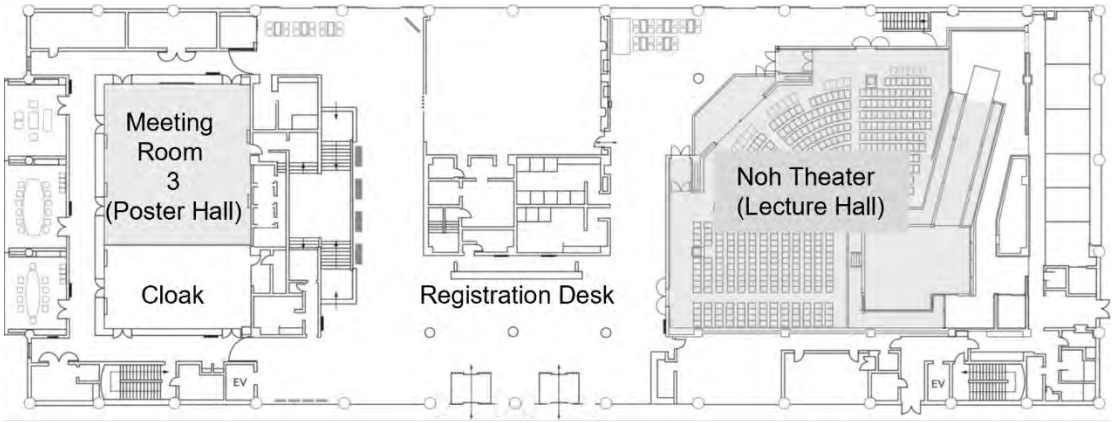
17:00 Presentation Awards and Closing Remarks
Shizuo Akira
Director of the Organizing Committee of the 3rd Annual Meeting of International Society of Nucleic Acid Immunity (NAIS26)
WPI Immunology Frontier Research Center (IFReC), The University of Osaka, Japan
Center for Advanced Modalities and Drug Delivery System Center(CAMaD), The University of Osaka, Japan

Floor Plan

Nara Kasugano International Forum I・RA・KA



2nd Floor



1st Floor

Keynote Lecture

Physiological functions of RIG-I-like receptors

T.Fujita,[1, 2]
[1] Center for advanced Modalities and DDS (CAMaD), The University of Osaka, 1-10 Yamadaoka, Suita, Osaka, 565-0871, JAPAN
[2] Institute for Life and Medical Sciences, Kyoto University, 53 Shogoin Kawaharacho, Sakyo-ku, Kyoto, 606-8507, JAPAN

Keywords: RLR, viral RNA, antiviral innate immunity, non-self RNA, interferonopathy

Since the discovery of RIG-I like receptor (RLR) in 2004, studies revealed that it is crucial for pathogen detection and triggering immune responses and have immense physiological importance. In this talk, I first summarize the interferon system and innate immunity, which constitute primary and secondary responses. Next, the molecular structure of RLR and the mechanism of sensing non-self RNA are described. Usually, self-RNA is refractory to RLR; however, there are underlying host mechanisms that prevent immune reactions. Studies have revealed that the regulatory mechanisms of RLR involve covalent molecular modifications, association with regulatory factors, and subcellular localization. Viruses have evolved to acquire antagonistic RLR functions to escape the host immune reactions. Finally, the pathologies caused by the malfunction of RLR signaling are described.

L01

Targeting the Poxvirus RNA Cap Maturation Enzyme as an Antiviral Strategy Against Mpox

Hiroki Kato
Institute of Cardiovascular Immunology, Medical Faculty, University Hospital Bonn, University of Bonn, Bonn, Germany

Keywords: RNA cap maturation, mpox, monkeypox virus, orthopoxvirus, antiviral drug screening

Mpox is an infectious disease caused by monkeypox virus (MPXV), a member of the genus *Orthopoxvirus*, which also includes variola virus, the causative pathogen of smallpox. While smallpox was declared eradicated in 1980, MPXV continues to cause recurrent outbreaks. For example, in 2024, outbreaks of clade I MPXV, which has been linked to more severe disease, spread across Central and Eastern Africa. Although smallpox-derived vaccines can protect against mpox, vaccination is primarily targeted at individuals at elevated risk rather than being included in routine immunization programs. Therapeutic options also remain limited. Therefore, developing new therapeutic options for mpox is an urgent priority.

Poxviruses are large double-stranded DNA viruses that replicate in the cytoplasm and use their own virus-encoded enzymes to mature the 5’ cap structure of their mRNA. The poxvirus cap maturation enzyme VP39 is conserved among orthopoxviruses and uses *S*-adenosyl-L-methionine (SAM) for its activity. In mammalian cells, cap maturation of mRNA is important not only for mRNA stability and efficient translation, but also for preventing the recognition by innate immune sensors, which leads to the interferon response and translational shut-off. We experimentally confirmed that a VP39 mutant of the commonly used *orthopoxvirus* vaccinia virus (VACV) induced higher interferon responses and showed reduced replication. This finding prompted us to investigate whether inhibiting viral cap maturation could suppress poxvirus replication in general. We screened our unique library of nucleoside- and non-nucleoside-derived small molecules against the SAM-binding pocket of VACV VP39 and have identified several chemical compounds with potent antiviral activity against VACV and mild toxicity. These VACV VP39 inhibitors also suppressed the replication of other poxviruses in cell culture, including MPXV, and exhibited synergistic antiviral effects when combined with tecovirimat, an approved smallpox antiviral. Treatment with the compound also suppressed a tecovirimat-resistant virus. Notably, treatment with each compound provided protection against a lethal VACV or MPXV challenge in a rodent model. We are currently investigating their antiviral activities in a non-human primate model using common marmosets. Together, these findings support the targeting of poxvirus RNA cap maturation as a promising antiviral strategy.

L02

Editing-Dependent and Editing-Independent Functions of ADAR1 In Regulation of “Self” vs “Non-Self” Discrimination of Cellular dsRNA.

Carl Walkley[1]
[1]Centre for Innate Immunity and Infection Diseases, Hudson Institute of Medical Research; Clayton, Victoria 3168, Australia
[2] Department of Molecular and Translational Sciences, Monash University; Clayton, 3168, Victoria, Australia

Keywords: ADAR1, RNA modification, MDA5, Innate immune sensing, self-tolerance

Adenosine deaminase acting on RNA 1 (ADAR1) is an RNA binding and editing enzyme. ADAR1 converts adenosine nucleotides to inosine (A-to-I) in regions of double stranded RNA (dsRNA). A-to-I editing results in a permanent change to the sequence of the RNA from that encoded in the DNA. A-to-I editing is a prevalent feature of the transcriptome and can result in diverse consequences for the transcript, from changing the protein coding sequence through to modifying interactions with microRNAs and changing the secondary structure of the RNA. Most A-to-I editing in mammals occurs in short interspersed nuclear elements (SINEs, also referred to as repetitive elements), including *Alu* elements (humans/primates) and B1/B2 SINEs (rodents). The physiologically most important function of ADAR1 *in vivo* is editing of cellular derived dsRNA. This serves as a critical immune checkpoint and “self” vs “non-self” discrimination mechanism in both human and mouse by marking cellular derived dsRNA, via A-to-I editing, as self-derived and therefore not ligands for the cytoplasmic dsRNA sensor melanoma differentiation-associated protein 5 (MDA5). Genetic studies have demonstrated that it is the cytoplasmic ADAR1 isoform, ADAR1p150, that is essential to achieving self-tolerance to cellular dsRNA.
Whilst now established that A-to-I editing by ADAR1p150 is key in achieving self-tolerance it remains less clear if there are functions of the ADAR1 proteins independent of their A-to-I editing action. Using an allelic series of viable adult ADAR1 mutant animals, rescued by loss of both MDA5 and PKR, that allow comparison of full ADAR1 deficiency (*Adar1*$^{-/-}$, p110 and p150), a loss of editing but retained protein expression (*Adar1*$^{E861A/E861A}$) and loss of ADAR1p150 (*Adar1*$^{p150-/-}$) we have compared the *in vivo* editing-dependent and editing-independent functions of ADAR1. We found that the protein expression of the cytoplasmic ADAR1p150 isoform is essential for maintaining peripheral T cell numbers and for the normal differentiation of hematopoietic stem and progenitor cells (HSPCs), independent of editing activity. In bone marrow transplants ADAR1p150 protein, but not its editing activity, was crucial for T cell regeneration and HSPC repopulation, demonstrating an essential cell-intrinsic function in hematopoiesis. Treatment of purified HSPCs *in vitro* with IFNβ or administration of IFNAR1 neutralizing antibodies *in vivo* demonstrated hypersensitivity to tonic type I interferon (IFN) signaling due to loss of ADAR1p150 protein was the driver of the *in vivo* phenotype. Using cell lines derived from the mouse models we demonstrate that RNA binding by ADAR1p150 protein was required to suppress activation of the OAS-RNaseL pathway following type I IFN exposure. These results implicate tonic type I IFN as an inducer of immunogenic cellular dsRNAs. ADAR1p150 suppresses immune sensing of self-dsRNA through editing-dependent (MDA5) and RNA binding editing-independent (PKR, OAS-RNaseL) mechanisms. Thus, ADAR1p150 protein levels define the cellular threshold for tolerance to self-derived dsRNA.

L03

Ancestral Immunity: the Immune Modules Conserved Across Domains of Life

E. Poirier[1], A. Bernheim[2], J. Cury[2], M. Haudiquet[1], X. Lahaye[3], N. Manel[3], H. Vaysset[2]
[1]Innate Immunity in Physiology and Cancer Team, Institut Curie, PSL Research University, INSERM U932, Paris, France
[2]Institut Pasteur, Université Paris-Cité, CNRS UMR3525, Molecular Diversity of Microbes, Paris, France
[3]Innate Immunity Team, Institut Curie, PSL Research University, INSERM U932, Paris, France

Keywords: Innate immunity, antiphage system, evolution, immunotherapy

Bacterial antiphage defense systems and eukaryotic immune systems were long considered unrelated. However, a growing number of central immune proteins, including cGAS, STING, TIR-domain receptors, and gasdermins, have now been recognized as evolutionary descendants of bacterial immune proteins. Our team formalized this connection through the concept of ancestral immunity, defined as protein domains conserved across the tree of life that mediate immune functions in both prokaryotes and eukaryotes (Bernheim, Cury & Poirier, PLOS Biology, 2023). We demonstrated that this evolutionary framework can directly drive the discovery of previously unrecognized immune components. Using a homology-based approach, we identified SIRal, a human descendant of bacterial SIR2 antiphage proteins, as a critical regulator of Toll-like receptor signaling (Bonhomme, Vaysset et al., Science, 2025). To extend this strategy beyond individual examples, we developed the Ancestral Immune Score, a quantitative metric that measures the extent to which a eukaryotic protein is composed of domains associated with bacterial immunity. High-scoring human genes are significantly enriched for immune functions and virus-induced expression programs. Functional characterization of high-scoring candidates lacking prior links to immunity uncovers novel human immunomodulatory proteins. Together, these findings establish ancestral immunity as a systematic, evolution-guided framework for the discovery of immune genes and therapeutic targets across eukaryotes, including previously unknown components of the human innate immune system.

S01

Beyond DAMPs: HMGB1 as the Molecular Courier of Nucleic Acid Immunity

Daolin Tang[1], Michael T. Lotze[2].
[1] University of Texas Southwestern, Dallas, TX
Daolin.Tang@UTSouthwestern.edu
[2] University of Pittsburgh Medical Center, Hillman Cancer Center, Pittsburgh, PA lotzemt@upmc.edu

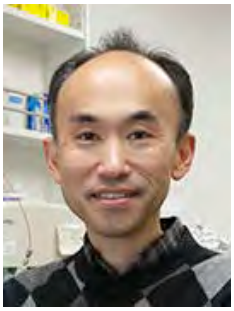
Keywords: DAMPs, HMGB1, Cancer, Immunity, Nucleic Acids

High mobility group box 1 (HMGB1) is widely recognized as a prototypical damage-associated molecular pattern (DAMP) molecule that alerts the immune system to tissue injury. Accumulating evidence suggests that this classical view is incomplete. Beyond functioning as an extracellular alarmin, HMGB1 is an evolutionarily conserved DNA- and RNA-binding protein capable of stabilizing nucleic acids, facilitating their cellular uptake, and directing their delivery to innate immune sensors. We propose that HMGB1 functions as a molecular courier of nucleic acid immunity, escorting endogenous and exogenous nucleic acids from sites of tissue damage to pattern-recognition receptors including TLR7, TLR8, TLR9, RAGE, and the cGAS–STING pathway. This model provides a unifying framework for understanding how regulated cell death, infection, sterile injury, and cancer generate immunostimulatory nucleic acid signals.
During apoptosis, pyroptosis, necroptosis, ferroptosis, alkaliptosis, and other forms of regulated cell death, HMGB1 is released together with intracellular DNA, RNA, and nucleosomes, forming immune-active complexes that amplify type I interferon production, inflammatory cytokine expression, antigen presentation, and adaptive immune priming. The immunological consequences of these complexes are further shaped by the redox state and post-translational modifications of HMGB1, providing multiple regulatory checkpoints that determine whether nucleic acid sensing promotes protective immunity, chronic inflammation, or autoimmunity. Repositioning HMGB1 from a passive danger signal to an active molecular courier (a 'shlepper') shifts the conceptual focus from DAMP release to nucleic acid trafficking as a fundamental mechanism of innate immune surveillance. This perspective identifies HMGB1-dependent nucleic acid transport as a potentially druggable process for cancer immunotherapy, infectious diseases, and autoimmune disorders.

References.

1: Dai E, … Lotze MT, Klionsky DJ, Stockwell BR, Kroemer G, Tang D.A guideline on the molecular ecosystem regulating ferroptosis. Nat Cell Biol. 2024 Sep;26(9):1447-1457. doi: 10.1038/s41556-024-01360-8. Epub 2024 Feb 29. PMID: 38424270; PMCID: PMC11650678.
2: Tang D, Kang R, Zeh HJ, Lotze MT. The multifunctional protein HMGB1: 50 years of discovery. Nat Rev Immunol. 2023 Dec;23(12):824-841. doi: 10.1038/s41577-023-00894-6. Epub 2023 Jun 15. PMID: 37322174.
3: Chen R, Huang Y, Quan J, Liu J, Wang H, Billiar TR, Lotze MT, Zeh HJ, Kang R, Tang D. HMGB1 as a potential biomarker and therapeutic target for severe COVID-19. Heliyon. 2020 Dec 7;6(12):e05672. doi: 10.1016/j.heliyon.2020.e05672.PMID: 33313438; PMCID: PMC7720697.
4: Galluzzi L, Vitale I, Warren S, … Lotze MT, Manic G, Merghoub T, Melcher AA, Mossman KL, Prosper F, Rekdal Ø, Rescigno M, Riganti C, Sistigu A, Smyth MJ, Spisek R, Stagg J, Strauss BE, Tang D, Tatsuno K, van Gool SW, Vandenabeele P, Yamazaki T, Zamarin D, Zitvogel L, Cesano A, Marincola FM. Consensus guidelines for the definition, detection and interpretation of immunogenic cell death. J Immunother Cancer. 2020 Mar;8(1):e000337. doi: 10.1136/jitc-2019-000337. PMID: 32209603; PMCID: PMC7064135.
5: Zeh HJ, Bahary N, Boone BA, Singhi AD, Miller-Ocuin JL, Normolle DP, Zureikat AH, Hogg ME, Bartlett DL, Lee KK, Tsung A, Marsh JW, Murthy P, Tang D, Seiser N, Amaravadi RK, Espina V, Liotta L, Lotze MT. A Randomized Phase II Preoperative Study of Autophagy Inhibition with High-Dose Hydroxychloroquine and Gemcitabine/Nab-Paclitaxel in Pancreatic Cancer Patients. Clin Cancer Res. 2020 Jul 1;26(13):3126-3134. doi: 10.1158/1078-0432.CCR-19-4042. Epub 2020 Mar 10. PMID: 32156749; PMCID: PMC8086597.

Bridging Innate and Adaptive Immunity through Neoself Antigens



Hisashi Arase[1,2]
[1]Laboratory of Immunochemistry, Immunology Frontier Research Center, The University of Osaka, Japan.
[2]Department of Immunochemistry, Research Institute for Microbial Diseases, The University of Osaka, Japan.

Activation of the innate immune response is often followed by activation of adaptive immunity, and excessive activation can ultimately result in autoimmunity. Cytokine production and/or upregulation of co-stimulatory molecules are thought to contribute to the activation of adaptive immunity. However, how activation of innate immunity induces antigen-specific autoimmune responses has remained unclear. Self–nonself discrimination is a fundamental principle of the immune system that explains why immune cells respond to foreign antigens, such as viral antigens, while remaining tolerant to self. Recently, we discovered that self antigens can be further classified into self and neoself antigens, and that T cells can distinguish between them even when they are derived from the same self proteins (Mori et al., ***Cell***, 2024). Because normal T cells are not tolerant to neoself antigens, presentation of these antigens by MHC class II molecules leads T cells to recognize them as foreign, thereby triggering autoimmune responses. Further analyses of neoself antigens revealed that activation of innate immunity promotes the presentation of neoself antigens, thereby stimulating autoreactive T cells. These findings suggest that neoself antigens provide a mechanistic link between innate and adaptive immunity.

L04

Disease-driving TLR7 responses to lysosomal RNA/nucleoside stress

K. Miyake [1, 2] and Y. Murakami[3]

[1]Synergy Institute for Futuristic Mucosal Vaccine Research and Development, Chiba University, Chiba, Japan
[2] Medical Mycology Research Center, Chiba University, Chiba, Japan
[3] Faculty of Pharmacy, Department of Pharmaceutical Sciences & Research Institute of Pharmaceutical Sciences, Musashino University, Tokyo, Japan

RNA-sensing TLRs reside in the endosomal compartment and respond to pathogen-derived ssRNAs to initiate defense responses. Dysregulated RNA metabolism causes lysosomal storage of RNAs and nucleosides, leading to various diseases due to constitutive activation of RNA-sensing TLRs. For example, loss-of-function variants of the lysosomal nucleoside transporter SLC29A3 cause lysosomal nucleoside accumulation and constitutive activation of TLR7 and TLR8 in macrophages, leading to histiocytic diseases called SLC29A3 disorders. On the other hand, loss-of-function variants of the lysosomal RNase phospholipase D4 (PLD4) cause systemic lupus erythematosus due to TLR7 activation by lysosomal RNA accumulation. These findings suggest that macrophage responses to nucleosides cause histiocytosis whereas B cell responses to RNAs cause lupus. To understand disease-driving TLR7 responses, we are working on mice harboring D34A Unc93b1 mutation, in which TLR7 drives autoimmune hepatitis and autoantibody production. We analyzed TLR7 signals mediating type I interferon production in plasmacytoid DCs, B cell proliferation, and B cell maturation into age-associated B cells (ABCs). Our findings revealed the disease-driving TLR7 signaling pathway in pDCs and B cells in D34A Unc93b1 mice.

L05

mRNA Decay in the Regulation of Immune Cells

O. Takeuchi[1]
[1]Graduate School of Medicine, Kyoto University, Yoshida-Konoe-cho, Sakyo-ku, Kyoto 606-8501, Japan

Keywords: mRNA decay, Cytokines, Translation, Antisense-oligonucleotides (ASOs)

Immune cell activation is tightly regulated at both the transcriptional and post-transcriptional levels. In particular, mRNAs encoding cytokines and other immune regulators are controlled through selective degradation mediated by cis-elements in their 3′ untranslated regions (3′UTRs), such as AU-rich elements and stem–loop (SL) structures. These elements are recognized by RNA-binding proteins (RBPs) that regulate mRNA stability and translation. Regnase-1 is an endoribonuclease that destabilizes inflammatory mRNAs by targeting SL structures in 3′UTRs. Dysregulated Regnase-1 expression contributes to inflammatory and autoimmune diseases in mice and humans. We developed a therapeutic strategy to enhance Regnase-1 activity by blocking its autoregulatory mechanism using two antisense oligonucleotides (ASOs) targeting its 3′UTR. These Regnase-1-targeting ASOs increased Regnase-1 expression, reduced multiple proinflammatory transcripts in macrophages, and ameliorated inflammation in vivo. Beyond 3′UTR-mediated control, mRNA stability is also influenced by synonymous codon usage within coding sequences. Transcripts enriched in AU-ending non-optimal codons are inherently unstable. Through a genome-wide CRISPR screen, we identified the RNA-binding protein DHX29 as a key regulator of codon-dependent gene expression. Cryo–electron microscopy and selective ribosome profiling revealed that DHX29 associates with the A-site entrance of the translating 80S ribosome during decoding of non-optimal codons. DHX29 subsequently recruits the GIGYF2–4EHP complex to repress non-optimal mRNAs. Together, these findings uncover complementary post-transcriptional regulatory mechanisms operating through both 3′UTRs and coding sequences to fine-tune immune gene expression, providing new therapeutic opportunities for controlling inflammation.

S03

Self vs Non-self RNA discrimination by RIG-I like receptors

K. Kato[1], N. Kurihara[1] and Y. Isayama[2]
[1] Mechanistic Immunology Research Unit, Institute of Science Tokyo

Keywords: RIG-I-like receptors, LGP2, anti-MDA5 antibody, Antiviral immunity, Cryo-EM

"Self" and "non-self" discrimination is a fundamental feature of the immune system, enabling organisms to mount effective defenses against invading pathogens while avoiding damage to their own cells. This distinction is particularly critical in the recognition of viral RNA within the cytoplasm, where a diverse array of self-RNAs, including messenger RNAs and non-coding RNAs, coexist. RIG-I-like receptors (RLRs), a family of cytosolic RNA sensors, play a crucial role in this process by specifically recognizing viral RNA while avoiding self-RNA. Recent studies have demonstrated that the antiviral immune response mediated by RLRs is tightly and hierarchically regulated by various co-factor proteins and post-translational modifications. These regulatory mechanisms ensure precise immune activation while preventing inappropriate or excessive responses. In this presentation, I will discuss our recent findings on the molecular mechanisms underlying RLR-mediated antiviral responses, with a particular focus on how co-factor proteins modulate RLR function to maintain immune homeostasis.

Meet the Editor of *JITC* at NAIS26

How to Write a Great Paper and Get it Published

Michael T. Lotze, MD, FACS, FAIO
Director DAMP Laboratory; G.27b University of Pittsburgh Medical Center Hillman Cancer Center; lotzemt@upmc.edu

Keywords: *JITC*, Original Research, Reviews, Commentaries

Publishing scientific research is an essential component of advancing the field of nucleic acid immunity, yet navigating the publication process can be challenging for investigators at all career stages. In this interactive Meet the Editor session, the Editor-in-Chief of the *Journal for ImmunoTherapy of Cancer (JITC)*, Dr. Michael T. Lotze, will provide practical guidance on transforming high-quality research into successful publications. Attendees will gain insight into what editors and reviewers look for in submitted manuscripts, common reasons for rejection, strategies for effectively communicating scientific findings, and best practices for responding to peer review. Participants will also learn about *JITC*'s scope, priorities, and commitment to advancing translational, clinical, and basic immunotherapy research. Through discussion and audience questions, attendees will leave with actionable strategies to strengthen their future submissions, better understand the editorial decision-making process, and identify appropriate publication pathways for their work. This session is designed for clinicians, researchers, trainees, and allied health professionals seeking to maximize the impact, visibility, and dissemination of their scientific contributions.

The Journal for ImmunoTherapy of Cancer (JITC) is the open access, peer reviewed, online journal of the Society for Immunotherapy of Cancer (SITC). The journal publishes articles on all aspects of tumor immunology and cancer immunotherapy and, in doing so, aims to enrich communication and advance scientific understanding among the many stakeholders in this rapidly evolving field. Topics of interest range widely across the basic science-translational-clinical spectrum and include tumor-host interactions, the tumor microenvironment, animal models, predictive and prognostic immune biomarkers, novel pharmaceutical and cellular therapies, vaccines, combination immune-based therapies, mechanistic case reports and series, local and oncolytic virus therapies, and immune-related toxicity. Acceptance rate currently is ~21% with a 2025 Impact Factor (JCR):11.7. The IF rank (Oncology) is 30/333 total journals and IF rank (Immunology) of 9/183 total journals. The Citescore is16.6. Here at the 3rd NAIS Meeting we will discuss the importance of publishing findings from our research and the role of the *Journal* to: 1) decide whether it is suitable; 2) improve it through rigorous peer review; and 3) promote the paper through all available social media channels and SITC.

JITC also welcomes applications from interested individuals who would like to take advantage of the many benefits of serving as a reviewer. Benefits include: 1) A **10% APC discount** on papers authored by active reviewers; 2) Automated **ORCiD credit** for reviewer activity; 3) Credit toward **SITC's Path to Leadership; 4)** Continuing medical education (**CME**) credit; and 5) **Recognition** through annual acknowledgement and reviewer recognition services.

L06

Regnase-1: An Endoribonuclease Linking Immunity and Metabolism

S. Akira,[1,2] A. Murata,[1] H. Sato[1] and K. Maeda[1]
[1] WPI Immunology Frontier Research Center, The University of Osaka, Japan
[2] Center for Advanced Modalities and Drug Delivery System Center, The University of Osaka, Japan

Keywords: RNA binding protein, inflammation, autoimmunity, T cell, MASH

Regnase-1 is an RNA-binding endoribonuclease that functions as a key post-transcriptional regulator of inflammation by promoting the degradation of mRNAs encoding inflammatory cytokines, including IL-6.. Under steady-state conditions, Regnase-1 is constitutively expressed and suppresses inflammatory gene expression, thereby maintaining immune quiescence. Following immune stimulation, Regnase-1 is rapidly inactivated, permitting robust induction of inflammatory responses. Subsequently, Regnase-1 expression is re-established, forming a negative feedback loop that terminates inflammation and restores immune homeostasis.

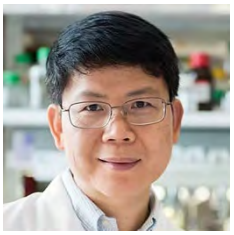
Cell type–specific genetic studies have demonstrated that Regnase-1 plays indispensable roles in immune regulation. Deletion of Regnase-1 in T cells results in spontaneous immune activation, systemic autoimmunity, and enhanced effector function. Likewise, Regnase-1 deficiency markedly augments the antitumor activity of CAR T cells, and NK cells, underscoring its role as a critical checkpoint that restrains immune activation.

Beyond the immune system, Regnase-1 is evolutionarily conserved and regulates diverse biological processes. In Caenorhabditis elegans, it controls lipid metabolism, whereas in Drosophila it is essential for developmental transitions. In mammals, Regnase-1 contributes to intestinal homeostasis and has been implicated in inflammatory bowel disease, where disease-associated variants alter its function. More recently, we identified a previously unrecognized role for Regnase-1 in hepatic metabolism, demonstrating that hepatocyte-specific deficiency of Regnase-1 confers resistance to metabolic dysfunction–associated steatohepatitis (MASH).

Together, these findings establish Regnase-1 as a central regulator that coordinates immune responses, inflammation, and metabolic homeostasis through post-transcriptional control of gene expression. These diverse functions highlight Regnase-1 as a promising therapeutic target for both immune-mediated and metabolic diseases.

L07

A DNA-activated cGAS-like Enzyme in Bacterial Anti-Phage Immune Defense



Zhijian ‘James’ Chen
HHMI
University of Texas Southwestern Medical Center

The cGAS-STING pathway has evolved for billions of years from bacteria to humans. A large family of cGAS-like enzymes has been shown to play a key role in the arms race between bacteria and their viruses, the bacteriophage, but the mechanisms by which the bacterial cGAS-like enzymes are activated remain poorly understood. We have identified a bacterial cGAS-like enzyme that is activated by DNA, suggesting an evolutionary conservation of cGAS activation mechanism. The similarities and differences of cGAS activation between humans and bacteria will be discussed.

L08

Immune sensing of DNA – mechanisms of (in)activation

A.Ablasser,[1]
[1]Swiss Federal Institute of Technology Lausanne, EPFL, CH

The life of any organism depends on the ability of cells to detect and to respond to pathogens. In order to detect the immense variety of pathogenic entities, the innate immune system of mammals has evolved a range of distinct sensing strategies. One major mechanism is based on the recognition of microbial DNA - an invariant and highly immunogenic pathogen-associated molecular pattern. Host cells, however, contain abundant sources of self-DNA. In the context of cellular damage or metabolic derangement, “out-of-the-context” self-DNA can elicit potentially damaging inflammatory responses. Our research focuses on the so-called cGAS-STING system - an evolutionary highly conserved innate DNA sensing system. On DNA binding, cGAS is activated to produce a second messenger cyclic dinucleotide (cyclic GMP-AMP), which stimulates the adaptor protein STING to induce innate immune responses. While this process was originally discovered as a crucial component of the immune defense against pathogens, recent work has elucidated a pathogenic role for innate DNA sensing in a variety of sterile inflammatory diseases. In this talk I will discuss recent findings on molecular regulation of DNA sensing and highlight opportunities for harnessing cGAS-STING for therapeutic purposes.

L09

Double-stranded RNA triggered antiviral immunity in insects

J.L. Imler,[1,2]
[1]Sino-French Hoffmann Institute, Guangzhou Medical University, Guangzhou, China
[2]University of Strasbourg, Institut de Biologie Moleculaire et Cellulaire, Strasbourg, France

Keywords: cGAS-like receptors, STING, evolution, RNA interference, cyclic dinucleotides.

RNA interference (RNAi) plays a major role in the control of viral infections in insects. This mechanism is activated upon sensing of viral double stranded (ds) RNA produced during viral replication by the enzyme Dicer-2, composed of a RLR-like helicase domain coupled to two RNaseIII domains. Cleavage of dsRNA by Dicer-2 generates small interfering RNAs, which are loaded onto the RNaseH-like enzyme Argonaute-2 and guide it towards viral RNAs. Work in the model insect *Drosophila melanogaster* further revealed that NF-κB mediated induced responses also participate in the control of viral infections. Here, viral dsRNAs are sensed by cGAS-like receptor (cGLR)-1 or -2, leading to the production of up to 4 different cyclic dinucleotides and activation of STING signalling. This raises two questions that will be addressed here. The first is about the contribution of the cGLR/STING pathway to antiviral immunity in other insects and I will present data showing the importance of this pathway in bees, with intriguing differences compared to flies. The second is the respective contributions and added values of RNAi and STING, two pathways triggered by dsRNA. Our data suggest that RNAi provides an efficient first line defence in flies, with cGLR/STING acting as a powerful back-up, associated with activation of systemic immunity.

S04

Activation of the cGAS-STING pathway

N. Manel[1]
[1]Institut Curie, INSERM U932, PSL University, Paris, France

Keywords: cGAS, STING

In this talk, I will present our recent work on mechanisms that control activation of the cGAS-STING pathway.

Distinct transmembrane mechanisms of STING activation

Conggang Zhang[1], Xiangyu Liu[1], Shuhao Zhang[1], Jing Han[1], and Yang Zhao[1]
[1] School of Pharmaceutical Sciences, Tsinghua University, Beijing, 100084, China

Keywords: STING, cGAS-STING pathway, Antitumor immunity, Protein assembly

The cGAS–STING pathway is a central innate immune signaling pathway, in which STING functions as the key adaptor protein linking cytosolic DNA sensing to type I interferon production. Although STING has traditionally been viewed as a membrane scaffold, recent studies suggest that its transmembrane domain serves as an active regulatory hub for ligand recognition and signal transduction.

Here, we report two structurally distinct small-molecule agonists, GNE-6468 and GZR, that directly engage the STING transmembrane region. Using cryo-EM, biochemical and functional analyses, we show that these agonists induce distinct transmembrane rearrangements that converge on enhanced TM1–TM3 interactions, thereby promoting STING oligomerization and activation. GNE-6468 engages a transmembrane pocket and cooperates with membrane lipids to remodel transmembrane helices, whereas GZR engages the transmembrane domain through a previously unrecognized activation mechanism. Despite their mechanistic differences, both agonists activate STING-dependent interferon responses and exhibit potent antiviral and antitumor activities.

These findings establish the STING transmembrane domain as a central signaling platform rather than a passive membrane anchor and provide new opportunities for therapeutic modulation of innate immunity through transmembrane-targeted agonists.

L10

Integrative multiomics analysis and CRISPR screening identify functional noncanonical translation loci in the mouse immune system

Eric Malekos[1], Valeriy Smaliy[2], Susan Carpenter[2]
[1]Department of Biomolecular Engineering and Bioinformatics, University of California, Santa Cruz
[2]Department of Molecular and Cellular Biology, University of California, Santa Cruz

Keywords: noncoding RNAs, short open reading frames, peptides, inflammation, CRISPR screening

Ribosome profiling has revealed thousands of noncanonical translation events across mammalian genomes, yet functional characterization has overwhelmingly focused on proliferative fitness in cancer cell lines. Here, we present a comprehensive survey of noncanonical translation in the mouse immune system and its functional consequences in macrophages. By performing a unified Ribo-seq meta-analysis across 20 public mouse leukocyte datasets - spanning macrophages, dendritic cells, neutrophils, B cells, and T cells - we define a compendium of 22,276 noncanonical coding sequences (CDSs), including upstream ORFs (uORFs), downstream ORFs, and ORFs on noncoding RNAs and pseudogenes (ncORFs). Proteogenomic integration with reanalyzed mass spectrometry data prioritizes a high-confidence subset with detectable protein products, including pseudogene-encoded and lncRNA-encoded zinc finger proteins. To move beyond cataloging, we carried out two orthogonal CRISPR screens in immortalized bone marrow-derived macrophages: a fitness screen identifying noncanonical CDSs required for macrophage viability, and a TLR1/TLR2-NFκB reporter screen uncovering CDSs that modulate innate immune signaling. These screens nominate uORFs, several conserved between mouse and human, that exhibit knockout phenotypes on par with their cognate coding sequences. We unexpectedly discovered a family of endogenous retroviral envelope-derived proteins translated in adult myeloid cells. Among these, SYNIR - SYNcytin-like Immune Regulator - is a novel envelope-derived transmembrane protein that positively regulates NFκB-responsive transcription, while SEMR - Secreted Envelope-like Metabolic Regulator - is a novel secreted protein with structural homology to the feline leukemia virus accessory protein FeLIX that drives broad transcriptional remodeling of macrophage gene programs upon knockout. Updated single-cell RNA-seq annotations and an interactive UCSC Genome Browser session integrating Ribo-seq, proteomics, and CRISPR screen data are provided as community resources. Together, these findings expand the functional landscape of noncanonical translation in immunity and establish endogenous retroviral proteins as previously unrecognized regulators of macrophage biology.

L11

MyD88 is a pattern recognition receptor for cytosolic dsDNA

H. Wu[1,2]
[1]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston MA
[2]Program in Cellular and Molecular Medicine, Boston Children's Hospital, Boston MA

MyD88 is a universal adaptor that connects to Toll-like receptors (TLRs) and interleukin-1 receptors (IL-1Rs) through its Toll/IL-1R homology (TIR) domain, activating inflammatory signalling via its death domain (DD). However, how $MyD88^{TIR}$ serves as a platform for these functions and whether it possesses additional roles remain unclear. Here, we report cryo-EM structures of $MyD88^{TIR}$ and its cancer-specific mutant L252P (previously L265P) in ring and spiral forms, respectively. Both assemblies consist of antiparallel double-filaments utilizing canonical TIR interfaces within each strand, alongside unique inter-strand surfaces. Surprisingly, these filaments are bound to dsDNA. In vitro assays and cellular studies confirm the role of MyD88 in direct dsDNA binding and inflammatory cytokine expression upon dsDNA transfection or viral infection. A structure-guided MyD88 mutant designed to disrupt dsDNA binding specifically compromises DNA sensing and punctum formation while maintaining normal TLR signalling. Finally, RNA-seq reveals that MyD88 plays a crucial role in inducing gene expression upon dsDNA sensing in a TLR9-independent manner, and that nearly half of all cGAS-dependent genes are also MyD88-dependent. Thus, our findings identify MyD88 as a cytosolic dsDNA sensor that cooperates with cGAS.

S06

Identification of ZAIP as a negative regulator of inflammatory enhancer activation

Taro Kawai[1]
[1]Laboratory of Molecular Immunobiology, Graduate School of Science and Technology, Nara Institute of Science and Technology (NAIST), Nara, Japan

Keywords: Gene expression, epigenetics, non-coding RNA

Precise control of inflammatory gene expression is essential for effective host defense while preventing excessive inflammation. However, the mechanisms that restrain enhancer activation during inflammatory responses remain incompletely understood. Here, we identified a previously uncharacterized inhibitory factor, designated ZAIP, through a CRISPR/Cas9 knockout screen based on LPS-induced cytokine production in macrophages. Loss of ZAIP caused an approximately 100-fold increase in IL-6 production, identifying ZAIP as a potent negative regulator of inflammatory gene expression. Upon LPS stimulation, ZAIP accumulated in the nucleus, suggesting a role in stimulus-dependent transcriptional regulation. ZAIP deficiency preferentially enhanced the expression of Il6 and multiple chemokine genes without affecting Tnf expression. Integrated transcriptomic and epigenomic analyses revealed widespread accumulation of H3K4me1 at inflammatory regulatory regions, accompanied by increased enhancer-associated non-coding RNAs and promoter upstream transcripts (PROMPTs). Guided by these genome-wide analyses, we identified H3K4me1-enriched regulatory elements upstream of Il6 and demonstrated that genetic deletion of these regions markedly reduced Il6 induction. CRISPR interference targeting the associated enhancer RNAs similarly attenuated Il6 expression, establishing the functional importance of enhancer-associated transcription. Together, our findings identify ZAIP as an epigenetic regulator that coordinates enhancer chromatin and enhancer-associated transcription to fine-tune stimulus-induced inflammatory gene expression.

L12

From B- and Z-Form Nucleic Acids to Immune Control

Ken J. Ishii[1,2,3]
[1]Division of Vaccine Science, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo (IMSUT)
[2] International Vaccine Design Center (VDesC), IMSUT, Minato-ku, Tokyo 108-8639, Japan
[3] The University of Tokyo Pandemic Preparedness, Infection and Advanced Research Center (UTOPIA), the University of Tokyo

Keywords: B-DNA, STING, TLR9, Z-NA, mRNA

Nucleic acids are fundamental signals of innate immunity whose sequence, structure, localization, and delivery determine whether they promote host defense or immunopathology. Our studies began with the recognition that cytosolic B-form double-stranded DNA activates Toll-like receptor-independent antiviral immunity, establishing nucleic acids themselves as potent danger signals. More recently, we have revisited this concept from the perspective of nucleic-acid structure, including Z-form nucleic acids and ZBP1-mediated responses, and have explored approaches to manipulate the conformational transition between Z- and B-form nucleic acids as a new means of controlling innate immunity.

These principles are directly relevant to modern vaccinology. In LNP-mRNA vaccination, we identified a stromal–immune TBK1–IL-33–ST2–MyD88 circuit in which RNA sensing and ionizable lipid composition cooperate to induce the optimal immunity. This pathway enhances antigen-specific $CD8^+$ T-cell immunity but simultaneously promotes type-2 immune responses, illustrating how nucleic-acid sensing can couple protective to potentially unseen responses. In parallel, the Dectin-1 targeting TLR9 agonist induces prolonged, antigen-independent protection against respiratory viruses through sequential activation of macrophages and epigenetically reprogrammed innate lymphoid cells, suggesting a strategy for broad immunoprophylaxis during the interval before pathogen-specific vaccines become available.

Our recent analysis of the live attenuated vaccine as well as adjuvanted protein vaccines in the clinical use further demonstrates how appropriately programmed innate immunity supports robust germinal-center, T-cell, and cross-clade antibody responses, providing mechanistic insights relevant to on- and off-target efficacy of adjuvanted vaccines. Conversely, excessive nucleic-acid-induced inflammation can be restrained using a sequence- and structure-specific antagonists that suppresses innate immune responses downstream of multiple DNA- and RNA-sensing pathways.

Together, these findings define nucleic acid immunity as a programmable system that can be activated, shaped, or suppressed to prevent infection, enhance vaccination, treat cancer, and control allergy and inflammation.

L13

Novel Exosome-Hijacking Drug Delivery System using Antibody-Oligonucleotide Conjugates

A. Yamayoshi
Department of Life Science and Technology, Institute of Science Tokyo, 4259 Nagatsuta-cho, Midori-ku, Yokohama, Kanagawa, 26-8501 JAPAN

Keywords: antibody-oligonucleotide conjugate, exosome, nucleic acid therapeutics

Recently, mRNAs and microRNAs (miRNAs) have been identified in exosomes, which can be taken up by neighboring or distant cells. It has also been reported that such miRNAs (exosomal-miRNAs) regulate gene expression in the recipient cells. MiRNAs are a type of non-coding RNA that induce post-transcriptional gene silencing of their target genes and regulate a wide range of biological processes, including apoptosis, differentiation, metabolism, and cell proliferation. According to recent reports, the aberrant expression of miRNAs is associated with most pathological disease processes, including carcinogenesis. Therefore, circulating onco-miRs are considered as significant therapeutic targets for cancer therapy. However, there is no report to regulate the function of miRNAs in exosomes.

In this study, we attempted to develop novel drug delivery system using anti-exosome antibody-oligonucleotide conjugates (ExHijack-Oligo) for functional inhibition of circulating miRNAs. We found that ExHijack-Oligo can bind to the surface of exosomes and that the complexes are introduced into exosome-recipient cells then inhibit the activity of exosomal-miRNA *in vitro* and *in vivo*. We also found that ExHijack-Oligo can accumulate to not only primary tumors but also metastatic tumors after intravenous administration. With existing technologies, it is difficult to introduce anti-miRs into exosomes, and the likelihood of delivering anti-miRs to exosome-receiving cells after administration into the body (e.g., *via* intravenous injection) is extremely low. The development of an inhibition technology targeting exosomal miRNAs had not been achieved until now. In this study, we demonstrated that ExHijack-Oligo has established an inhibition technology targeting exosome-derived miRNAs. Furthermore, we will also report the immune responses induced by artificial oligonucleotides delivered into cells using ExHijack-Oligo.

L14

Tlr7-biallelism defines a hyperfunctional state of female B lymphocytes

Jean-Charles Guéry[1]
[1]Univ Toulouse, INSERM, CNRS, Infinity – Toulouse Institute for Infectious and Inflammatory Diseases, Toulouse, France.
jean-charles.guery@inserm.fr

Keywords: TLR7, X chromosome inactivation, female-biased immunity, B cell systemic autoimmunity, SLE.

Sex disparities in systemic autoimmunity have long been associated with enhanced dosage of the X-linked Toll-like receptor *TLR7*. Indeed, SLE and others rheumatic disorders are more prevalent in women and XXY Klinefelter men, underlining that the number of X chromosome is clearly associated with a greater risk of autoimmunity [1]. In XX female cells, one X chromosome is normally silenced, through a process called X Chromosome Inactivation (XCI), to equalize the dosage of X-linked gene expression between sexes, resulting in cellular mosaicism. As many immune-related genes are carried on the X chromosome, it has been proposed that gene dosage effects due to XCI escape contribute to sex differences in autoimmune responses. We previously showed that *TLR7* escapes XCI in immune cells and that B cells expressing *TLR7* bi-allelically were positively selected over mono-allelic cells at various stages of B cell activation and differentiation involving signalling via TLR7 [2]. Moreover, dysregulation of XCI maintenance at the *TLR7/Tlr7* locus is associated with the development of spontaneous autoimmunity in mice [3] and humans [4]. Together, these findings suggest a crucial role for the regulation of XCI in driving sex-related differences in immunity. However, it remains unresolved whether such *TLR7/Tlr7* "bi-allelic" cells represent a traceable B-cell population with enhanced cell-intrinsic functional properties that contribute to female-biased autoimmune diseases. Using novel genetically engineered female mice that report expression of the lupus-associated X-linked gene *Tlr7*, we identify a distinct hyperfunctional B cell subset that expresses *Tlr7* from both X chromosomes, which is required for the development of systemic B cell-mediated lupus-like disease. Collectively, this study supports future efforts to target X chromosome inactivation maintenance as a broad therapeutic strategy in sex-biased autoimmune disorders.

1 Miquel, C. H., Faz-Lopez, B. and Guery, J. C., Influence of X chromosome in sex-biased autoimmune diseases. *J Autoimmun* 2023: 102992. 10.1016/j.jaut.2023.102992
2 Souyris, M., Cenac, C., Azar, P., Daviaud, D., Canivet, A., Grunenwald, S., Pienkowski, C., Chaumeil, J., Mejia, J. E. and Guery, J. C., TLR7 escapes X chromosome inactivation in immune cells. *Sci Immunol* 2018. 3: eaap8855. 10.1126/sciimmunol.aap8855
3 Huret, C., Ferraye, L., David, A., Mohamed, M., Valentin, N., Charlotte, F., Savignac, M., Goodhardt, M., Guery, J. C., Rougeulle, C. and Morey, C., Altered X-chromosome inactivation predisposes to autoimmunity. *Sci Adv* 2024. 10: eadn6537. 10.1126/sciadv.adn6537
4 Du, Y., Faz-Lopez, B., Ah Kioon, M. D., Cenac, C., Pierides, M., Lakin, K. S., Spiera, R. F., Chaumeil, J., Truchetet, M. E., Gordon, J. K., Guery, J. C. and Barrat, F. J., Altered X-chromosome inactivation of the TLR7/8 locus and heterogeneity of pDCs in systemic sclerosis. *J Exp Med* 2025. 222: e20231809. 10.1084/jem.20231809

S07

Identification of an unconventional and potent small double-strand RNA MDA5 agonist

JJ Kim[1], YH Kwon[1], F Bender[3], S Sechi[3], T Zillinger[3], P Dua[2], G Hartmann[3], DK Lee[1,4]
[1]OliX Pharmaceuticals Inc, Seongnam-si, South Korea
[2]OliX US, Cambridge, 1 Broadway, MA, USA
[3]University of Bonn, Venusberg-Campus 1, Bonn, Germany
[4]Sungkyunkwan University, Suwon, Korea

RIG-I-like receptors (RLRs) are activated by distinct stimulatory RNA with defined features. While RIG-I is activated by short blunt end RNA duplexes with 5 -triphosphate ends, MDA5 activation requires long dsRNA fragments (>1 kb). Contrary to the current understanding in this field, we identified a 30-bp blunt-ended MDA5 agonist that is heavily modified by 2'OMe and 2'F ribose modifications. This dsRNA agonist is ten times more potent than poly I:C and do not require LGP2 for MDA5 binding and activation. MDA5 activation was seen in broad cell types ranging from immune cells to cancer cells of both mouse and human origin. Anticancer studies in syngeneic mouse tumor models confirmed activation of MDA5 pathway, downstream type 1 interferon production and a robust inhibition of B16F10 mouse melanoma. To our information this is the first of its kind non-classical MDA5 stimulatory RNA with significant translational and therapeutic value.

Therapeutic Applications of Nucleic Acid Self-nonself Discrimination Through TLR7/8/9

Arthur M Krieg[1]
[1]Zola Therapeutics, Needham, MA USA

Keywords: TLR7, TLR9, dendritic cell, immunotherapy, lipid nanoparticle

Tumors program immune suppression by releasing “damage associated molecular patterns” (DAMPs) that recruit and program immune suppressive myeloid cells and tumor microenvironment (TME) that kill or suppress anti-tumor CD8+ T cells, limiting response to immune checkpoint inhibitors (ICI). Many efforts to reprogram the TME with innate immune agonists have proven to be toxic or ineffective in humans, despite strong efficacy in mouse tumor models.

Nucleic acid immunity implies that CD8+ T cells evolved at least in part, for the purpose of killing retroviral-infected cells. The minimal plausible PRRs able to distinguish retroviral particles from normal cellular debris are TLR7/8 (GU-rich RNA) and TLR9 (CpG DNA). To mimic the natural structures of retroviral replicative complexes, we developed Z-007, a first-in-class TLR7/8/9 agonist that combines GU-rich RNA and CpG-A DNA within a single synthetic oligonucleotide, packaged in a noninflammatory lipid nanoparticle for intravenous (IV) administration.

We have evaluated Z-007 and comparator TLR agonists across various experimental systems including: i) *ex vivo* in human PBMC and tumor-associated immune cells (e.g., ascites); and *in vivo* in ii) several mouse models; iii) in pet dogs with spontaneous tumors; and iv) cynomolgus monkeys.

Unlike prior TLR agonists, Z-007 strongly induces IFN-α secretion with only low inflammatory cytokines in human immune cells, and markedly upregulates gene expression programs associated with human clinical response and survival.

In mouse models IV Z-007 significantly reduced tumor growth and reprogrammed the TME into an antiviral state with increased activated tumor-infiltrating dendritic cells, myeloid cells, and CD8+ T cells, as well as gene expression programs associated with ICI response and human and murine survival.

In eleven pet dogs with spontaneous tumors, weekly IV Z-007 (0.05–0.4 mg) was well-tolerated, with five dogs achieving >30% tumor shrinkage. Remarkably, 3/3 dogs with refractory hemangiosarcoma experienced complete or >90% resolution of lung, skin, and cardiac metastases within 2 months of monotherapy, without drug-related toxicity over >8 months of dosing.

Cynomolgus monkeys tolerated weekly IV Z-007 (0.02–2.0 mg) with no fever, liver enzyme elevations, or clinical toxicity. Serum IFN-α (>1000 pg/mL) and gene expression programs associated with ICI response and human and murine survival were dose-dependently induced in liver, spleen and lymph nodes, without increased serum IL-6 or TNF-α (<25 pg/mL).

Unlike prior TLR agonists, IV Z-007 uniquely drives high IFN-α secretion with negligible systemic inflammation, successfully reprogramming the TME *in vivo* and *ex vivo*. Additional canine trials and IND-enabling safety studies are underway to support human clinical development starting in 2027.

L16

A secreted decoy receptor buffers type I interferon signalling

J. Rehwinkel
MRC Translational Immune Discovery Unit, MRC Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, UK

Keywords: type I interferon, IFNAR, IFNAR2A, alternative mRNA processing, influenza A virus

Type I interferons (T1-IFNs) mediate antiviral immunity, yet when produced chronically drive immunopathology and autoinflammation. T1-IFNs signal through IFNAR, a dimeric receptor. We interrogated the function of IFNAR2a, a secreted isoform of IFNAR2 that binds T1-IFNs but lacks signalling domains. Loss of IFNAR2a in mice caused stronger induction of interferon-stimulated genes (ISGs) after T1-IFN stimulation. Similarly, IFNAR2a-deficiency elevated T1-IFN responses when *Adar1* was mutated. Following intranasal flu infection, lung ISG induction was comparable in wild-type and *Ifnar2*$^{\Delta a/\Delta a}$ mice; however, IFNAR2a-deficiency caused increased ISG responses in uninfected tissues including heart and lung. In humans, genetic variants associated with reduced IFNAR2a expression also associate with increased risk of severe COVID-19. Thus, secreted IFNAR2a may buffer systemic T1-IFN spread, preventing T1-IFN-driven disease.

Natural human STING variation determines cysteine-dependent regulation and sensitivity to the inhibitor H-151

T. Zillinger[1], B. Putschli[1], K. Fischer[2], M. Nastaly[1,4], R. Behrendt[1], C. Guenther[3], E. Bartok[4] and G. Hartmann[1]

[1]Institute of Clinical Chemistry and Clinical Pharmacology, University Hospital Bonn, University of Bonn
[2]Department of Dermatology, University Hospital Dresden, Technische Universität Dresden
[3]Department of Dermatology, University Hospital Tübingen, Eberhard Karls University Tübingen
[4]Institute of Experimental Haematology and Transfusion Medicine

Keywords: innate immunity, DNA sensing, Sting, genetic variation, type I IFN

Aberrant activation of the cGAS-STING pathway contributes to inflammatory and autoimmune disease, making STING an attractive therapeutic target. Covalent STING antagonists, including the widely used inhibitor H-151, act through regulatory cysteines. Whether natural genetic variation of human STING alters the functions of these cysteines or inhibitor responsiveness is unknown. Here, we show that STING haplotype, activation mode and species jointly determine cysteine requirements and H-151 sensitivity. H-151 responsiveness varied markedly among primary human donors and was associated with STING diplotype. In isogenic THP-1 cells expressing common haplotypes from the endogenous *TMEM173/STING1* locus, inhibition of 2′3′-cyclic GMP-AMP (cGAMP)-induced responses differed by more than an order of magnitude (IC_{50}, 0.21 μM for RGHR and >4 μM for the prevalent RGRR haplotype), whereas double-stranded DNA-induced responses were less sensitive. Moreover, at 4 μM, H-151 suppressed RIG-I- and MDA5-driven responses independently of STING. Systematic cysteine substitution revealed context-specific requirements: SAVI mutants were strongly dependent on C88/C91, while common haplotypes differed in their use of C64, C91 and C148. Notably, C91, the covalent target of H-151, was largely dispensable for RGRR signaling but remained essential for inhibitor activity, uncoupling drug action from palmitoylation inhibition. Murine STING differed from every common human haplotype in both cysteine usage and mode-dependent H-151 sensitivity. Thus, natural STING variation determines pharmacological susceptibility and limits extrapolation from murine models, underscoring the need to account for STING genotype in preclinical studies and therapeutic development.

S09

Mouse TLR8 is a Functional Sensor of Nucleic Acid Fragments

M.P. Gantier,[1, 2] L. Hinkelmann, M. Brehm, E. Rupasinghe,[1, 2] W.S.N. Jayasekara[1, 2], S. Sapkota[1, 2], A.L. McAllan[1, 2] and S. Lehnardt[3]
[1]Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, Australia
[2]Department of Molecular and Translational Science, Monash University, Clayton, Australia
[3]Neuroscience Research Center, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany

Keywords: Murine TLR8, TLR7, natural antagonism, RNA sensing

Activation of human Toll-like receptor 8 (hTLR8) depends on cooperative binding of RNA degradation products to two sites: site 1, which recognizes uridine or small-molecule agonists, and site 2, which binds short nucleic acid fragments. We recently identified a third regulatory site that binds 2′-O-methyl RNA fragments and mediates allosteric inhibition, thereby limiting autoinflammation [1].
In contrast, the function of murine TLR8 (mTLR8) remains poorly understood. Here, we identify 2’,3’-cyclic guanosine monophosphate (2’,3’-cGMP) and 2’,3’-cyclic uridine monophosphate (2’,3’-cUMP) as endogenous site 1 ligands of mTLR8 that activate the receptor independently, analogous to uridine-mediated activation of hTLR8. These ligands synergized with selected DNA and RNA fragments binding to site 2, resulting in cooperative activation of mTLR8 signalling independently of TLR7.
Using a novel mouse model expressing mTLR8 under control of the *Tlr7* promoter, we show that mTLR8 partially restores TLR7-dependent gene expression in B cells, establishing basal mTLR8 activity and physiological function at steady state. Notably, the mTLR8 antagonistic site contains substitutions equivalent to human disease-associated mutations, resulting in loss of detectable antagonistic-site activity. These findings suggest that, although mTLR8 can be activated by RNA degradation products, it lacks the endogenous 2′-O-methyl RNA-mediated inhibitory mechanism that constrains hTLR8.
Together, our results establish mTLR8 as a functional sensor of nucleic acid degradation products with activity partially overlapping that mTLR7. We propose that the comparatively weak activity of mTLR8 in mice reflects an evolutionary adaptation to the absence of endogenous antagonism.

[1] Alharbi AS, Sapkota S, Zhang Z, Jin R, et. al. 2'-O-Methyl-guanosine RNA fragments antagonize TLR7 and TLR8 to limit autoimmunity. *Nat Immunol.* 2026 27(4):762-775.

L17

The ART Of Defense: Endogenous Reverse Transcription In Antiviral Immunity

L. Muhandes*, K. L. Kleemann*, Patrick Müller and R. Behrendt
University Bonn, University Hospital Bonn, Nucleic Acid Immunity & Genome Defense, Institute of Clinical Chemistry and Clinical Pharmacology, 53127 Bonn, Germany.
*equal contribution

Keywords: retroelements, NRTI, innate priming, antiviral immunity

In order defend against infections the immune system rests in a stand-by state rather than being completely switched off. This primed state is maintained by constant low-level exposure to immunogenic molecules. For antiviral immunity, tonic activation of nucleic acid sensors like the intracellular DNA sensor cGAS has been shown to underlie innate priming in several tissues. This prompted the hunt for endogenous cGAS ligands that would enable rapid defense responses. Previously, it has been suggested that cGAS can be activated by products of reverse transcription. Intriguingly our genome hosts a significant number of retroelements, which utilize reverse transcript intermediates for mobilization. Excessive de-repression of endogenous retroelements and subsequent activation of cGAS has been implicated as a driving mechanism in a range of inflammatory conditions, including aging. In all these model systems, pharmacologic inhibition of reverse transcription using FDA-approved nucleoside reverse transcriptase inhibitors (NRTI) used in antiretroviral therapy (ART) reduced inflammatory effects and proximal signatures of cGAS activation. Consequently, NRTI have been suggested to mitigate retrotransposon driven inflammation and to potentially slow down aging. While ample data on the effects of NRTI in several mouse models and HIV-infected humans are available, controlled data of how NRTI affect the unchallenged human immune system are spares. Moreover, if and how the activity of endogenous retroelements can shape human immunity remains an area of intense research.

L18

Mismatch repair deficiency activates CD8 T cell tumor immunity without tumor antigens

J. Lieberman,[1],[2]
[1]Program in Cellular and Molecular Medicine, Boston Children's Hospital, Boston MA
[2]Department of Pediatrics, Harvard Medical School, Boston MA

Keywords: mismatch repair, chromosomal instability, interferon g, tumor immunity

Mismatch repair (MMR)-deficient tumors respond to immune checkpoint blockade, while MMR-proficient tumors are typically refractory, suggesting that inhibiting MMR could induce protective antitumor immunity. However, there are no MMR inhibitor drugs. We have found a way to induce MMR in tumor cells that rapidly induces chromosomal instability, micronuclei and cytosolic DNA, causing genotoxic stress and profound anti-tumor immunity. MMR-deficient tumors drive potent $CD8^+$ T cell–mediated tumor suppression or even rejection in colorectal, breast, and melanoma models. Notably, immune control does not require T cell receptor antigen recognition as it is preserved or even enhanced in *B2m*-deficient tumors. (MMR)-deficient tumors induce durable systemic immunity, surprisingly conferring protection against unmanipulated immunologically cold MMR-proficient homologous and unrelated heterologous tumors. Together, these findings uncover an unexpected, potent, non-canonical, TCR-independent $CD8^+$ T cell mechanism of immune control and establish a potential strategy to convert immunologically "cold" tumors into immunotherapy responsive tumors. I will discuss what we know so far about the mechanisms behind immune protection.

P01 Defining the Immunomodulatory Determinants of 3-mer Oligonucleotides on TLR7 and TLR8 sensing

S. Sapkota[1,2], Z. Zhang[3], C. Wolf[4], M.A. Lee-Kirsch[4], J.I. Ellyard[5], O.F. Laczka[6], T. Shimizu[3] and M.P. Gantier[1,2].
[1]Hudson Institute of Medical Research, Clayton, Australia
[2]Monash University, Clayton, Australia
[3]Faculty of Pharmaceutical Sciences, The University of Tokyo, Japan
[4]Department of Pediatrics, Technische Universität Dresden, Dresden, Germany
[5]John Curtin School of Medical Research, The Australian National University, Australia
[6]Noxopharm Limited, Castle Hill, New South Wales, Australia

Chemical modifications such as 2′-O-methyl (2′-OMe) are central to the efficacy and tolerability of RNA therapeutics. We recently identified that 2′-OMe RNA fragments as short as three nucleotides can exert opposing effects on Toll-like receptor 8 (TLR8) sensing in a motif-dependent manner. Here, leveraging their short length, we systematically map how base and sugar modifications within 3-mer oligonucleotides regulate TLR7 and TLR8 responses, and we resolve the structural basis for both TLR8 potentiation and TLR7/8 antagonism by nucleic acid fragments. Building on these insights, we report the development of a dual TLR7/8 inhibitory oligonucleotide with therapeutic potential in autoimmune disease. Together, these findings expand our understanding of the immunomodulatory activities of oligonucleotides degradation fragments on TLR7 and TLR8 and provide design principles for oligo-therapeutics.

P02 Investigating the Chemical Repertoire of Lysosomal and TLR7/8-bound RNA Ligands

T. Komar,[1] M. Bérouti,[1] A. Yikilmazsoy,[1] M. Wagner,[2] K. Halter,[2] K.-P. Hopfner,[1] T. Carell[2] and V. Hornung[1]
[1]Gene Center and Department of Biochemistry, Ludwig-Maximilians-Universität, Munich, Germany
[2]Department of Chemistry, Ludwig-Maximilians-Universität, Munich, Germany

Toll-like receptors TLR7 and TLR8 are key components of the innate immune system for RNA recognition. As such, they are crucial for anti-viral immunity, but are also linked to autoimmune disease when aberrantly activated. Our recent studies revealed how RNase T2 and PLD exonucleases generate the precise fragments that activate TLR7 and TLR8, and how pseudouridine silences immune recognition. These findings suggest that the immunogenicity of RNA is not determined solely by sequence but emerges from a combination of the chemical space of RNA fragments generated in lysosomes and the modification-dependent masking or unmasking of these fragments. We therefore hypothesize that endogenous and pathogen-derived RNAs give rise to distinct fragment repertoires, some stimulatory and others inhibitory, and that disruption of this balance underlies autoimmune disease.
In this study, we will systematically profile RNA fragments present in lysosomes and those directly bound to TLR7 and TLR8 under steady-state and stimulatory conditions. Using a combination of bottom-up and top-down LC-MS approaches, as well as receptor pulldowns and recombinant ectodomain binding assays, we aim to identify both stimulatory and inhibitory ligands, including candidates that may occupy the proposed third binding pocket.

P03 2‘,3‘-cUMP Is the Endogenous Ligand of TLR8

Marleen Bérouti[1], Aysenur Yikilmazsoy[1], Matthias Heiss[2], Wilhelm Greulich[1], Jan Stöckl[1], Tobias Komar[1], Thomas Fröhlich[1], Thomas Carell[2], Karl-Peter Hopfner[1], Veit Hornung[1,*]
[1]Gene Center and Department of Biochemistry, Ludwig-Maximilians-Universität, Munich, Germany
[2]Department of Chemistry, Ludwig-Maximilians-Universität, Munich, Germany

Toll-like receptor 8 (TLR8) is a highly expressed pattern-recognition receptor in the human myeloid compartment that detects bacterial and viral RNA. TLR8 contains two ligand-binding pockets that recognize distinct RNA degradation products: pocket 1 senses the nucleoside uridine, whereas pocket 2 engages short purine-terminated RNA fragments generated by the enzyme RNase T2.
Using cell-based and structural approaches, we found that TLR8 preferentially binds 2’,3’-cyclic uridine monophosphate (2’,3’-cUMP) over uridine in pocket 1. This cyclic nucleotide is produced by members of the RNase A family, including RNase 1, RNase 2, and RNase 6. Although these enzymes are primarily characterized as endoribonucleases, we found that they possess 5’exonuclease activity. Furthermore, we show that PLD3 and PLD4 trim RNase T2-generated RNA fragments to produce ligands capable of engaging TLR8 pocket 2. Together, our findings provide a mechanistic framework for how RNA degradation products are generated and detected by TLR8.

P04 Revisiting the functionality of murine TLR8 *in vivo*

E. Rupasinghe,[1, 2] W.S.N. Jayasekara[1, 2], S. Sapkota[1, 2], A.L. McAllan[1, 2] and M.P. Gantier[1, 2]

[1]Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, Australia
[2]Department of Molecular and Translational Science, Monash University, Clayton, Australia

Toll-like receptor 8 (TLR8) activation depends on cooperative binding of RNA degradation products to two sites: site 1, which binds uridine or small-molecule agonists (e.g., TL8-506), and site 2, which engages short nucleic acid fragments. We recently identified a third regulatory site in human TLR8 that binds 2′-O-methyl RNA fragments and mediates allosteric inhibition, limiting autoinflammation. Here, we revisited murine TLR8 (mTLR8), whose characterization predates this framework. Using defined site 1 and 2 ligands, we confirmed cooperative activation of mTLR8 *in vitro*. To assess function *in vivo* while minimising TLR7 interference, we generated pTlr7-mTlr8 mice in which *Tlr7* was deleted to express *mTlr8* under the *Tlr7* promoter, and mice expressing a humanised TLR8 variant under the *Tlr7* promoter. Primary cells from these mice showed functional site 1 and 2 responses, though mTLR8 was less sensitive to site 1 agonists than human TLR8. RNA-seq of several tissues revealed basal mTLR8 activity at steady state in these mice. Notably, mTLR8 lacked detectable antagonistic site activity, suggesting that mTLR8 can be activated by RNA fragments but, unlike human TLR8, its function is not constrained by endogenous 2′-O-methyl RNA-mediated inhibition. This may have conditioned its lower activity in mice.

P05 TLR7 Reprograms Macrophages to Drive Cytotoxic T Cell–Mediated Submandibular Sialadenitis

T. Shibata[1], R. Sato[2], E. Nishimura[1], and K. Miyake[2]
[1]Division of Aging and Regeneration, The Institute of Medical Science, The University of Tokyo
[2]Synergy Institute for Futuristic Mucosal Vaccine Research and Development, Chiba University

Submandibular sialadenitis causes severe xerostomia, which leads to oral dysfunction and a reduced quality of life. Although sialadenitis is more prevalent in aged individuals, as well as patients undergoing radiotherapy or immune checkpoint therapy, and those suffering from autoimmune diseases and chronic GVHD, its molecular pathogenesis remains unclear.

We recently identified TLR7 as a driver of submandibular gland (SMG) sialadenitis. We demonstrated that deficiency of the nucleoside transporter SLC29A3 causes endolysosomal accumulation of guanosine analogues in macrophages, resulting in TLR7-dependent sialadenitis. TLR7 activation in SMG macrophages upregulated cytotoxic T lymphocyte (CTL)-attracting chemokines such as Cxcl9, promoted interactions with CTLs, and increased the expression of molecules involved in cross-presentation, including MHC class I and CD80. Consequently, activated CTLs selectively destroyed saliva-producing acinar cells.

These findings suggest that TLR7 hyperactivation reprograms SMG macrophages to promote CTL activation, thereby disrupting immune privilege and driving sialadenitis.

P06 2′-O-Methyl Guanosine RNA Fragments Inhibit Toll-Like Receptor 3 and 13 Activation

R. Gao[1,2]; L. Wilkinson-White[3], S. Sapkota[1,2], and M. P. Gantier[1,2]
[1]Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, Victoria, Australia
[2]Department of Molecular and Translational Science, Monash University, Australia
[3]Sydney Analytical Core Research Facility, University of Sydney, Australia

Toll-like receptors (TLRs) 3 and 13 are endosomal immune sensors that detect double-stranded and bacterial single-stranded RNA, respectively, and share structural similarities. We previously showed that specific 2′-O-methyl (2′-OMe) guanosine RNA fragments act as allosteric inhibitors of TLR7/8, limiting autoimmunity. We demonstrate here that select short 2′-OMe guanosine-containing RNA fragments can also antagonize both TLR13 and TLR3. Notably, distinct structural requirements were observed: TLR13 inhibition required a penultimate 3′-end 2′-OMe guanosine, presumably involving RNase T2 processing, whereas TLR3 inhibition depended on 5′-end 2′-OMe guanosine modification, similar to TLR7/8. Antagonism was sequence- and length-dependent, with fragments shorter than five nucleotides showing minimal effects on TLR3 but retaining activity on TLR13. These findings extend the role of 2′-OMe RNA fragments as endogenous regulators of endosomal TLRs, supporting their importance in maintaining immune homeostasis and preventing autoinflammation.

P07 CpG/Alum-Adjuvanted Multivalent Dengue Vaccine Confers Protection under Metabolic Stress

SW. Wan,[1,2] and C. Tan [1]
[1]Department of Microbiology & Immunology, College of Medicine, National Cheng Kung University, Taiwan
[2]Center of Infectious Disease and Signaling Research, National Cheng Kung University, Taiwan

Dengue virus (DENV) infection may progress to severe dengue, especially in individuals with metabolic comorbidities. High-fat diet (HFD)-fed mice exhibit increased DENV susceptibility and inflammation, making them a relevant model for evaluating vaccine efficacy under metabolically stressed conditions. We previously developed a multivalent dengue fusion protein vaccine candidate, ΔcNS1-cEDIII-ΔnNS3. Here, we evaluated this vaccine formulated with CpG oligodeoxynucleotide (CpG) and Alum in HFD-induced obese and diabetic mice. Although vaccine-induced innate, humoral, and T cell responses were weaker in HFD-fed mice than in chow diet (CD)-fed mice, vaccination still reduced DENV-induced viremia and bleeding tendency after challenge. Further immune profiling showed impaired antigen-specific T cell activation and central memory T cell responses in vaccinated HFD-fed mice. However, effector memory T cell responses were maintained after DENV infection, suggesting rapid functional recall upon viral challenge. Together, these findings indicate that HFD-associated metabolic stress dampens vaccine-induced immunity but does not eliminate protection, highlighting the immunomodulatory potential of CpG as a nucleic acid-based adjuvant to improve dengue vaccine efficacy under metabolically compromised conditions.

P08 Macrophage-Intrinsic TLR9 Activation by Lysosomal DNA Stress Drives Emergency Myelopoiesis and Liver Fibrosis

Ryota Sato[1], Takuma Shibata[2], Kiyoshi Yamaguchi[3], Yoichi Furukawa[3], Kenta Nakano[4], Tadashi Okamura[4], Ryutaro Fukui[1], Yuji Motoi[1], Kensuke Miyake[1]
[1]Miyake Lab, cSIMVa, Chiba University, Chiba, Japan.
[2]Nishimura Lab, IMSUT, The University of Tokyo, Tokyo, Japan.
[3]Division of Clinical Genome Research, IMSUT, The University of Tokyo, Tokyo, Japan.
[4]Department of Laboratory Animal Medicine, JIHS, Tokyo, Japan.

Lysosomal nucleic acid stress occurs when RNA/DNA fragments are not degraded in lysosomes. Although RNA-driven stress induces protective macrophage programs via TLR7/8/13, lysosomal DNA stress remains poorly understood. PLD3/4 are lysosomal DNases whose deficiency causes single-stranded DNA accumulation and TLR9 activation. We examined *Pld3*$^{-/-}$*Pld4*$^{-/-}$ (Pld3/4-dKO) mice. Pld3/4-dKO mice developed splenomegaly, hepatomegaly, thrombocytopenia, skin inflammation, and liver fibrosis with macrophage expansion. Bulk RNA-seq of splenic Ly6C^{+} macrophages revealed proliferative gene induction. Liver scRNA-seq showed expansion of neutrophils and effector CD8^{+} T cells, Kupffer cell activation, and proliferating monocytes with inflammatory/fibrotic signatures.
TLR9 deficiency, but not Sting deficiency, rescued inflammation and fibrosis. Loss of B cells, CD4^{+} T cells, or CD8^{+} T cells failed to rescue disease. Myeloid-lineage deletion of Pld3/4 was sufficient to induce splenomegaly and liver fibrosis.
Thus, myeloid-intrinsic TLR9 hyperactivation under lysosomal DNA stress drives macrophage expansion and liver fibrosis.

P09 Single-Nuclei Multiomics Reveal Heterogeneous Regulatory Circuits Underlying Human Dendritic Cell Response to Nucleic Acid Stimuli

F. Füchsl[1,2], M. Bourdon[1,2], B. Puzek[1,2], I. Namara[1,2], J. Klein[1,2], J. Jin[1,2], A. Jucha[1,2], J. Wiesel[1,2], D. Eser[1], S. Padmarasu[3], F. Schweiger[1], S. Grotz[1], C. Geiger[1], E. Herold[1], C. Klein[1], S. Kim-Hellmuth[1,2]
[1]Dr. von Hauner Children's Hospital, LMU University Hospital, Munich, Germany
[2]Institute of Translational Genomics, Helmholtz Munich, Neuherberg, Germany,
[3]Genomic Core Facility, Helmholtz Munich, Neuherberg, Germany

Dendritic cells (DCs) are key immune gatekeepers linking innate and adaptive immunity, yet the functional relevance of heterogeneous DC subtypes during activation remains elusive.
We generated a single-cell multiomic dataset, capturing gene expression and chromatin accessibility, from 170,000 DC-enriched PBMC from healthy donors. Unstimulated cells were compared to a time-course stimulation with TLR7/8, TLR9, or STING agonists.
Within lineages, TLR-stimulated cDCs and pDCs clustered together, with TLR7/8 inducing NF-κB/IL-1-driven inflammatory cytokine programs and TLR9 inducing type I interferon-producing (pDC) and lymphocyte-priming (cDC) states. Meanwhile, STING activation elicited a distinct interferon-stimulated gene program. Mapping eRegulons (linking transcription factors, chromatin peaks, and target genes) revealed distinct stimulus-specific regulatory circuits associated with these DC states. Integration with GWAS signals showed that multiple autoimmune-relevant circuits were enriched only in stimulated, but not resting, DC subsets. Our results define stimulus-specific DC activation programs and highlight disease-relevant regulatory circuits that emerge upon immune activation.

P10 BATF Deficiency Impairs CpG-enhanced Antigen-specific CD8$^+$ T Cell Proliferation in Association with Altered Dendritic Cell Responses

Tomoko Asatsuma-Okumura and Yoshiko Iwai
Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo, Japan

BATF (Basic leucine zipper transcription factor, ATF-like) is a member of the AP-1 subfamily. While it suppresses AP-1–mediated transcription via heterodimerization with c-Jun, it can also promote transcription by cooperating with interferon regulatory factors (IRFs), thereby affecting the differentiation and function of immune cells such as T cells. Our group previously generated Batf knock mice (BATF KO) and demonstrated its role in CD8$^+$ T cell differentiation. In contrast, while BATF3 is known to be essential for cDC1 development, the function of BATF itself in dendritic cells (DCs) has not been well characterized.
We found that stimulation of splenic or bone marrow–derived DCs with various TLR ligands increased Batf expression, particularly with TLR7 or TLR9 ligands. Among DC subsets, pDCs exhibited relatively high BATF expression. The production of inflammatory cytokine production, such as interleukin-12 (IL-12) and interferon-α (IFN-α), was decreased in Batf KO DCs. To examined the role of Batf in Ag-specific CD8 T cell response, OVA antigen was injected into Batf KO or WT mice transferred with OT-I Tg CD8 T cells. The proliferation of OT-I T cells was impaired in Batf KO mice.
These results suggest that Batf plays may play a role in the regulation of T cell responses by controlling DC function.

P11 Starvation Licenses the pDC Cell Line CAL-1 for IFN-∝ Production

N. B. Kegel[1], J. Kreitmeier[1], L. Staliunaite[2], J. Arnold[3], F. Venegas Solis[1], T. Maeda[4], F. Meissner[3], A. Kaufmann[1], and S. Bauer[1]
[1]Institute of Immunology, Philipps-Universität Marburg, Marburg Germany
[2]Leibniz Institute of Virology, Martinistraße 52 (N63), Hamburg, Germany
[3]Institute of Innate Immunity, University Hospital Bonn, Bonn, Germany
[4]Nagasaki University Graduate School of Biomedicine, Nagasaki, Japan

Plasmacytoid dendritic cells (pDCs) are the main producers of IFN-∝ but the molecular basis for their unique IFN-∝ production capacity remains elusive. We here describe how starvation of the pDC cell line CAL-1, which is considered dysfunctional for IFN-∝ production, enables IFN-∝ production similar to primary pDCs. Mechanistically, starvation induced an IFN signature independent of IFNAR signaling and a drastic downregulation of cell cycle-associated genes, suggesting a cell-intrinsic maturation program. We are currently investigating the role of epigenetic changes in generating *IFNA* promotor accessibility, which stands in line with current advances in the field. Our findings suggest a regulatory link between cell growth and cytokine production in pDCs. Moreover, we present a convenient, cost-efficient protocol to license CAL-1 cells for IFN-∝ production, enabling further in-depth research of pDC biology with possible applications in the fields of immunology, virology, and tumor biology.

P12 The SLC15A4-TASL Complex Is Essential for Lupus Development

A. Drobek[1], M. Delacrétaz[1], A. Vasilakou[2], M. Monguió-Tortajada[1], L. Bernaleau[1], M. Dubois[2], V. Sisirak[2], M. Rebsamen[1].
[1] Department of Immunobiology, University of Lausanne, Epalinges, Switzerland
[2] CNRS-UMR 5164, Immunoconcept, Bordeaux University, Bordeaux, France

Nucleic acid sensing by endolysosomal Toll-like receptors (TLRs) is critically involved in systemic lupus erythematosus (SLE) and related autoimmune diseases. Downstream of TLR7, 8 and 9, the SLE-associated SLC15A4-TASL complex selectively mediates IRF5 activation, while being dispensable for NF-κB and MAPK pathways. Here we show that the SLC15A4-TASL signalling axis is broadly required for disease development and pathogenesis across three complementary genetic SLE models, reflecting different aetiologies. Genetic ablation of *Tasl* (*Tasl*^{KO}) and its paralogue *Tasl2* (*Tasl*^{DKO}) ameliorated or fully prevented autoimmune manifestations resulting from *Fas*^{lpr} loss-of-function, respectively. Furthermore, SLC15A4-TASL complex deficiency was sufficient to protect from disease, including splenomegaly, immune activation and autoantibody formation, driven by the patient-derived *Tlr7*^{Y264H} gain-of-function mutation, even in the presence of functional NF-κB and MAPK responses. Lastly, *Tasl*^{DKO} splenocyte transfer into lymphopenic *Dnase1l3*-deficient mice demonstrated that protection extends to DNA-driven autoimmunity, in which TLR7 and TLR9 act redundantly, strongly supporting a B cell-intrinsic role. Notably, SLC15A4-TASL complex deficiency blunted the generation of pathogenic age-associated B (ABC) cells in all three autoimmune models as well as in aged animals. These data demonstrate the central role of the SLC15A4-TASL complex in SLE, supporting its potential as therapeutic target.

P13 Disrupting MAVS Filament Extension Abrogates Antiviral Immunity

[1]J.L. Guzmán Martín, [1]S. Pritzl, [1]S. Normann, [2]T. Stähler, [3]H. Beckert, [4]T. Ebert, [4]J. Schmid-Burgk, [1]F.I. Schmidt
[1] University of Bonn, Medical Faculty, Institute of Innate immunity
[2] University of Bonn, Core Facility Nanobodies
[4] University of Bonn, Institute of Clinical Chemistry and Clinical Pharmacology

The RIG-I like receptors (RLR) detect viral infections and are activated by cytosolic viral RNA. RLRs nucleate mitochondrial antiviral signaling protein (MAVS) through caspase recruitment domain (CARD) interaction. MAVS signalosomes serve as a central hub of the signaling pathway and therefore represent a potential target for therapeutic intervention. Yet, tools to interfere with and visualize MAVS filament assembly on different organelles are scarce. To study endogenous $MAVS^{CARD}$ filaments assembly, we generated single domain antibodies (VHHs) against human $MAVS^{CARD}$ and found three MAVS VHHs that inhibit interferon and interferon-stimulated genes (ISGs) when expressed in the cytosol of cells. Electron-microscopy and co-immunoprecipitation studies as well as Alphafold3 protein structure predictions revealed that the inhibitory VHHs are able to disrupt the extension of MAVS filaments without affecting the initial RLR:MAVS interaction. They thus stabilize a critical intermediate of MAVS activation and prove that cooperative assembly is critical for the early interferon response. They provide exciting tools for the visualization of MAVS and for therapeutic intervention in human primary cells.

P14 MAVS-IRF1 activation triggered by cholesterol deficiency in T cells dampens T cell anti-tumor function

Zhang Zhihan,[1] Zheng Lin[1]
[1]Center of Excellence in Molecular Cell Science, Shanghai

Cholesterol homeostasis is crucial for T cell function, yet how cholesterol availability regulates T cell fitness remains poorly understood. Here, we identify an unexpected role of cholesterol deficiency in activating a RIG-I/MDA5-MAVS-dependent innate immune program in T cells. Mechanistically, cholesterol depletion disrupts mitochondrial homeostasis, leading to mitochondrial RNA leakage and subsequent MAVS aggregation. Notably, we identify MAVS as a potential cholesterol-binding protein whose activation is directly modulated by mitochondrial cholesterol availability. Importantly, we demonstrate that IRF1 represents a major functional downstream effector of MAVS in T cells. Sustained MAVS activation induces IRF1-dependent transcriptional suppression of mTORC1 and cell-cycle programs, thereby limiting anabolic activity and clonal expansion. Consistent with this mechanism, IRF1 overexpression impairs CAR-T cell persistence, whereas IRF1 ablation enhances long-term expansion and antitumor efficacy. In clinical settings, IRF1 activity correlates with T cell dysfunction and poor responses to immunotherapy. Together, our findings establish a MAVS-IRF1 metabolic checkpoint that couples cholesterol insufficiency to T cell functional impairment and identify IRF1 as a potential therapeutic target for improving T cell-based immunotherapies.

P15 Annexin A2 Mediates RIG-I-like Receptor Responses to Viral Infection

G. Wray-McCann[1,2], D. Michalík [3], A. L. McAllan[1,2], L. J. Gearing[1,2], R. Liou[1,2], N. K. Campbell[1,2], S. Straub[1,2], H. Hosseni-Far[1,2], A. Matthews[1,2], M. D. Tate[1,2], S. Davidson[1,2], R. Fisher[4], C. Bernecky[3], J. Rehwinkel[5], P. J. Hertzog[1,2], N. G. Sampaio[1,2,*]
[1] Hudson Institute of Medical Research, Australia, [2] Monash University, Australia [3] Institute of Science and Technology Austria (ISTA), Austria, [4] Target Discovery Institute, University of Oxford, UK, [5] MRC Weatherall Institute of Molecular Medicine, University of Oxford, UK

RIG-I and MDA5 are critical sensors of virus infection and detect many important human pathogens including SARS-CoV-2, influenza A virus (IAV), and West Nile virus (WNV). MDA5 is activated by double-stranded RNA generated during infection and induces strong pro-inflammatory and antiviral responses. Activation of MDA5 is also implicated in monogenic (e.g. Aicardi-Goutières syndrome) and complex polygenic (e.g. systemic lupus erythematosus) autoinflammatory diseases, demonstrating the importance of appropriate pathway regulation. Yet, how MDA5 is regulated is poorly defined. We employed SILAC-mass spectrometry to discover MDA5 binding partners in virus-infected cells. Our screen revealed Annexin A2 (ANXA2) as an interactor of MDA5 during infection. Direct binding of ANXA2 and MDA5 was recapitulated in vitro and required Ca2+. Loss of ANXA2 blunted responses to EMCV, WNV and IAV, demonstrating a role for ANXA2 in activation of both MDA5 and RIG-I. Using human induced pluripotent stem cells, we found that ANXA2 was required for potent neuronal cell response to WNV and macrophage response to multiple viral pathogens. Thus, we find that ANXA2 is a critical mediator of RIG-I-like receptor pathways.

P16 G3BP1 Methylation by PRMT5 Facilitates Its Recruitment of TBK1 for IRF3 Activation during Host Response to Foreign Ribonucleic Acids

W. Song,[1] S.S.Y. Kim,[1] C. Yam,[1] J. Teo,[1] J.Y. Lim,[1] X. Bi,[2] H.H. Lim [1] and K.P. Lam [1,3,4]
[1]Singapore Immunology Network (SIgN), A*STAR, Singapore
[2]Bioprocessing Technology Institute (BTI), A*STAR, Singapore
[3]Department of Microbiology & Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
[4]School of Biological Sciences, Nanyang Technological University, Singapore

G3BP1 is an RNA-binding scaffolding protein implicated in stress granules and innate immune responses through RIG-I and cGAS. Here, we uncover its role in binding TBK1 and IRF3. G3BP1 deficiency reduced TBK1–IRF3 complex formation and affected IRF3 phosphorylation, nuclear translocation, and IFN-β induction upon p(I:C), while TBK1 phosphorylation was preserved. We further show that G3BP1 binds IRF3 constitutively and recruits TBK1 upon p(I:C). These findings suggest that G3BP1 scaffolds activated TBK1 to activate IRF3. Domain mapping experiments indicated that the C-terminal RGG region mediates IRF3/TBK1 binding. LC-MS/MS analysis revealed R435/R460 methylation, and mutagenesis showed R460 supports TBK1 binding. We further show that PRMT5 associates with G3BP1 and promotes symmetric arginine dimethylation, whereas PRMT5 inhibition impaired G3BP1–TBK1 interaction. Together, our data reveal G3BP1 as a signaling hub that constitutively binds IRF3 and is arginine-methylated by PRMT5 to recruit TBK1 for IRF3 phosphorylation and the induction of interferon production during host antiviral response.

P17 The spatiotemporal interplay between RIG-I-like receptors and endosomes

Chien-Ming Chen,[1] Kuan-Wei Lee,[1] Yu-Kai Hsu,[1] and Pin Ling[1,2]
[1]Department of Microbiology and Immunology, College of Medicine, National Cheng Kung University, Tainan, Taiwan.
[2] Department of Medical Laboratory Science and Biotechnology, College of Medicine, National Cheng Kung University, Tainan, Taiwan.

RIG-I-like receptors are cytosolic viral RNA sensors that are critical for triggering type I IFN-driven antiviral defense against RNA virus infections. Our recent work revealed a novel RIG-I action mode in which RIG-I is recruited onto endosomes, the “gateway” of virus entry into a host cell, to detect viral RNA upon viral breaching endosomes. To better understand this novel RIG-I action mode, we further explored the spatiotemporal regulation of RIG-I ubiquitination and activation by endosomes and two E3 ligases Riplet and Trim25. While published work indicates a key role for the endosomal adaptor TAPE in the formation and polyubiquitination of the RIG-I-Trim25 complex, our recent results showed that TAPE was not essential for the formation of the RIG-I-Riplet complex. Neither TAPE nor endosomes were critical for the polyubiquitination of Riplet and the RIG-I C-terminal domain. MDA5 detects non-enveloped RNA viruses, such as enteroviruses, which invade cells via membrane fusion. In this regard, it remains unclear whether endosomes are essential for MDA5 ubiquitination and activation. Our preliminary results showed that TAPE and endosomes were implicated in ubiquitination and activation of MDA5. Insights from these studies might reveal the early action modes of RIG-I and MDA5.

P18 A Paramyxovirus V Protein–Inspired Peptide Strategy for Selective MDA5 and LGP2 Inhibition and Blockade of Type I/III Interferon–Driven Inflammation

L.R. ter Haar*[1], A.C. Kooij*[1], R.Y. Sanchez David*[2], R.A. Cordfunke[1], N. Dolezal[1], H. Le[2], T. van Strijen[1], A. Ramlal[1], J.W. Drijfhout[1], P.V. Maillard*[2], A.G. van der Veen*[1]
[1]Department of Immunology, Leiden University Medical Center, NL
[2]Centre for Immunobiology, Queen Mary University of London, UK
Inadvertent activation of the RNA sensors MDA5 and LGP2 results in chronic type I/III interferon (IFN-I) signalling and underlies a spectrum of autoinflammatory diseases. There is currently no standardized therapeutic strategy to limit IFN-driven autoinflammation without broadly suppressing immune function. To circumvent this, we aimed to develop a targeted approach to inhibit the upstream RNA sensors MDA5 and LGP2 using Paramyxoviridae-encoded V proteins and synthetic peptides derived from these proteins. We show that various full-length V proteins, as well as their C-terminal domains (CTDs), effectively suppress IFN responses in cellular models of IFN-driven autoinflammation. Guided by structural insights, we designed peptides that mimic the interaction interface between the V-protein’s CTD and MDA5/LGP2. We identified several peptides that strongly inhibit human MDA5 and LGP2 in vitro. To enable intracellular delivery, selected peptides were conjugated to the cell-penetrating peptide TAT. We are currently investigating whether these peptides can effectively reduce autoinflammation in cellular models, including human iPSC-derived cortical neurons and astrocytes. Altogether, our V protein-inspired peptide strategy represents a promising avenue for developing a novel class of selective MDA5/LGP2 inhibitors with therapeutic potential for autoinflammatory disorders driven by aberrant RNA sensing.

P19 The Assembly of Stress Granules is Dispensable for Innate Immune Activation Following dsRNA Sensing and FMDV Infection

M. Marques,[1] Z. Sun[2], A. Ruggieri[2], J. P. Taylor[3], T. J. Tuthill[1] and N. Locker[1]
[1]The Pirbright Institute, Pirbright, UK.
[2]Heidelberg University, Centre for Integrative Infectious Disease Research, Germany
[3]Dpt of Cell and Molecular Biology, St. Jude Children's Research Hospital, Memphis, USA

Recognition of viral double-stranded RNA (dsRNA) intermediates during infection is a central trigger of cellular antiviral responses and stress adaptation pathways. We investigated how dsRNA sensing coordinates the assembly of biomolecular condensates with innate immune signalling. Time-resolved analyses following poly(I:C) stimulation reveal that formation of G3BP1 condensates and RLR/MAVS/IRF3 activation occur within the same cells, yet these cells show reduced IRF1 expression. Pharmacological inhibition of G3BP1 condensation abolished granule formation without affecting the induction of interferon responses. Similarly, infection with foot-and-mouth disease virus (FMDV), a highly contagious RNA virus, triggers the formation of stress granules independently of activation of interferon signalling. These findings demonstrate that dsRNA-triggered formation of condensates is uncoupled from innate immune activation but may modulate downstream expression of antiviral factors or viral replication, in a process we hypothesize is crucial for cell survival. Overall, our work highlights stress-induced granules as context-dependent regulators rather than essential platforms for antiviral response initiation.

P20 Ribosomes Sequester dsRNA Sensors to Modulate Innate Immunity

C.-I Yang,[1] and R. Green[1,2]
[1]Johns Hopkins University School of Medicine, Baltimore, Maryland, USA.
[2] Howard Hughes Medical Institute, Chevy Chase, Maryland, USA

PKR senses virally-derived double-stranded RNA (dsRNA) and activates the integrated stress response to inhibit host translation. Accumulating evidence suggests endogenous dsRNAs can also regulate this pathway. Here, we show that ribosomes, an abundant source of cellular dsRNA, regulate the interplay between PKR and its negative regulator PACT, which binds dsRNA agonists to impede PKR dimerization. We find that both PKR and PACT bind ribosomes. Yet, despite high sequence homology between their dsRNA binding domains (DRBDs), PKR binds more tightly to perfectly base-paired dsRNA while PACT binds more tightly to imperfect duplexes on ribosomes. The tight ribosome-PACT binding suggests that in addition to regulating PKR directly, ribosomes may tune PKR activity indirectly by modulating PACT function. Indeed, depleting ribosomes activates PKR in PACT-deficient cells, indicating ribosomes directly suppress PKR activity. Moreover, ribosomes partially relieve PACT-mediated inhibition of PKR in vitro, suggesting they preferentially sequester PACT while PKR is released and activated by dsRNA. Our results demonstrate that ribosomes regulate PKR in multiple ways: first as a direct negative regulator, and second by an indirect mechanism that hinges on tight association with PACT. Taken together, we establish that ribosomes can sequester dsRNA sensors, tuning innate immune responses across different levels of stress.

P21 Uncovering New Mechanisms That Restrain Innate Sensing of Self Double-Stranded RNA

Jacki Heraud-Farlow,[1,2,3], Hanna Vine[2], Scott Taylor[2], Ankita Gupte[2] and Carl Walkley[2,3]
[1]Walter and Eliza Hall Institute, Parkville, Victoria, 3052, Australia
[2]Hudson Institute of Medical Research, Clayton, Victoria, 3168, Australia
[3]Department of Molecular and Translational Sciences, Monash University, Clayton, Victoria, 3168, Australia.

The ability to detect double-stranded (ds)RNA as a signature of viral infection is an evolutionarily conserved feature of innate immunity. However, animal genomes also express long dsRNA molecules capable of activating immune sensors. To avoid harmful inflammation, cells carefully control dsRNA levels, localisation and its exposure to sensors. The RNA editing enzyme, ADAR1, directly modifies dsRNA by converting adenosine to inosine (A-to-I editing), which suppresses dsRNA sensor activation to prevent autoinflammation. Using loss of editing by ADAR1 as a model and genome-wide CRISPR screening, we identified new cellular regulators of dsRNA (*Science Immunology*, 2024). We identified a new function for the cyclin-dependent kinase, CDK13, in dsRNA sensing. CDK13 is a regulator of transcription elongation, and we hypothesise that loss of CDK13 reduces the dsRNA content of cells and/or results in aberrant proteins or RNAs that suppress dsRNA sensing, allowing a cell to tolerate loss of A-to-I RNA editing. Understanding new mechanisms of dsRNA control present new opportunities to manipulate the immune response to RNA, of relevance to autoinflammatory disease and cancer immunotherapy.

P22 hnRNPM and ADAR1 Cooperate to Limit Endogenous dsRNA Access to MDA5

C. Gonzalez-Figueroa[1#], Y. Zhang[1#], T.H. Nguyen[1], J.H. Bahn[1], R. Varada[1], M. Dunlap[2], G. Bobkov[2], A. Khanbabaei[1], C. Cheng[2], X. Xiao[1]
[1]Department of Integrative Biology and Physiology, UCLA, California, USA, 90095
[2]Department of Molecular and Human Genetics, Baylor College of Medicine, USA, 77054

To prevent inappropriate innate immune activation, cells must limit access of endogenous double-stranded RNAs (dsRNAs) to cytosolic sensors such as MDA5. Although the RNA-binding protein hnRNPM suppresses interferon responses, the endogenous RNAs that engage MDA5 after hnRNPM knockdown have remained unclear. Here, we directly map the MDA5-associated RNA repertoire in hnRNPM-knockdown cells using crosslinking and immunoprecipitation (CLIP). We show that the interferon response in this setting is mediated primarily through the MDA5-MAVS axis and accompanied by cytoplasmic dsRNA accumulation. MDA5-bound RNAs are enriched for Alu-containing duplexes, with retained introns representing a major source of intronic ligands. We also identify cytoplasmic, MDA5-associated NEAT1 and candidate NEAT1-containing intermolecular RNA pairings. In parallel, RNA editing induction accompanies hnRNPM knockdown, yet the dsRNAs recovered by MDA5 are not the most highly edited species. Combined hnRNPM and ADAR1 depletion reveals an additional, compositionally distinct reservoir of MDA5-associated dsRNAs, consistent with editing-dependent masking of a subset of endogenous ligands. Together, these findings indicate that RNA processing and RNA editing cooperate to limit exposure of endogenous structured RNAs to cytosolic innate immune sensing.

P23 Loss of RMND1 Disrupts Mitochondrial RNA Processing and Triggers Type I Interferon Signalling

Yan de Souza Angelo,[1] Alexandre Pierga[1], Yanick J. Crow[1,2], Alice Lepelley[1]
[1] Université Paris Cité, Institut Imagine, INSERM UMR 1163, Paris, France
[2] MRC Human Genetics Unit, Institute of Genetics and Cancer, University of Edinburgh, Edinburgh, UK

RMND1 is an inner mitochondrial membrane protein involved in mitochondrial ribosome assembly. Mutations in RMND1 cause a mitochondrial cytopathy presenting a phenotypic overlap with certain type I interferonopathies. Thus, we hypothesised that RMND1 dysfunction might result in aberrant innate immune signalling. Here we show that RMND1 deficiency is accompanied by an activation of type I interferon signalling, induced by the sensing of mitochondrial nucleic acids through both MDA5/MAVS and STING. Loss of RMND1 results in a relocalization of mitochondrial DNA and the accumulation of mitochondrial double-stranded RNA within enlarged mitochondrial structures, leading to their release into the cytosol. Notably, the accumulation of non-coding and unprocessed mitochondrial transcripts, including ribosomal mitochondrial RNA *MT-RNR2*, suggests a previously unrecognised role of RMND1 in mitochondrial RNA processing.

P24 SALDOR-Seq: a novel workflow for full-length sequencing of long dsRNA

F. Bender[1], S. Dudek[1], M. Hega[1], K. Maser[1], A. L. Awaragi[1], B. Putschli[1], J. Wieseler[2], R. Behrendt[1], B. M. Kümmerer[2,3], G. Hartmann[1,3], E. Bartok[4], T. Zillinger[1]
[1]University Hospital Bonn, Institute of Clinical Chemistry and Clinical Pharmacology, Germany
[2]University Hospital Bonn, Institute of Virology, Germany
[3]German Centre for Infection Research, Partner Site Bonn-Cologne, Germany
[4]University Hospital Bonn, Institute for Experimental Hematology and Transfusion Medicine, Germany

Long double-stranded RNA (dsRNA) is a hallmark of viral replication and a prototypical pathogen-associated molecular pattern, yet it remains difficult to sequence because of its relative rarity, thermostability and poor compatibility with conventional sequencing workflows. We introduce Size-Accurate Long Double-stranded RNA-Sequencing (SALDOR-Seq), a dual-selection approach that recovers intact dsRNA and preserves native length. SALDOR-Seq achieves high specificity and ~15,000-fold higher dsRNA recovery than short-read workflows, enabling full-length sequencing of viral dsRNA genomes, resolving replication-associated dsRNA intermediates, identification of A-to-I editing within dsRNA, and, through metagenomic analysis, screening for occult virus infections. SALDOR-Seq therefore provides a dedicated method for high-confidence dsRNA profiling in complex samples.

P25 A Fluorescent RNA Imaging Platform for Visualizing ADAR1 Z-Domain Interactions in Cells

Soyoung Park[1]
[1]Earth-Life Science Institute (ELSI), Institute of Science Tokyo, Tokyo, 152-8550, Japan

Proteins containing Z-nucleic acid-binding domains, including ADAR1 and ZBP1, play critical roles in innate immunity and antiviral responses through recognition of noncanonical left-handed nucleic acid structures. However, direct visualization of Z-domain–RNA interactions in living cells remains challenging. Here, we report a fluorogenic RNA imaging platform based on the molecular rotor fluorescent nucleoside 2′-*O*-methylated thiophene-expanded uridine (2′-*O*Me-$^{\mathbf{Thex}}$**U**). Incorporation of 2′-*O*Me-$^{\mathbf{Thex}}$**U** into fully 2′-*O*-methylated RNA generated a fluorescent RNA analogue suitable for cellular imaging while retaining binding to both the ADAR1 Zα domain and the Zα–linker–Zβ module. Live-cell imaging demonstrated intracellular colocalization between the fluorescent RNA and mCherry-tagged ADAR1 Zα or Zα–linker–Zβ domains, and fluorescence derived from 2′-*O*Me-$^{\mathbf{Thex}}$**U**-labeled RNA was also detected in RAW 264.7 macrophages expressing endogenous ADAR1. These findings establish 2′-*O*Me-$^{\mathbf{Thex}}$**U**-labeled RNA as a fluorescent RNA analogue for monitoring ADAR1 Z-domain interactions in living cells and provide a versatile platform for investigating RNA-mediated cellular recognition processes.

P26 Non-Invasive Real-Time Tracking of Immunogenic RNA Emergence During Influenza Virus Infection

E. Horton,[1] J. Ranum,[1] S. Baker,[1] M. Ledworth,[1] and A. Mehle[1]
[1]Department of Medical Microbiology, University of Wisconsin-Madison

Influenza virus infection triggers innate immune responses that antagonise viral replication. This is largely driven by RIG-I agonists generated during replication of the viral genome, particularly deletion-containing viral genomes (DelVGs). DelVGs are aberrant replication products that retain sequences necessary for replication, transcription, and packaging, but no longer encode essential viral proteins. Consequently, DelVGs parasitise wild-type virus replication. The transcriptional landscape of infected cells driving DelVG production remains unknown. Whether innate-immune primed cells instigate DelVG production is of key interest, as DelVG-containing virions are being developed as antiviral therapeutics. We developed a novel system for non-invasive, real-time measurement of DelVG production in living cells. We built on our recent discovery that DelVGs express a new class of cryptic viral proteins, DelVG-encoded proteins (DPRs), and used these as a readout for DelVG formation. We engineered viruses to express GFP11 only when a DPR is generated. These reporter viruses were inoculated onto cells expressing GFP1-10 resulting in bimolecular fluorescence complementation in cells where DelVGs were formed. Quantification of DPR accumulation over serial passaging revealed increasing DelVG burden in the population, while visualising viral plaques revealed *de novo* DPR emergence from a clone. We have generated a system to systematically track and analyse the interactions between the innate immune response and influenza virus that contribute to the formation and enrichment of immunogenic RNAs.

P27 Structure-guided discovery of ubiquitin ligase control in antiviral nucleic acid sensing

I. Syed[1], S. Chen[2] and D. Gonçalves-Carneiro[1]
[1]Department of Infectious Disease, Faculty of Medicine, Imperial College London. London, United Kingdom

Ubiquitin ligases are critical regulators of antiviral nucleic acid sensing, yet many remain undiscovered because ligase-substrate interactions are transient and often coupled to substrate degradation. To systematically identify these regulators, we used AlphaFold to screen for interactions between the entire ubiquitin ligase repertoire against known human nucleic acid sensors. This proteome-scale approach recovered established immune regulatory pairs, including cGAS-SPSB3 and RIG-I-Riplet. We then prioritized uncharacterized predictions involving the OAS-RNase L pathway, where ubiquitin ligase regulation remains poorly defined. Among OAS1-interacting candidates, we identified TRIM58 as a previously unrecognized regulator of OAS1. TRIM58 bound OAS1, promoted its ubiquitination and drove proteasome-dependent degradation. Upon interferon signalling, OAS1 was selectively degraded, whereas OAS2 and OAS3 remained comparatively stable, revealing specific post-translational control within the OAS family. Loss of TRIM58 stabilized OAS1 and enhanced RNase L activation, demonstrating that TRIM58 acts as a brake on the OAS1-RNase L antiviral effector pathway. Together, our work establishes structure-guided prediction as a powerful strategy to uncover transient ubiquitin-dependent immune regulatory interactions and reveals a new mechanism controlling the intensity of RNase L activation during antiviral nucleic acid sensing.

P28 OASL regulation of NA sensing pathways

T. S. Franz[1] and C. C. de Oliveira Mann[2]
[1,2] Department of Bioscience, School of Natural Sciences, Technical University of Munich (TUM)

The OAS-RNaseL pathway plays a critical role in virus restriction via dsRNA sensing during infection and in immune homeostasis, but its complex regulation remains poorly understood. This is especially true for OASL, a member of the OAS protein family that possess two ubiquitin-like domains but is catalytically inactive. OASL has however been described as a modulator of other NA sensing pathways in which it can exert pro- and antiviral activity. We thus focus on OASL and its distinct interactions with NA ligands, with an emphasis on the underlying biochemical mechanisms. To achieve this, we study OASL and its individual domains via biochemical assays and cryo-EM to elucidate their effects on NA sensing and interactions with other NA sensing proteins as well as to characterize their ability to oligomerize and undergo liquid-liquid phase separation. Our preliminary data has provided insights into OASL structure and its antiviral function, stressing once again the importance of this protein in NA sensing modulation. These findings can then be placed within the broader context of innate immune pathways to provide a better understanding of their complex regulatory mechanisms.

P29 NSUN2 Shapes the Innate Immune Program of A549

P. Schult[1], K. Paeschke[1]
[1] Institute of Clinical Chemistry and Clinical Pharmacology, University Hospital Bonn, 53127 Bonn, Germany.

RNA is not merely a template for translation but fulfills diverse regulatory roles heavily exploited by viruses. RNA functionality is enhanced via post-transcriptional modifications (PTMs) – the “epitranscriptome” – which modulate pro- and antiviral processes. A common PTM is 5-methylcytosine (m5C), primarily deposited by host methyltransferases like NSUN2. However, mechanisms of target selection and functional impact during viral infection remain enigmatic. We hypothesized that RNA secondary structures like G-quadruplexes (G4), enriched in viral genomes, might influence m5C deposition. Thus, we investigated how m5C affects the Yellow Fever Virus (YFV) life cycle and how G4s modulate NSUN2-mediated methylation by analyzing the impact of NSUN2 knockout and inhibition. Additionally, we profiled host and viral transcriptomes to examine global m5C changes without NSUN2 activity. Immune-related transcripts dysregulated upon knock-out were used to infer regulatory effects of m5C, and predicted G4s were matched to mapped m5C sites. Our results indicate a proviral role for NSUN2, as genetic and chemical inhibition reduced early viral replication. Bisulfite sequencing suggests NSUN2-dependent m5C deposition adjacent to viral G4s. Furthermore, NSUN2 modulates host immunity, as knock-out cells displayed shifted immune responses during infection. These findings underscore the multifaceted role of NSUN2 in viral replication and immune regulation.

P30 Innate immune sensing of human influenza A virus infection: challenging established paradigms

Xiaohui Wang[1], Ralph Patrick[1], Max Salmons[1], Xichun Li[1], Tyron Esposito[1], Brittany Hill[1], Di Xia[1], Jennifer Stow[1], Kirsty Short[2], Kate Schroder[1], Christian Nefzger[1], Larisa I Labzin[1]
[1]Institute for Molecular Bioscience (IMB), The University of Queensland, Brisbane, QLD, Australia, 4072
[2]School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD, Australia, 4072

Effective innate immune sensing protects against influenza A virus (IAV), although dysregulated macrophage responses can drive immunopathology. Current understanding of IAV sensing in human macrophages is largely extrapolated from murine immune cells or human epithelial cell lines, despite important species- and cell-specific difference
Here, we define how primary human monocyte-derived macrophages sense H1N1 Auck/09 and H3N2 SA/19 IAV. Using pharmacological inhibition and CRISPR/Cas9-mediated depletion, we show that RIG-I but not TLR7/8 drive early cytokine responses to replicating IAV. Unexpectedly, RIG-I, but not MAVS, is dispensable for antiviral responses at 24 hours. Instead, an alternative MAVS-dependent pathway compensates for RIG-I loss and sustains antiviral cytokine production. These findings revise current models of IAV sensing in human macrophages and reveal pathways for modulating antiviral and inflammatory responses.

P31 dsRNA Shielding by Viral Proteins Enhances Exogenous RNA Expression

Youngran Park[1,2], Junyoung Park[1,2], Ji Yoon Han[2], Sun-min Ju[1,2], Minseok Jung[1,2] Hyeshik Chang[1,2], Soo Seok Hwang[1,2], Jong-Seo Kim[1,2], V. Narry Kim[1,2,*]
[1] Center for RNA Research, Institute for Basic Science,
[2] School of Biological Sciences, Seoul National University, Seoul 08826, Republic of Korea
* To whom correspondence should be addressed. Email: narrykim@snu.ac.kr

Exogenous RNA platforms, including in vitro–transcribed (IVT) mRNA and self-amplifying RNA (saRNA), are limited by dsRNA-mediated innate immune activation. Here, we demonstrate that the viral dsRNA-binding protein dsXP enhances exogenous RNA expression by shielding dsRNA from innate immune sensing. dsXP selectively enhances protein expression from exogenous, but not endogenous, mRNA. This effect is abolished upon removal of dsRNA byproducts, demonstrating its dsRNA dependence. Mechanistically, dsXP binds dsRNA and limits recognition by cytosolic pattern-recognition receptors, thereby delaying innate immune activation. This effect is particularly pronounced in saRNA, which generates dsRNA replication intermediates. Co-delivery of dsXP enhances saRNA-driven protein expression by more than 50-fold across multiple immune-competent cell lines. *In vivo*, dsXP increases both the magnitude and duration of saRNA-mediated protein expression. Together, these findings establish viral protein-mediated dsRNA shielding as a strategy for robust and sustained exogenous RNA expression.

P32 Anti-fibrotic Effects of Hairpin RNA, a RIG-I Ligand, in Hepatic Stellate Cells

F. Sakurai,[1,2] I. Ishigami,[2] K. Nakagawa,[2] Y. Imamura,[1] A. Shimada,[1] A. Kawase, H.[1] Mizuguchi.[2,3]
[1]Faculty of Pharmacy, Kindai University, Higashi-Osaka, 577-8502, Japan
[2]Graduate School of Pharmaceutical Sciences, [3]Global Center for Medical Engineering and Informatics, The University of Osaka, Osaka, 565-0871, Japan

In fibrosis, activated fibroblasts (myofibroblasts) produce excessive amounts of extracellular matrices, leading to severe tissue damages and disfunction. We have previously found that reovirus double-stranded RNA genome and polyI:C, which are a RIG-I ligand, exhibited potent anti-fibrotic effects in activated hepatic stellate cells. In this study, we examined the anti-fibrotic effects of RIG-I ligand single-stranded hairpin RNA (hpRNA) with approximately 90 bases long. We found that hpRNA reduced the expression of several fibrosis marker genes approximately 100-fold more efficiently than conventional siRNAs without exhibiting apparent cytotoxicity in the human hepatic stellate cell line LX-2 cells. Functions of activated LX-2 cells, including lipid droplet accumulation, were significantly restored by hpRNA. The anti-fibrotic effects of hpRNA were cancelled when the 5'-triphosphate group was removed from hpRNA or when RIG-I was knocked down. These findings suggest that RIG-I plays a critical role in the anti-fibrotic effects of hpRNA.

P33 A CREB3-Dependent Golgi Stress Response Sustains Antiviral Signaling During HSV-1 Infection

Toyodome R[1], Ori D[1,2], Kano N[1] and Kawai T[1]
[1] Laboratory of Molecular Immunobiology, Division of Biological Science, Graduate School of Science and Technology, Nara Institute of Science and Technology (NAIST), 8916-5 Takayama-cho, Ikoma, Nara 630-0192, Japan
[2] Department of Immunology, Faculty of Medicine, Kagawa University, 1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0793, Japan

The Golgi apparatus functions as a signaling platform for innate immune responses. Disruption of Golgi homeostasis induces Golgi stress, which is counteracted by the Golgi Stress Response (GSR), although its role in antiviral immunity remains unclear. Here, we investigated the GSR during herpes simplex virus type 1 (HSV-1) infection. HSV-1 infection induced marked Golgi fragmentation in mouse embryonic fibroblasts. Transcriptome analysis revealed suppression of Golgi-associated gene programs following HSV-1 infection. HSV-1 induced cleavage and nuclear translocation of the Golgi stress-responsive transcription factor CREB3. CREB3 deficiency impaired Ifnb1 induction following HSV-1 infection. In addition, CREB3-deficient cells exhibited sustained Golgi fragmentation, defective recovery of Golgi-related gene expression and enhanced HSV-1 replication. These findings identify the GSR as an adaptive host pathway that preserves Golgi integrity to sustain antiviral signaling during HSV-1 infection.

P34 Innate Sensor Responses Set a Supra-Unitary Threshold for Viruses to Successfully Infect Cells

Kojiro Mukai[1], Dongya Jia[2], Grégoire Altan-Bonnet[2], Nihal Altan-Bonnet[1]
[1]Laboratory of Host-Pathogen Dynamics, National Heart Lung and Blood Institute, National Institutes of Health, Bethesda, MD, USA
[2]Laboratory of ImmunoDynamics, National Cancer Institute, Bethesda, MD, USA

Cells resist viral infection by triggering rapid and sensitive innate immune responses, initiated by cytoplasmic viral sensor proteins (CyVSP), RIG-I, MDA5, and PKR. Our single-cell analysis revealed that viral replication requires a supra-unitary viral genome input, significantly more than one, which we defined as the virion input threshold for replication (VITREP). This threshold varies depending on viruses and cell types, independently of viral protein synthesis. Through mathematical modeling and experimental testing, we uncovered that supra-unitary viral genome quantities activate negative feedback regulators (NFR) of CyVSP including Death-Associated Protein Kinase 1 (DAPK1), Rio Kinase 3 (RIOK3) and Protein Phosphatase 2 Regulatory Subunit Alpha (PPP2R2A), that suppress innate immune responses and allow replication. We validated key predictions of our model, namely downregulating NFR or coinfecting cells, reduced or promoted replication respectively. Together, these findings reveal a fundamental mechanism governing viral infectivity and tissue tropism.

P35 Intratumoral IL-2 and RIG-I immunotherapy increases survival in melanoma tumor bearing mice

A. Heilmann[1], D. A. Rodriguez[1], W. Liu[1], A. A. Pandyra[1], and G. Hartmann[1].
[1] Institute for Clinical Chemistry and Clinical Pharmacology, University Hospital Bonn

Interleukin-2 (IL-2) is a well-established cytokine used in cancer immunotherapy, including high-dose systemic administration. However, this approach is limited by severe toxicity and modest efficacy across many tumor types. While intratumoral delivery reduces systemic side effects, its therapeutic impact remains constrained in immunologically “cold” tumors due to poor immune infiltration and active immunosuppression. In addition, IL-2 can promote regulatory T cell (Treg) expansion, further limiting antitumor responses. Activation of retinoic acid-inducible gene I (RIG-I) has emerged as a strategy to convert “cold” tumors into “hot” tumors by inducing type I interferon responses and enhancing T-cell recruitment. Here, we combined cyclophosphamide-mediated lymphodepletion with intratumoral IL-2 and a RIG-I agonist to overcome these barriers. Monotherapy with IL-2 or RIG-I only showed a small improvement on survival compared with untreated controls. In contrast, the combination therapy significantly reduced tumor growth and prolonged survival. These findings demonstrate that coordinated Treg depletion, localized IL-2 signaling, and RIG-I–mediated innate immune activation can overcome immunosuppressive tumor microenvironments.

P36 MyD88 is a Pattern Recognition Receptor for Cytosolic dsDNA

P Fontana[1,2,6,7], AB Tufan[1,2,6] ,S Vora [1,2], J Paulo[3,4],Oh H[5], S Gygi[3], D Knipe[5], H Wu[1,2,*]
[1]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston MA
[2]Program in Cellular and Molecular Medicine, Boston Children’s Hospital, Boston MA
[3]Department of Cell Biology, Harvard Medical School, Boston, Massachusetts.
[4]Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, Massachusetts.
[5]Department of Microbiology, Blavatnik Institute, Harvard Medical School, Boston, MA
[6]These authors contributed equally

MyD88 is a universal adaptor connecting TLRs and IL-1Rs via its TIR domain, activating inflammatory signaling through its death domain (DD). To elucidate its platform mechanisms, we report cryo-EM structures of MyD88TIR and its cancer-specific L252P mutant, forming ring and spiral assemblies, respectively. Both are antiparallel double-filaments utilizing canonical intra-strand and unique inter-strand TIR interfaces, unilaterally aligning MyD88DDs to facilitate self-oligomerization. Surprisingly, these assemblies encapsulate dsDNA on their inner concave surfaces. *In vitro* and cellular studies confirm MyD88 directly binds dsDNA, driving inflammatory cytokine expression during viral infection or dsDNA transfection. Furthermore, a structure-guided mutant disrupting dsDNA binding specifically abolishes DNA sensing and punctum formation while preserving normal TLR signaling. Finally, multi-omics reveal MyD88 critically induces TLR9-independent gene expression upon dsDNA sensing, and that nearly half of all upregulated cytokine expression.

P37 Regulation of Influenza A virus (IAV) induced inflammation by PYHIN1 in epithelial cells

A. Swirk[1] and A.G. Bowie[1]
[1]Trinity College Dublin, Ireland

Human airway epithelial cells (HAECs) are the primary targets of respiratory RNA viruses. However, compared to myeloid cells, we know a lot less about the innate immune response of HAECs to viruses. Furthermore, the role of pyrin and HIN domain (PYHIN) proteins in HAECs during RNA virus infection is unclear. Although expressed in HAECs, PYHIN1 is the least characterised out of the five known human PYHINs. Here, I found that PYHIN1 was required for restricting IAV infection of HAECs. Following infection with IAV, PYHIN1 protein expression was increased and the protein relocalised from the cytoplasm to the nucleus (the site of IAV replication). PYHIN1 knockout in HAECs increased viral replication and viral gene expression, underscoring the importance of this protein in the antiviral response to IAV. The absence of PYHIN1 in HAECs also disrupted virus-stimulated IFN-λ induction and secretion. Since type III IFN has recently been identified as a critical factor in the antiviral response of epithelial cells, a role for PYHIN1 in type III IFN induction may contribute to the mechanism by which PYHIN1 restricts IAV. In addition, RNA-seq data of *PYHIN1*$^{-/-}$ HAECs revealed a shift in the host antiviral response towards a proviral cell state. Ongoing experiments are exploring this and other mechanisms that would account for this novel anti-viral role of PYHIN1.

P38 IFI16 regulation of cytokine induced responses in human keratinocytes

M. Baran[1] and A.G. Bowie[1]
[1]School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland

IFI16, a Pyrin and HIN domain-containing protein (PYHIN), functions as an intracellular nucleic acid sensor involved in both dsDNA-induced STING activation and the restriction of DNA and RNA viruses. In addition, a more downstream role in transcriptional regulation of immune genes has emerged, and here we sought to better understand this role of IFI16. Human keratinocytes are characterised by high IFI16 expression, and its association with psoriasis has been reported. Stimulation of IFI16 wild type and knock out (*IFI16*$^{-/-}$) HaCaT cells with psoriasis-associated cytokine cocktail (TNFα, IL17, IFNγ and IL22) resulted in impaired expression of psoriasis-related genes such as CCL20 and CXCL2, as measured by qPCR and ELISA. Single-cytokine stimulation experiments identified TNF as the primary driver of the observed IFI16-dependent responses. Cytosolic signalling pathways leading to NF-κB and MAPK activation were not impaired in *IFI16*$^{-/-}$ HaCaTs, suggesting a gene promoter-proximal role for IFI16. RNAseq performed in TNFα-stimulated wild type and IFI16$^{-/-}$ HaCaTs, revealed a subset of early-induced genes strongly dependent on IFI16. Analysis of regulatory elements within the promoters of these affected genes demonstrated a significant enrichment of AP-1 regulatory elements.
Current work focusses on further validation of the RNA-seq findings and further investigation of the relationship between IFI16 and AP-1.

P39 Regulation of Dendritic Cell Functions by PYHIN Proteins

S. Hayes,[1] A.G. Bowie[1] and C.P. McEntee[1]
[1]School of Biochemistry and Immunology, Trinity College Dublin, Ireland

Murine PYHIN proteins include AIM2 and IFI204, an ortholog and functional homolog of human AIM2 and IFI16 respectively. These proteins form the AIM2-like receptor (ALR) family which sense intracellular DNA. However, the role of PYHIN proteins in dendritic cells (DCs), which link innate and adaptive immunity, is unknown. We show that in response to transfected DNA, but not other stimuli, that DCs from *Aim2*$^{-/-}$ and ALR$^{-/-}$ mice, but not *Ifi204*$^{-/-}$, exhibit enhanced pro-inflammatory and type I IFN responses compared to WT mice. Additionally, autocrine type I IFN signalling causes increased phosphorylation of STAT1 in *Aim2*$^{-/-}$ DCs which promotes enhanced co-stimulatory molecule expression to allow *Aim2*$^{-/-}$ DCs to drive enhanced cytotoxic and helper T cell responses. Mechanistically, the absence of AIM2 causes increased activation of the cGAS-STING DNA sensing pathway through increased phosphorylation of TBK1, IRF3 and NFκB. Interestingly, while STING inhibition completely ablates DNA-induced pro-inflammatory cytokine production, it only partially reduces the IFN-I response. This dichotomy led us to discover that DNA-PK, primarily involved in the DNA damage response, also contributes to DNA induced, IRF3-dependent IFN-I responses. Consequently, inhibiting DNA-PK fully reduces the increased IFN-I response and IRF3 phosphorylation we see in *Aim2*$^{-/-}$ and ALR$^{-/-}$ DCs. This work places PYHINs, particularly AIM2, as negative regulators of the DNA-induced responses in DCs and provides evidence of how targeting of AIM2 could promote effector cell responses.

P40 Exploring the Role of PYHIN Proteins in Adjuvant-Induced Immunity

J. Davis[1], C. McEntee[1]
[1]The School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland

Vaccine adjuvant-induced damage-associated molecular pattern (DAMP) release largely underpins their efficacy. How this release is regulated to shape innate sensing and adaptive responses remains unclear. We previously showed that AIM2$^{-/-}$ and total PYHIN knockout (ALR$^{-/-}$) bone marrow-derived dendritic cells (BMDCs) exhibit heightened cGAS STING-dependent responses to transfected DNA, suggesting PYHINs negatively regulate DNA-driven immunity. Here, we explored whether this phenotype is recapitulated using endogenous mitochondrial DNA, a well-characterised DAMP released following chitosan-based adjuvant treatment. AIM2$^{-/-}$ murine BMDCs secreted modestly enhanced IFN-β and CXCL10 compared with WT cells following chitosan stimulation. TBK1 phosphorylation was elevated, consistent with enhanced cGAS–STING activation. AIM2$^{-/-}$ BMDCs also displayed characteristics of enhanced maturation, including increased CD86 and MHC-I expression. OT-I/OT-II T cells co-cultured in the presence of chitosan-treated AIM2$^{-/-}$ BMDCs appeared to express greater levels of the activation marker CD69. These exploratory findings highlight variability in adjuvant-induced DAMP release and necessitate normalisation of responses to endogenous cytosolic DNA levels. Additionally, they point toward a role for PYHINs in regulating DNA sensing pathways that shape adjuvant immunogenicity.

P41 TRAP Loss-of-function and Type I Interferon Signalling in SPENCD

C. C. Y. Wong[1]
[1]MRC Human Genetics Unit, Institute of Genetics and Cancer, University of Edinburgh, Edinburgh, United Kingdom

Spondyloenchondrodysplasia (SPENCD) is an immuno-osseus disorder caused by loss-of-function mutations in *ACP5,* resulting in deficiency of tartrate resistant acid phosphatase (TRAP). Patients with SPENCD demonstrate persistent upregulation of type I interferon (IFN) signalling. However, despite the description of an *Acp5* KO mouse in 1996, and detailed studies of the role of TRAP in bone and immune homeostasis, the link between TRAP deficiency and enhanced type I IFN signalling remains unclear.

In six patients with SPENCD we recorded a positive IFN score and extremely high levels of IFN alpha (IFNa) protein in blood and serum. These data confirm SPENCD as a type I interferonopathy. Based on gene and protein expression, we hypothesized that macrophages and dendritic cells likely represent the cell types contributing to this type I IFN signalling upregulation. To explore this possibility, we have derived *ACP5* KO macrophage cell lines in which to study the response to stimulation with natural and artificial ligands of endosomal Toll-like receptors and the cGAS-STING pathway. We have also generated data through the ex vivo stimulation of patient-derived cells.

Here I will summarise the results of our experiments to date.

P042 Loss of the SUMO protease SENP7 dysregulates nucleic acid immunity

S. Bhattacharjee,[1] A. Eicher[2], N. Rieber[3], F. Hauck[2], M. Wagner[3], J. Hauer[3], J. Klughammer[2], C. Wolf[1], M.A. Lee-Kirsch[1]
[1] Technische Universität Dresden, Germany, [2] Ludwig-Maximilians-Universität München, Germany, [3]Technical University of Munich, Germany

Type I interferonopathies have uncovered fundamental mechanisms of nucleic acid immunity. Here, we identify loss of the SUMO-specific protease SENP7 as a novel cause of immune dysregulation associated with excessive type I IFN activation. We studied a child with autoinflammation, agammaglobulinemia, and a strong IFN signature carrying a homozygous nonsense variant in *SENP7*. Patient fibroblasts lacked detectable SENP7 protein and exhibited globally increased SUMOylation, consistent with defective deSUMOylation. Whole-blood stimulation and activation of cGAS and RIG-I pathways in patient fibroblasts induced exaggerated IFN responses and delayed signal termination. Cycloheximide-chase experiments demonstrated impaired protein turnover, suggesting prolonged stability of innate immune signaling components in the absence of SENP7. Single-cell transcriptomic profiling of peripheral blood mononuclear cells demonstrated broad interferon activation across innate immune populations and impaired B-cell maturation. Together, these findings identify SENP7 as a previously unrecognized regulator of nucleic acid immunity. Our data suggest that defective deSUMOylation promotes sustained activation of nucleic acid sensing pathways through impaired signaling termination and protein turnover, resulting in chronic IFN production and immune dysregulation.

P43 Characterization of a novel invertebrate DNA sensor with ancient roots in bacterial immune defense

L. Drees[1], S. Naskar[1], F. Kagan[1], I. Bickmeyer[1], J. Rink[1]
[1]Max Planck Institute for Multidisciplinary Sciences, Goettingen, Germany

The planarian flatworm *Schmidtea mediterranea* can regenerate its entire body from small tissue pieces, a process facilitated by an abundant population of adult pluripotent stem cells. While regeneration in *S. mediterranea* is extensively studied, its innate immune system remains poorly understood. In an ongoing effort to identify components of the planarian innate immune system, we discovered a novel DNA-sensing protein.
This protein is highly conserved across Protostomia but absent in Deuterostomes. By RNAi-mediated knockdown in planarians and CRISPR/Cas9 mediated knockout in Drosophila, together with *in vivo* infection experiments, we demonstrate that it is a central regulator of the innate immune response. Interestingly, this protein evolved from a bacterial immune effector Purine Nucleoside Phosphorylase (PNP), which halts phage infection by depleting cellular ATP in infected cells. While bacterial PNP is activated by upstream phage-sensing proteins, our biochemical assays and structural analyses revealed that catalytic activity of the planarian protein is directly regulated by DNA binding. We therefore propose that this protein represents a novel DNA sensor with a conserved function in invertebrate innate immunity.

P44 The cGAS N-Terminus Enables Full cGAS Activation by Short dsDNA

T. Schorpp,[1] A. Kirchhoff[1], C. Wallerath[1], C. Hunkler[1] and M. Schlee[1]
[1]Institute of Clinical Chemistry and Clinical Pharmacology, University Hospital Bonn, Bonn
The double stranded (ds) DNA receptor cGAS detects dsDNA of invading pathogens or endogenous dsDNA released during cell stress. cGAS is composed of a highly conserved dsDNA binding catalytic domain (CD) and a less conserved DNA binding N-terminal domain (NTD). Upon stimulation, the CD synthesizes the second messenger cGAMP initiating a signalling cascade leading to transcriptional type-I IFN gene and ISG induction. Human cGAS is activated by long (≥44bp) blunt ended dsDNA. dsDNA recognition by cGAS has been considered to be length but not sequence dependent so far and research of cGAS recognition motifs has been largely neglected.
Our lab has discovered an additional motif, short (≤20mer) Y-form DNA with unpaired guanosine overhangs (G-YSD). While the structure and DNA binding of the CD is well understood the function and DNA interaction of the poorly conserved cGAS NTD has not been well studied. Using THP-1 cells expressing different human cGAS derivates with altered NTDs, we found that the NTD is required for recognition of G-YSD and medium-length (44-65bp) dsDNA with blunt ends, while the recognition of very long dsDNA (≥150bp) is completely NTD independent. Further, we identified a minimal region within the NTD that is critical for recognition of G-YSD and medium-length dsDNA. Unexpectedly, we discovered that the recognition of G-YSD by the CD alone depends on the dsDNA sequence and that the NTD compensates this sequence dependence *in cellulo*. Our results demonstrate different activation modes of cGAS depending on DNA length and structure.

P45 Redox-Dependent Self-Tetramerization of Human cGAS Links Metabolic Stress to Innate Immune Activation

A. Yikilmazsoy,[1] J. Kamper[1], C. Jung[1], K. Lammens[1], V. Hornung[1] and K.P. Hopfner[1]
[1]Department of Biochemistry, Ludwig-Maximilians-Universität, Munich, Germany.

The cyclic GMP-AMP synthase (cGAS) acts as a primary cytosolic sentry of the innate immune system, recognizing foreign or misplaced double-stranded DNA to produce the second messenger cGAMP, which subsequently triggers downstream antiviral and inflammatory signaling via STING. The prevailing model holds that cGAS remains inactive until physical binding to DNA scaffolds drives its dimerization and activation. However, accumulating evidence suggests that cGAS is subject to additional layers of regulation beyond simple DNA binding, particularly in response to metabolic stress and cellular redox environments. In this study, we uncover a novel, DNA-independent regulatory mechanism demonstrating that human cGAS can pre-assemble into stable apo-tetramers through a redox-sensitive pathway. By combining single-particle cryo-electron microscopy, biophysical profiling, and species-specific functional assays, we show that oxidation drives disulfide bridge formation via Cysteine 199, creating a distinct structural state unique to human cGAS. Cellular investigations in differentiated THP-1 macrophages further reveal that this redox-primed state tunes downstream immune outputs such as IP-10 production. Together, our findings establish a paradigm where metabolic and oxidative stress directly interface with nucleic acid sensing, providing new insight into how innate immunity is coordinated with the broader cellular environment.

P46 Modulation of Cyclic GMP-AMP Synthase Activity by RNA and RNA-Binding Protein G3BP1

M. Aleksejczuk,[1] R. Dittmer[1,] T. Gutmann[2] and S. Alberti[1]
[1]Biotechnology Center, Center for Molecular and Cellular Bioengineering, Dresden University of Technology, 45/47 Tatzberg, Dresden, Germany
[2]Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstraße 108, Dresden, Germany

Stress granules are assemblies of RNAs and RNA-binding proteins, formed in response to different stress stimuli. The main event leading to their formation is the release of mRNA to the cytoplasm, and its subsequent binding by Ras GTPase-activating protein-binding proteins 1 and 2 (G3BP1/2). Recent studies indicate that cyclic GMP-AMP synthase (cGAS), protein which detects accumulation of pathological DNA in the cytoplasm, colocalizes with G3BP1 in cells and that its activity is significantly inhibited in G3BP1/2 knock-out cells. Here, using *in vitro* reconstitution assays, we measured interactions of stress granules' main components – RNA and G3BP1 – with cGAS. We determined that folded RNAs, like mRNA and tRNA, can inhibit cGAS activity and condensate formation. Furthermore, we show that the inhibition by mRNA can be reversed by G3BP1 sequestering away mRNA. Contrary to previous reports, we show using both *in vitro* reconstitution assays and cell-based assays that in absence of mRNA G3BP1 doesn't affect cGAS activity. Our research offers a framework for understanding the interactions of cGAS with stress granules and their importance in sustaining cGAS-dependent immune response.

P47 Structural Analysis of Innate Immune DNA Sensor – Chromatin Complexes

T. Kujirai[1,2], R. Pickering[3], T. Ito[1], C. Zierhut[3], and H. Kurumizaka[1,2,4,5]
[1]Institute for Quantitative Biosciences, The University of Tokyo, Tokyo, Japan
[2]RIKEN Center for Integrative Medical Sciences, RIKEN, Yokohama, Japan
[3]Institute of Cancer Research, London, United Kingdom
[4]Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan
[5]Graduate School of Science, The University of Tokyo, Tokyo, Japan

The innate immune system serves as a mechanism for responding to foreign DNA. Intracellular DNA sensors detect cytosolic DNA and activate subsequent signalling pathways, triggering antiviral responses. In eukaryotic cells, some DNA sensors are present not only in the cytoplasm but also within the nucleus. Nuclear DNA sensors must be inactivated to avoid autoreactivity against genomic self-DNA. The eukaryotic genomic DNA is packaged into chromatin, in which the nucleosome is the basic unit. We and others previously revealed that cGAS is inactivated by nucleosomes, suggesting that nucleosomes are a marker of self-DNA.

To obtain deeper insights into nuclear DNA sensors, we performed chromatin immunoprecipitation (ChIP)-mediated cryo-EM analyses of DNA sensors. In this method, the extracted chromatin from human cells is digested by micrococcal nuclease into mono-nucleosome units, and the DNA sensor is immunoprecipitated. Using this method, we visualized a cGAS structure in complex with chromatin in human cells. In addition, we found an interaction between the DNA sensor AIM2 and chromatin, and analyzed it by cryo-EM.

At this meeting, I will discuss the interaction between DNA sensors and chromatin.

P48 Ceramide retains STING in the endoplasmic reticulum to control activation of signaling

Jian Zhao[1,2], Emil A Thomsen[1,2], Marc R Duque[1,2], Line S Reinert[1,2], Maria João Amorim[3], Jacob G Mikkelsen[1,2], Søren R Paludan[1,21]
[1] Department of Biomedicine, Aarhus University, Denmark
[2] Center for Immunology of Viral Infections, Aarhus University, Denmark
[3] Universidade Católica Portuguesa, Católica Medical School, Católica Biomedical Research Centre, Lisbon, Portugal.

Regulation of innate immune responses is essential to avoid pathological inflammation. The cGAS-STING pathway is important for antiviral defense and tumor surveillance, but also contributes to senescence and autoinflammation. Trafficking of STING from the endoplasmic reticulum (ER) to the Golgi leads to activation of the pathway, but it remains poorly understood how STING activation is regulated on the ER. From a genome-wide CRISPR screen we identified Ceramide synthase 6 (CerS6) and its enzymatic product C16 ceramide to restrict STING activation and expression of interferon and inflammatory genes. CerS6 reduced STING oligomerization and trafficking to the Golgi, suggesting the activity to take place on the ER. Interestingly, C16 Ceramide interacted directly with STING in the resting state, but this association was decreased upon STING agonist treatment, correlating with trafficking and activation. Importantly, CerS6-deficient mice exhibited enhanced *Ifnb* expression after HSV-1 inoculation and increased resistance to brain infection, supporting a physiological role for CerS6-mediated suppression of cGAS-STING signaling *in vivo*. To further elucidate the underlying mechanism, we are currently developing structural models of the ceramide–STING interaction to molecularly define how C16 ceramide restrains STING activation

P49 Proteome-wide spatiotemporal Dissection of STING trafficking and signal transduction

J. Arnold[1], K.R. Balka[1], A. Voulgari[1] A.U. Lindner[1], and F. Meissner[1]
[1]Institute of Innate Immunity, University Hospital Bonn, Germany

The DNA sensor STING orchestrates antiviral immunity, inflammation, and autophagy which uniquely modulate the outcome of STING responses in different inflammatory contexts. However, the mechanisms that govern its distinct downstream signaling outputs remain incompletely understood. Here, we combine subcellular fractionation with high-resolution mass spectrometry-based spatial proteomics to systematically map the spatiotemporal remodeling of the human proteome during STING activation in THP-1 monocytes. With this approach we resolve the coordinated redistribution of thousands of proteins across cellular compartments and capture proteins that co-traffic with, converge on, or diverge from the STING pathway. To functionally dissect these trafficking networks, we further integrate spatial proteomics with loss-of-function STING variants and alternative STING ligands that selectively modulate interferon, inflammatory, or autophagic responses. Beyond recapitulating the dynamic redistribution of established STING-associated proteins, our analyses also identify previously unrecognized regulators linked to endosomal sorting, ESCRT machinery, and autophagy-related processes, including candidates associated with specific STING signaling branches. Together, our study establishes a systems-level framework connecting STING trafficking dynamics to downstream immune functions and provides a rich resource for identifying novel regulators of innate DNA sensing.

P50 Different Routes of STING Agonist Delivery Dictate Distinct Requirements for Signaling Molecules

R. Narita[1,2], S. R. Paludan[1,2]
[1] Department of Biomedicine, Aarhus University, Denmark
[2] Center for Immunology of Viral Infections, Aarhus University, Denmark

The cGAS–STING pathway is essential for detecting invading DNA pathogens. While intracellular cGAMP (i-cGAMP) directly activates STING, growing evidence supports that extracellular cGAMP (e-cGAMP) can also enter cells to trigger STING activation. However, whether the route of STING agonist delivery impacts the cellular responses and the molecular signaling machinery involved remains unclear. Here, we characterized STING activation induced by i- and e-cGAMP. Both delivery modes successfully activated STING, inducing type I interferons and inflammatory cytokines. Crucially, these different entry routes dictate distinct molecular mechanisms. While STING ER exit protein (STEEP) is essential for i-cGAMP-mediated STING activation, e-cGAMP stimulation does not require STEEP. Furthermore, TBK1, but not IKKε, is indispensable for STING activation by i-cGAMP, whereas TBK1 and IKKε play redundant roles upon e-cGAMP stimulation. Taken together, our results demonstrate that different routes engage distinct molecular mechanisms for STING activation. We are currently investigating the precise functional nature of these route-dependent cellular responses. Our discoveries provide new insights into the molecular mechanisms of STING activation, offering novel strategies for targeting the STING pathway in cancer immunotherapy and other STING-related diseases.

P51 Identification of genes that upon over-expression suppress STING-mediated inflammatory signalling

C. Stappenbelt[1], V. Pantaxi, A[1]. García López[1], W. Lutjeboer[1], M.J.C. Broekhuis[2], R. Wardenaar[2], M. Losito[2] and B.L.W. van de Kooij[1], F. Foijer[2], M.A.T.M. van Vugt[1].
[1] Department of Medical Oncology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
[2] European Research Institute for the Biology of Ageing, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

Despite the success of immunotherapy in some types of cancer, the majority of patients do not benefit. In particular, tumors characterised by high levels of genomic instability, including high-grade ovarian cancer or triple-negative breast cancer show minimal responses. One possible explanation is that tumors downregulate innate signalling, leading to decreased immune cell activation, a requirement for proper immune checkpoint response. For example, MYC amplification was previously described as a mechanism to suppress tumor cell-intrinsic immune responses. The aim of this project is to identify genes that regulate inflammatory signalling. To do so, THP-1 cells harbouring an interferon (IFN)-driven fluorescent reporter were engineered to express dCas9-VP64 to allow CRISPR-A-mediated gene activation. This system was validated using sgRNAs targeting MYC, which downregulated the IFN type I response. Subsequently, a genome-wide flow cytometry-based CRISPR activating screen was performed. This screen identified several genes that upon overexpression suppress interferon signalling, including *MED7, ABCC4, TMA7, OR2T12, TMEM131L, SETX, WNK1, VDR, KCTD19, LPAR6, LPIN1* and *TRAF2*. Validation of the top performing identified genes will be presented.

P52 In vivo deconvolution of STING-driven inflammation

M. Hega[1], P. Müller[1], A. Michel[1], D. Bejarano[2], A. Schlitzer[2] and R. Behrendt[1]
[1]Department for Nucleic Acid Immunity & Genome Defense, Institute for clinical Chemistry and clinical Pharmacology, Bonn, Germany
[2]Departmenf for Biology of Inflammation, Life & Medical Science Institute, Bonn, Germany

Chronic activation of the cGAS/STING pathway has been shown to drive type I interferonopathies, such as Aicardi-Goutières Syndrome (AGS). AGS can be caused by bi-allelic loss of function mutations in the 3' repair exonuclease 1 (TREX1) and also loss of Trex1 in mice drives lethal systemic autoimmunity. In mice, epistasis experiments established the cGAS-STING-IRF3-IFNAR axis as the driver of systemic inflammation. However, activation of STING simultaneous activates IRF3 and NFkB signalling, the contribution of the latter to cGAS driven immunopathology has not been evaluated. We found that inactivation of *Ifnb1* or *Ifnar1* genes delayed but not rescued lethal immunopathology in Trex1-deficient mice. In contrast to *Trex1*$^{-/-}$ mice, *Trex1*$^{-/-}$*Ifb1*$^{-/-}$ mice rapidly develop inflammation in lung and liver and only mild heart inflammation. A similar pattern developed later in life in *Trex1*$^{-/-}$*Ifnar1*$^{-/-}$ mice. Most importantly, the lung disease resembled observations made in STING gain-of-function mice, which develop lung inflammation independently of IRF3 and IFNAR1 signalling. Hence, we believe cGAS drives a distinct immunopathology in situations of compromised IFN signalling.

P53 Modulating Innate Immune Signaling Pathways in STING-Driven Inflammatory Diseases

Remzi Onur Eren[1,3§*], Simone Wolf[1*], Vagelis Rinotas[2], Merve Kavșur[1], Hailin Zhang[1], Marietta Armaka[2§], Manolis Pasparakis[1,3§]
[1]CECAD, University of Cologne, 50931 Cologne, Germany
[2]Biomedical Sciences Research Center 'Alexander Fleming', Vari, Greece
[3]Correspondance: Remzi Onur Eren and Manolis Pasparakis

TBK1 and IKKε are central mediators of type I IFN induction downstream of nearly all innate immune sensing pathways, making TBK1 an attractive therapeutic target in type I interferonopathies. Beyond their established roles in antiviral and IFN responses, TBK1 and IKKε also function as critical regulators of RIPK1-mediated cell death and inflammatory signaling. We previously demonstrated that simultaneous inhibition of TBK1 and IKKε induces both RIPK1-dependent and RIPK1-independent inflammatory responses, highlighting essential and partially redundant functions of these kinases in maintaining tissue homeostasis. Although concomitant inhibition of RIPK1 prevents the RIPK1-dependent inflammatory pathology triggered by loss of TBK1 and IKKε activity, it remained unclear whether combined inhibition of TBK1, IKKε, and RIPK1 would be sufficient to suppress STING-driven inflammatory diseases caused by defective nucleic acid homeostasis, particularly in settings characterized by both type I IFN-dependent and IFN-independent pathology. Here, we present our recent findings investigating how TBK1, IKKε, and RIPK1 kinase activities contribute to STING-driven inflammatory diseases and discuss the therapeutic potential of targeting these pathways in self-DNA-driven autoinflammation.

P54 Investigating the interplay between STING and cholesterol

Sebastián Montealegre[1,2], Melvin Le Bihan[1,2], Nicolas Manel[1,2]
[1] Institut Curie, INSERM U932, PSL Research University, Paris.
[2] Institut Curie, PSL Research University, Paris.

STING is a receptor protein for cyclic dinucleotides (CDNs) produced by bacteria and by the intracellular DNA sensor cGAS. STING is located in the endoplasmic reticulum, and following CDN binding, moves to the Golgi and recruits TBK1, leading to activation of IRF3 and expression of interferons. cGAS-independent (non-canonical) STING activation and functions have also been reported but are insufficiently understood.
Here, we characterize a non-canonical mechanism of interferon induction by STING. Using domain deletions and point mutations, we delineate amino acid determinants of this activation that are distinct from CDN-binding positions. We show that non-canonical interferon activation by STING requires TBK1 and IRF3, but no apparent trafficking to the Golgi compartment, in contrast to activation by CDN. Using functional annotation and mutagenesis, we observe that cholesterol metabolism is associated with the non-canonical activation of STING. Reporter assays and analysis of the STING pathway in primary cells indicate that cholesterol delivery can activate IRF3. Altogether, the results reveal a molecular basis for a non-canonical pathway of STING activation.

P55 Fatty acid desaturation controls STING activation via self-DNA Leakage

Toshio Kanno and Yusuke Endo[1]
[1]Kazusa DNA Research Institute 2-6-7 Kazusa-kamatari, Kisarazu, Chiba 292-0818, Japan

STING is a key innate immune sensor that recognizes pathogen-derived non-self DNA and induces I-IFN responses to initiate antiviral immunity. In recent years, STING has also been shown to contribute to antitumor immunity, leading to increasing interest in the development of STING-mediated therapeutics. However, excessive STING activation can cause severe autoinflammatory diseases, highlighting the need to understand the mechanisms that appropriately regulate STING activity.

We previously demonstrated that pharmacological inhibition or genetic deletion of stearoyl-CoA desaturase 2 (SCD2), a key enzyme involved in fatty acid desaturation, induces a STING-dependent antiviral response in $CD4^+$ T cells. Furthermore, administration of an SCD2 inhibitor markedly improved the survival of mice challenged with a lethal influenza virus infection, achieving an approximately 90% survival rate. Notably, SCD2 inhibition did not induce apparent autoinflammatory symptoms.

Interestingly, we found that STING activation was mediated by self-DNA derived from the nuclear genome. The accumulated DNA was enriched in retrotransposon-derived sequences. These genetic elements encode their own reverse transcriptase and are capable of genomic mobilization and amplification. Consistent with this finding, treatment with reverse transcriptase inhibitors significantly suppressed STING activation.

Collectively, these findings suggest that SCD2 inhibition induces the leakage of nuclear genome-derived DNA into the cytosol, thereby promoting fine-tuned STING activation.

P56 Nuclear-derived Extrachromosomal Circular DNA Fails to Trigger Productive cGAS-STING Signalling

H. Dushimyineza[1,2], J. Cai[2,3], M.T. Hayashi[2,4]
[1] Graduate School of Biostudies, Kyoto University; Kyoto, Japan
[2] IFOM-KU JRL, Graduate School of Medicine, Kyoto University; Kyoto, Japan
[3] TUM School of Life Sciences, Technical University of Munich; Munich, Germany
[4] Chromosome Instabilities Unit, IFOM ETS; Milan, Italy

The cGAS-STING pathway is a principal sensor of cytosolic DNA, yet whether nuclear-derived self-DNA productively engages this pathway remains unclear. Extrachromosomal circular DNA (eccDNA) accumulates in cancer and has been implicated in immune modulation; however, whether nuclear-derived eccDNA formed *de novo* within living cells is capable of activating cGAS-STING has not been directly examined. To address this, we developed a Cre-loxP-based eccDNA reporter coupled with an mRuby3-STING reporter to track eccDNA dynamics and STING activation in real time. Transfected naked DNA induced robust STING activation — marked by STING Accumulation Index (St-AI), pSTING phosphorylation, and interferon responses — whereas eccDNA-positive cells showed only marginal St-AI with no detectable pSTING or interferon induction. These results demonstrate that nuclear-derived eccDNA is insufficient to drive robust cGAS-STING signaling, suggesting that eccDNA possesses intrinsic properties limiting its immunostimulatory potential and providing a basis for understanding immune evasion in eccDNA-accumulating cancers.

P57 C9ORF72 safeguards against ageing by facilitating cytosolic DNA clearance via STING-mediated autophagy

Xiaofeng Qiu[1], Mengru Xie[2], Zhe Zhang[2] and Daxing Gao[1]
[1]State Key Laboratory of Immune Response and Immunotherapy, University of Science and Technology of China, Hefei 230027, Anhui, China.
[2]State Key Laboratory of Membrane Biology, Academy for Advanced Interdisciplinary Studies, School of Life Sciences, Peking University, Beijing, China

The cGAS-STING signaling pathway is an important mediator of chronic inflammation and functional decline during ageing, but how this pathway is restrained in ageing-associated senescence remains incompletely understood. Here we identify C9ORF72 as an ageing-associated regulator whose expression declines with age and in senescent cells. Genetic ablation of C9ORF72 induces cellular senescence and sensitizes cellular and murine models to DNA-induced senescence. Mechanistically, our data support a model in which C9ORF72 cooperates with LC3B and STING to promote autophagy-dependent routing and clearance of cytosolic DNA, thereby limiting persistent cGAS-STING inflammatory signaling. Conversely, C9ORF72 deficiency results in cytosolic DNA accumulation and cGAS-dependent senescence; cGAS ablation suppresses senescence despite persistent DNA accumulation, placing cGAS downstream of the DNA-clearance defect. We further identify theaflavin-3,3'-digallate (TFDG) as a candidate small-molecule modulator that binds C9ORF72 and enhances the LC3B-C9ORF72 interaction. TFDG treatment enhances cytosolic DNA clearance and ameliorates senescence and ageing-associated phenotypes in vitro and in vivo. These findings define C9ORF72 as a regulator of DNA-driven senescence and suggest that pharmacological modulation of this axis may provide a strategy to limit ageing-associated inflammatory decline.

P58 Doxorubicin drives cGAS sensing of mitochondrial DNA independently of STING

B. Ramos[1], J. Kohli[2], I. White[2], N. Zaman[2], D. Selwood[2] and G.J. Towers[1]
[1] Blizard Institute, Queen Mary University of London, UK.
[2] University College London, London, UK

Doxorubicin is a widely used anthracycline natural product that has remained a blockbuster anti-cancer chemotherapeutic since its clinical introduction in the late 1960s, owing to its broad efficacy across diverse malignancies. Although its activity is classically attributed to inducing DNA damage, its mechanisms of action extend to histone eviction and mitochondrial dysfunction, processes increasingly linked to activation of innate immune signalling. Here, we identify a cGAS-dependent, STING-independent interferon response driven by disruption of mitochondrial homeostasis. We show that doxorubicin induces collapse of the mitochondrial membrane potential ($\Delta\Psi m$) and promotes mitochondrial DNA release into the cytosol followed by cGAS-dependent interferon production. Inhibition of cyclophilin D, which regulates the mitochondrial permeability transition pore (mPTP), along with mitophagy induction or calcium chelation, suppresses IFN and ISG induction by doxorubicin, consistent with a role for mitochondrial DNA release. Notably, aclarubicin, an anthracycline incapable of inducing DNA double-strand breaks, retains IFN-inducing activity, supporting a mechanism independent of DNA lesions. Together, these data uncover a new feature of doxorubicin activity and cGAS activation, expanding current paradigms of nucleic acid sensing, with important implications for immunomodulation during cancer therapy.

P59 Broken Mitochondria, Unleashed Interferons: Insights from Genetic Diseases

A. Pierga[1], Y. de Souza Angelo[1], M. Marchais[1], M. Schiff[1,2], Y.J. Crow[1], and A. Lepelley[1]
[1]Université Paris Cité, Institut Imagine, INSERM UMR 1163, Paris, France
[2]Reference Center for Mitochondrial Diseases CARAMMEL, Necker Enfants Malades Hospital, APHP, Université Paris Cité, Paris, France

As endosymbiotic remnants of bacteria in eukaryotic cells, mitochondria lie at the border between self and non-self. Consistently, increasing evidence indicates that mitochondrial damage due to a variety of causes can result in the release of mitochondrial DNA and RNA into the cytosol, triggering innate immunity and type I interferon signalling, a phenomenon implicated in autoimmunity, neurodegeneration, cancer, and aging. Specifically, we and others have demonstrated that cytosolic mitochondrial nucleic acid sensing is responsible for chronic type I interferon signalling in type I interferonopathies caused by mutations in three mitochondrial protein encoding genes (*ATAD3A*, *PNPT1* and *REXO2*). These findings suggest the intriguing possibility that type I interferon signalling plays a role in the pathogenesis of mitochondrial diseases, monogenic disorders of mitochondrial energy metabolism. In a cohort of mitochondrial disease patients, we observed enhanced IFN signalling in a third of patients, primarily carrying pathogenic variants in eight nuclear-encoded genes. Notably, investigation of the link between deficiency of these genes and interferon signalling not only highlighted mitochondrial RNA perturbations as a clinically-relevant driver of innate signalling, but also identified previously unknown functions of mitochondrial proteins, improving our understanding of innate immune tolerance to mitochondria.

P60 cGAS-STING at the Intersection of Inflammaging and Tuberculosis

S. Jena[1], S. Mothe[1], S.R. Sahoo[1] and R.K. Nanda[1]
[1]Translational Health Group, International Centre for Genetic Engineering and Biotechnology, New Delhi, INDIA

The age-associated decline in immune cell function and chronic inflammation increases the susceptibility to tuberculosis (TB) in aged hosts. cGAS-STING, a nucleic acid sensing pathway activates inflammatory signalling in aging or *Mycobacterium tuberculosis* (Mtb) infection. However, how immunosenescence modulates cGAS-STING pathway and its protective or pathological responses against Mtb is poorly understood. We hypothesize that the primed nucleic acid sensing in aged hosts modulates the immune responses to Mtb. The transcriptomic profiling of bone-marrow derived macrophages (BMDMs) harvested from 6-8 weeks, male C57BL/6 mice infected with Mtb H37Rv (1:5 MOI) revealed upregulated nucleic acid sensing (cGAS, TBK1, IRF7, NFκB1, NFκB2, IL-6, IL-1β) and interferon-stimulatory genes (ISGs; ISG15, ISG20 and IFIT family) at 24 hours post infection. Similarly, single-cell RNA sequencing (scRNAseq) of lung immune cells harvested from healthy and aerosol Mtb infected male C57BL/6 mice revealed high module scores for cGAS-STING signalling related genes in monocyte-derived macrophages (mDMs), mature dendritic cells (mDCs), CD4 effector memory T (Tem) cells and granzyme k (Gzmk)+ CD8 T cells, suggesting a perturbation in myeloid and lymphoid cell lineages. Using senescent cells and aged mice models, we are attempting to decipher the role of cGAS-STING signalling upon Mtb infection. Modulating cGAS-STING pathway by micro molecules can improve mycobacterial clearance and alleviate disease pathology in the geriatric TB patients.

P61 Microbial Dysbiosis Fuels STING-Driven Autoinflammation Through Cyclic Dinucleotides

B. Temizoz[1,2,3], T. Shibahara[4], C. Coban[1,2,3,4], A. Kumanogoh[4] and K.J. Ishii[1,2,3,4]
[1]Division of Vaccine Science, IMSUT, The University of Tokyo, Tokyo, Japan
[2]VDesC, IMSUT, The University of Tokyo, Tokyo, Japan
[3]UTOPIA, The University of Tokyo, Tokyo, Japan
[4]IFReC, Osaka University, Osaka, Japan

Aberrant activation of the stimulator of interferon genes (STING) pathway drives autoinflammatory disorders such as STING-associated vasculopathy with onset in infancy (SAVI). Using SAVI mice carrying the N153S gain-of-function STING mutation, we identified a subset that developed severe colitis and diarrhea with elevated systemic inflammation. These mice exhibited marked gut dysbiosis characterized by depletion of short-chain fatty acid-producing bacteria and enrichment of segmented filamentous bacteria. Dysbiosis was associated with elevated levels of microbial- and host-derived cyclic dinucleotides (CDNs), potent STING agonists. Antibiotic treatment reduced inflammation, demonstrating a causal role for microbiota in disease progression. Elevated systemic CDNs were also detected in SAVI patients and a subset of patients with systemic lupus erythematosus (SLE), highlighting the heterogeneity of STING activation across autoimmune and autoinflammatory conditions. These findings position systemic CDNs as valuable biomarkers and therapeutic targets for STING-driven diseases.

P62 Extracellular cGAMP transmission drives neurodegeneration

Dayanne R. Carvalho[1,2], Rina Wang[1,2], Alessandra Sclip[2], Yu Li[2], Seula Shin[2], Nuo Liu[2], Hani Goodarzi[2,3], Christoph Thaiss[1,2], Silvana Konermann[1,2], Lingyin Li[1,2]
[1]Stanford University, Stanford, CA 94305, USA
[2]Arc Institute; Palo Alto, CA 94304, USA
[3]UCSF, San Francisco, CA 94143, USA

Neuroinflammation is a key driver of neurodegeneration and age-related cognitive decline, with STING-mediated inflammation emerging as a central contributor. In the brain, microglia are a major source of the second messenger cGAMP, synthesized by cGAS in response to cytosolic mitochondrial DNA. Beyond acting cell-autonomously, cGAMP is exported into the extracellular space in experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis, and in aging mice. In Enpp1^{H362A} mice, which cannot degrade extracellular cGAMP in the brain and spinal cord, this leads to heightened neuroinflammation, aggravated EAE paralysis, and premature aging, all of which are rescued by additional STING deletion. These findings reveal a critical role for the extracellular cGAMP–STING axis in driving neuroinflammation. Mechanistically, microglia release cGAMP through a volume-regulated anion channel, and once outside cells, cGAMP is taken up by astrocytes and neurons via glutamate and glutamine transport pathways, triggering STING-dependent type I interferon responses. Thus, the immunotransmitter cGAMP hijacks neurotransmitter glutamate signaling and the glutamate–glutamine cycle, exposing a selective vulnerability of the glutamatergic system that propagates neuroinflammation during aging and MS.

P63 Telomere Shortening Cause Aging and Cancer via cGAS-STING

M. G. Ferreira[1]
[1]Institute for Research on Cancer and Aging of Nice (IRCAN), CNRS UMR7284, INSERM U1081, Université Cote d'Azur, 06107 Nice, France

Telomere shortening is a hallmark of human aging. We use the zebrafish, a model organism that mirrors human telomere dynamics during aging. Telomerase-deficient zebrafish (*tert*) displays premature aging phenotypes, including reduced fertility, cachexia, chronic inflammation, and age-associated diseases. We recently showed that activation of the cGAS-STING pathway causes *tert* premature aging. *tert* mutants exhibit disrupted mitochondria, cytosolic DNA and micronuclei, resulting in the activation of the cGAS-STING pathway and type I interferon inflammation. Notably, double mutants lacking both *sting* and *tert* suppress age-associated phenotypes.
Cancer incidence is also anticipated to early life of *tert* zebrafish, similar to other age-associated phenotypes. This effect is also cGAS-STING dependent as *tert sting* double mutants delay the onset cancer to WT levels. Our previous results involved cancer progression and inflammation in early tumorigenesis. Therefore, we questioned if this was a result of cGAS-STING activation. Consistently, we show that STING inhibition by H151 significantly reduced metastatic dissemination in the prematurely aged microenvironment of *tert* zebrafish. This unexpected role of cGAS-STING in tumorigenesis challenges the conventional view of STING agonists in cancer therapy.

P64 Inherited loss-of-function mutation in the adaptor protein STING is associated with elevated infection rate and immune dysregulation

S. Rösing[1,2], T. Kaiser[3], Z. Abdullah[4], C. Baer de Oliveira Mann[3] and C. Günther[1,2]
[1]Department of Dermatology, University of Tübingen, Tübingen, Germany
[2]Department of Dermatology, Technical University Dresden, Germany
[3]Department of Bioscience, Technical University of Munich, Germany
[4]Institute of Molecular Medicine & Exp. Immunology, University Hospital Bonn, Germany

We report a 60-year-old woman with recurrent exanthema, fever and arthralgias. Her son experienced frequently severe infections during early childhood. Exome sequencing revealed a heterozygous STING deletion in both individuals and patient derived fibroblasts showed markedly reduced STING protein levels. To investigate the impact of the mutation on protein structure, the mutated cytosolic domain was expressed and purified from bacteria. However, the protein was not soluble, indicating no proper folding of the mutant. Functional assays in STING-deficient HEK293T and THP1 cells showed that the mutant failed to induce type I IFN and NF-κB signaling upon dsDNA stimulation, while heterozygous state led to reduced signaling. Consistently, patient fibroblasts exhibited diminished induction of interferon-stimulated genes after cGAS activation. Immunophenotyping additionally revealed reduced regulatory T cells in peripheral blood, that require STING signaling during differentiation. Together, these findings identify a STING loss-of-function mutation causing impaired protein expression and attenuated innate immune signaling, associated with increased infection susceptibility and impaired regulatory T cell function.

P65 Innate immune sensing of stalled replication forks in the nucleus

T.A. Ward,[1] A. Gamble[2], O.P.G. Wheeler[1], J.P. Morris[1], C.J. Staples[2] and L. Unterholzner[1].
[1] Faculty of Health and Medicine, Lancaster University, UK
[2] NorthWest Cancer Institute, Bangor University, UK

Replication stress and inflammation are intimately connected, and are hallmarks of cell ageing and cancer. When DNA replication is impaired and replication forks stall, this can lead to DNA damage and genomic instability. The innate immune system can sense the late effects of DNA damage through the detection of cytosolic DNA fragments or ruptured micronuclei by cyclic GMP-AMP synthase (cGAS) and its adaptor protein STING (Stimulator of Interferon Genes). We now find that stalled replication forks can also be sensed in the nucleus, even before DNA damage occurs. We show that the nuclear DNA binding protein IFI16 binds directly to stalled replication forks, then translocates to the cytosol to initiate the activation of NF-kB in a STING-dependent, but cGAS-independent manner. In addition to its innate immune sensing function, IFI16 also acts on replication forks directly, by protecting the newly-made DNA from degradation by nucleases. Using an optical tweezer / confocal microscopy approach, we can visualise single IFI16 molecules scanning along dsDNA in nuclear lysates, and we are using this method to probe the dynamic interactions with nucleic acid structures that may be detected by IFI16 at stalled forks. As IFI16 is itself a highly interferon-inducible gene, we hypothesise that replication stress may be sensed as an additional hallmark of viral infection, and that IFI16 acts as part of the interferon response to protect the host cell's genome from the deleterious effects of virus-induced replication stress.

P66 Nucleocytosis: Novel mechanism of Type I IFN Response by Nuclear DNA

H. Negishi[1], Y. Wada[1], Y. Shirasaki[2], (*Full authors listed in the presentation), KJ. Ishii[1]
[1]Division of Vaccine Science, The Institute of Medical Science, The University of Tokyo
[2]Division of Photonic Imaging, Research Center for Advanced Science and Technology, The University of Tokyo

Type I IFN is a central antiviral cytokine induced by nucleic acid-sensing pathways. While triggered by pathogen nucleic acids, self-derived nucleic acids from dying cells can also activate innate immunity, contributing to inflammatory and autoimmune diseases. However, whether and how extracellular self-DNA activates cytosolic sensors remains unclear. Here, we report a novel mechanism of self-DNA transfer mediated by a process we term "Nucleocytosis." We demonstrated that immune cells extend protrusions to directly access the nuclei of dying cells, enabling nuclear DNA transfer into the immune cell cytoplasm and subsequent cGAS–STING-dependent Type I IFN production. We also clarified the molecular mechanisms underlying Nucleocytosis.

Our findings identify Nucleocytosis as a novel pathway by which self-DNA activates innate immunity, providing crucial insights into nucleic acid sensing, related disease.

P67 Structural Basis of Specific Cytokine RNA Recognition by Regnase-1 and Its Binding Partners

Kaili Xu[1], Arthur Millius[1,2], Hiroya Oki[3], Christoph Gerle[4], Yuichiro Konishi[5], Kazuhiko Maeda[2], Hideki Shigematsu[4], Shizuo Akira[2], and Daron Standley[1]
[1]Laboratory of Systems Immunology, WPI-iFReC, The University of Osaka, Japan
[2]Laboratory of Host Defense, WPI-iFReC, The University of Osaka, Japan
[3]Department of Infection Metagenomics, RIMD, The University of Osaka, Japan
[4]Life Science Research Infrastructure Group, RIKEN, SPring-8 Center, Japan
[5]Laboratory of CryoEM Structural Biology, IPR, The University of Osaka, Japan

The endoribonuclease Regnase-1 maintains immune homeostasis in vivo by degrading cytokine mRNAs, such as interleukin-6, that contain stem-loop structures. In vitro, however, Regnase-1 can cleave both structured and unstructured RNAs. How Regnase-1 specifically targets structured RNAs in cells is unknown. Here, we identified a protein that both recognized RNA stem-loop structures and bound Regnase-1. Together with stem-loop RNA, this protein formed macromolecular filaments, which were resolved to 3Å resolution by cryoelectron microscopy. Addition of Regnase-1 altered the shape and size of the protein-RNA filaments, and these filamentous assemblies were consistent with protein co-localization to Regnase-1 speckles in cells. Furthermore, we are working to obtain a high-resolution structure of a Regnase-1-decorated protein-RNA filament, which may help provide a structural basis for developing therapeutic strategies targeting immune-related diseases.

P68 A Small-Molecule Inhibitor of Regnase-1 Activates Immune Responses

Arthur Millius[1,2,3], Chee-Hong Yew[1], Yuko Misumi[4], Jian Yu[5], Tatum Andini[1], Soyoung Park[2], Kazuhiko Maeda[3,8], Genji Kurisu[5], Shizuo Akira[3,8,9], and Daron Standley[1,2]
[1]Department of Genome Informatics, RIMD, The University of Osaka
[2]Laboratory for Systems Immunology, WPI-iFReC, The University of Osaka
[3]Laboratory of Host Defense, WPI-iFReC, The University of Osaka
[4]TechnoPro, Inc. TechnoPro R&D, Company, Kobe, Japan
[5]Protein Crystallography Laboratory, Institute for Protein Research, The University of Osaka
[8]Department of Host Defense, RIMD, The University of Osaka
[9]Center for Advanced Modalities and Drug Delivery System, The University of Osaka

Regnase-1 is an essential RNase that inhibits immune cell activation by targeting the mRNA of pro-inflammatory cytokines such as interleukin 6 (IL6). Although genetic suppression of Regnase-1 activity in CD8+ T cells has been shown to enhance antitumor immunity, no molecular inhibitors of Regnase-1 have been reported. By exploiting a library of nucleic acid analogs in conjunction with an RNA displacement assay, we identified multiple candidate competitors of Regnase-1 RNA substrates. Among these, one compound inhibited Regnase-1–mediated degradation of RNA in vitro, and enhanced *Il6* mRNA levels and IL6 production in mouse embryonic fibroblasts. A 2.62 Å crystal structure revealed the inhibitor bound to the Regnase-1 active site, coordinating with the catalytic Mg^{2+}. These findings uncover the first small-molecule inhibitor of Regnase-1–mediated RNA decay and suggest a novel mode of pharmacologically activating immune responses.

P69 Residual Regnase-1-deficient CD4+ T cells enhance autoantibody production

H. A. Sato[1], K. Maeda[1], A. Murata[1], Y. Ophinni[1,2], A. Millius[1,3], and S. Akira[1,4]
[1] Laboratory of Host Defense, WPI-IFReC, The University of Osaka, Osaka, Japan
[2] The Hakubi Center for Advanced Research, Kyoto University, Kyoto, Japan
[3] Laboratory of Systems Immunology, WPI-IFReC, The University of Osaka, Osaka, Japan
[4] CAMaD, The University of Osaka, Osaka, Japan

Regnase-1 is an endoribonuclease that regulates inflammatory responses by recognizing specific stem–loop structures within the 3′ untranslated regions (3′UTRs) of target mRNAs and promoting their degradation. Global deletion of Regnase-1 in mice results in severe autoimmune-like disease accompanied by autoantibody production, and similar phenotypes have also been reported following conditional deletion in either T or B cells. While the mechanisms and direct targets of Regnase-1 in T cells have been partially characterized, how B cell-specific deletion promotes autoimmunity remains largely unclear.

Here, we employed multiple B cell-specific Cre driver lines in combination with the $R26^{RFP}$ reporter system to investigate the cellular basis of this autoimmune phenotype. Unexpectedly, lineage-tracing analyses revealed that autoimmunity was not primarily caused by Regnase-1 deficiency in B cells. Instead, disease development was driven by unintended Cre-mediated recombination in a small population of T cells. These findings demonstrate the extraordinary sensitivity of immune homeostasis to Regnase-1 loss in T cells and emphasize the essential role of Regnase-1-mediated RNA regulation in preventing autoimmune pathogenesis.

P70 Endoribonuclease Regnase-1 Deficient Hepatocytes are Resistant to MASH Development

Akihiko Murata[1], Takatoshi Sakaguchi[1], Kazuhiko Maeda[1], Shizuo Akira[1]
[1] Laboratory of Host Defense, Immunology Frontier Research Center (IFReC), The University of Osaka, Suita 565-0871, Japan

Regnase-1 is an endoribonuclease that destabilizes a broad spectrum of mRNAs, including its own. By targeting inflammation-associated transcripts such as IL-6, it acts as a critical suppressor of inflammatory responses. While systemic Regnase-1 deficiency causes severe autoimmunity, specific deletion in the intestinal epithelium promotes tissue regeneration and dampens inflammation, reflecting its role in regulating mTOR signaling and nucleic acid metabolism. Here, we investigated its contribution to liver fibrosis during the progression of metabolic dysfunction-associated steatohepatitis (MASH). Hepatocyte-specific Regnase-1 deficiency was established by injecting an AAV-Cre vector into *Regnase-1*$^{fl/fl}$ mice at week 4 of an 8-week MASH-inducing diet. Regnase-1-deficient livers exhibited reduced steatosis and fibrosis. Pathway analysis indicated increased expression of mitochondrial function genes. Indeed, Regnase-1-deficient hepatocytes exhibited not only elevated oxygen consumption rates but also restored normal mitochondrial morphology. Finally, administration of GalNAc-conjugated Regnase-1 siRNA, targeting hepatocytes via the asialoglycoprotein receptor, successfully reduced fibrosis and steatosis, mirroring the knockout phenotype. These findings suggest that hepatocyte Regnase-1 exacerbates MASH by inducing mitochondrial dysfunction and promoting fibrosis. Therefore, targeted silencing of hepatic Regnase-1 using GalNAc-siRNA represents a promising therapeutic strategy for MASH.

P71 *MALAT1*, a Canonical Nuclear lncRNA, Controls Human Macrophage Inflammation through Mitochondrial HADHB

A. Yuxiang Liu,[1,2] B. Yoshiko Iwai[2] and C. Katsuhito Fujiu[1,3]
[1] Department of Advanced Cardiology, The University of Tokyo, Tokyo, Japan.
[2] Department of Cell Biology, Institute for Advanced Medical Sciences, Nippon Medical School, Tokyo, Japan.
[3] Department of Integrative Physiology, Institute of Science Tokyo, Tokyo, Japan.

Long noncoding RNAs (lncRNAs) are critical regulators of immune responses and cellular metabolism. Here, we report a previously unrecognized interaction between *MALAT1* and HADHB, which reveals additional regulatory roles for *MALAT1* in human macrophages. Our findings demonstrate that HuR-MTCH2-mediated mitochondrial targeting of *MALAT1* enables its interaction with HADHB, thereby enhancing HADHB thiolase activity during the late phase of inflammation. *MALAT1* also negatively regulates pro-inflammatory macrophage activation via HADHB. Knockdown of *MALAT1* induces metabolic reprogramming, characterized by enhanced glycolysis, increased fatty acid synthesis, and reduced fatty acid oxidation, suggesting that *MALAT1* suppresses inflammatory metabolic pathways. This study uncovers the *MALAT1*–HADHB interaction and demonstrates that *MALAT1* regulates macrophage metabolic reprogramming, offering new insights into the metabolic control of inflammation and highlighting *MALAT1* as a potential therapeutic target for inflammatory diseases.

P72 Synthesis and Evaluation of Modified Nucleoside Triphosphate-Containing mRNA Molecules

M.A. Islam[1,2], T.R. Chowdhury[1,2], H. Nakanishi[1,3], K. Itaka[1,3], and S. Obika[1,2]
[1]Center for Advanced Modalities and DDS (CAmaD), The University of Osaka
[2] Graduate School of Pharmaceutical Sciences, The University of Osaka
[3]Institute of Science Tokyo

Messenger RNA (mRNA) therapeutics have emerged as a promising platform for disease treatment and prevention. Chemical modification of nucleosides is an effective strategy for improving mRNA stability, reducing immunogenicity, and enhancing translational efficiency. In our previous studies, a library of modified purine and pyrimidine nucleosides was synthesized using Sonogashira cross-coupling chemistry and subsequently converted into the corresponding nucleoside triphosphates (NTPs). Because synthesis of modified NTPs remains challenging due to low yields and purification difficulties, various synthetic approaches were investigated to optimize NTP preparation. In this study, 5-alkyne-modified uridine and thymidine triphosphates bearing ethynyl and propynyl substituents were synthesized and incorporated into mRNA by in vitro transcription. The transcriptional and translational performance of the resulting mRNAs was evaluated and compared with unmodified controls. Preliminary results indicate that small alkyne modifications are compatible with efficient protein expression, highlighting their potential utility in the development of next-generation mRNA therapeutics.

P73 Design of immunosilent mRNA adjuvanted by a RIG-I-agonist for tunable innate immune activation

S. Dudek,[1] C. Wuebben,[1] T. Zillinger[1], M. Katzmarzyk[2], L. Cicin-Sain[2], A. Baumgart[3], C. Kurts[3], I. Trus[4], G. Michlewski[4] and G. Hartmann[1]
[1] Institute of Clinical Chemistry and Clinical Pharmacology, University Hospital Bonn
[2] Department of Viral Immunology, Helmholtz Centre for Infection Research, Braunschweig
[3] Institute for Molecular Medicine and Experimental Immunology, University Hospital Bonn
[4] International Institute of Molecular and Cell Biology, Warsaw

Despite the clinical success of mRNA vaccines, unbalanced innate immune activation by exogenous RNA can dampen the translation of mRNA or lead to immune-mediated side effects of mRNA therapeutics. However, effective immunization requires a certain degree of innate immune activation for the induction of a potent adaptive immune response against the presented antigen. Achieving a finely tuned balance between these opposing effects remains a central challenge in mRNA vaccine design. To address this, we developed new sequence design principles to avoid uncontrolled RLR activation by reducing the dsRNA byproducts from the mRNA in vitro transcription. In parallel, we introduced a novel RIG-I-agonist that is hybridized to the 3'UTR of the mRNA for tunable adjuvant activity. We demonstrate that the magnitude of the type I interferon response could be controlled by varying the number of RIG-I agonists occupying the hybridization sites on the mRNA. The RIG-I-adjuvanted mRNA vaccine elicited increased expression of costimulatory molecules on dendritic cells while maintaining antigen presentation. Consequently, antigen-specific T cell activation was enhanced compared to the non-adjuvanted vaccine, indicating improved induction of adaptive immunity.

P74 IL-33 Links Cytosolic RNA Sensing and Adaptive Responses in mRNA Vaccination

K. Liu,[1] K. Kobiyama,[1,2] N. Satoh-Takayama,[3] T. Hayashi,[1] B. Temizoz,[1] H. Negishi,[1] A. J. Tobuse,[1] M. Onaga,[1] A. Iwama,[4] P. D. Katsikis,[5] C. Coban,[6] and K. J. Ishii[1]
[1] Division of Vaccine Science, the Institute of Medical Science, the University of Tokyo
[2] Division of Rheumatology, Department of Medicine, University of California San Diego
[3] Precision Immune Regulation RIKEN ECL Research Unit, RIKEN Center for Integrative Medical Sciences
[4] Division of Stem Cell and Molecular Medicine, Center for Stem Cell Biology and Regenerative Medicine, the Institute of Medical Science, the University of Tokyo
[5] Department of Immunology, Erasmus University Medical Center
[6] Division of Malaria Immunology, the Institute of Medical Science, the University of Tokyo

Lipid nanoparticle (LNP)-mRNA vaccines have transformed vaccinology, yet the mechanisms linking innate sensing to adaptive immunity remain incompletely defined. Here, we show that a FDA-licensed vaccine establishes a distinct spatially organized immune-stromal circuit driven by TBK1–interleukin (IL)-33 and ST2–MyD88 intercellular signaling, coupling potent $CD8^+$ T cell responses to unexpected IgE induction. We identify the mRNA cargo and ionizable lipid as cooperative inducers of IL-33. Mechanistically, cytosolic RNA sensing activates TBK1, while LNP-mediated perturbabtion extracellular lipid metabolism IL-33 expression and necroptosis faciliates the IL-33 release. Functionally, IL-33 promotes antigen-specific $CD8^+$ T cell responses, while concurrently driving IgE production through mast cell-derived type 2 cytokines.

P75 AI-Guided Translation Efficiency Prediction Modeling for Balanced Multivalent mRNA Vaccine Design

N. Abdelkhalek[1] and S. Yamasaki[1]
[1]RNA Informatics Laboratory, Department of Biological Informatics, Research Institute for Microbial Diseases, University of Osaka, Osaka, Japan

Multivalent mRNA vaccines can broaden protection against variable pathogens, but balanced intracellular expression of multiple LNP-delivered antigen-encoding transcripts remains difficult to achieve. Current mRNA optimization approaches treat each transcript as an independent design problem, limiting their ability to address translational competition and antigen-expression balance. We therefore explored an interpretable machine-learning workflow to identify features associated with transcript-level translation efficiency (TE) as a first step toward the coordinated optimization of translation in multivalent mRNA vaccines. Quality-controlled transcript sequences from GRCh38 were paired with transcript-level translation efficiency scores from TEDD for model training. We evaluated biologically informed and computationally derived sequence features across multiple machine-learning models. Consistent with previous reports, feature-importance analysis highlighted the 5′UTR as a key determinant of translation efficiency, suggesting that 5′UTR engineering alone may enable coordinated optimization of multivalent mRNA vaccines. We are now improving model performance, incorporating ribosome elongation dynamics, and developing a coordinated optimization system. This presentation summarizes our current findings and outlines the system under development and its future directions.
Keywords: mRNA translation efficiency; codon usage; 5′UTR motifs; feature engineering; machine learning, multivalent mRNA vaccine

P76 IRES-modified ssRNA adjuvant elicits potent immune responses with enhanced safety in early-life mice

JY Bang[1],Y-J Seo[2] and S-H Hong[1,]
[1]Department of Microbiology, College of Medicine, Ewha Womans University, Seoul, 07804, Republic of Korea
[2]Department of Life Science, Chung-Ang University, Seoul, 06974, Republic of Korea

Infectious diseases remain a major cause of mortality in early life, while vaccine efficacy is often limited by immune immaturity. Here, we demonstrate that an internal ribosome entry site-derived single-stranded RNA (IRES-ssRNA) functions as a novel adjuvant for early-life vaccination. IRES-ssRNA enhanced antigen uptake and activated antigen-presenting cells in draining lymph nodes. Compared with alum, it induced more balanced IgG1/IgG2a responses and stronger T-cell immunity. Transcriptomic profiling revealed that IRES-ssRNA preferentially activated inflammatory and adaptive immune pathways, including T-cell activation and chemokine–cytokine signaling and consistent with these findings, IRES-ssRNA improved vaccine-induced protective efficacy.Notably, repeated administration of IRES-ssRNA did not cause granulomatous lesions or persistent injection-site inflammation, whereas alum induced localized granulomas and pro-inflammatory gene expression. These findings highlight IRES-ssRNA as a safe and effective adjuvant platform for improving vaccine efficacy in early-life immunization.

P77 Anti-infective Nanobodies Delivered by mRNA-LNP Inhibit Intracellular Rickettsial Pathogens

Yasuko Rikihisa[1], Wenqing Zhang[1], Mingqun Lin[1], Nan Duan[1], Qi Yan[1], Stephen Denton,[1] Jeffrey Lakritz[2], and Yizhou Dong[3]
[1]Department of Veterinary Biosciences and [2]Department of Preventive Medicine, The Ohio State University, 1925 Coffey Road, Columbus, OH 43210
[3]Icahn Genomics Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029

Intracellular bacterial infection is difficult to treat, as although disease-associated bacterial and host factors have been identified, tools to specifically target key molecules to inhibit such infection are not easily found. *Ehrlichia chafffeensis* is an obligatory intracellular bacterium that causes an emerging tick-borne disease called human monocytic ehrlichiosis. *E. chaffeensis* Type IV secretion system effectors (Etf-1, Etf-2, and Etf-3) are essential for infection: Etf-1 localizes to mitochondria and blocks mitochondria-mediated host cell apoptosis to keep the infected host cell alive for bacterial intracellular replication. Etf-1 also directly binds Beclin 1 (ATG6), and induces autophagy for *E. chaffeensis* to acquire catabolites as nutrients. Etf-2 directly binds Rab5-GTP on *Ehrlichia*-containing vacuole membranes and blocks Rab5 GTPase-activating protein (RabGAP-5) engagement with Rab5-GTP to prevent *Ehrlichia*-containing vacuoles from maturing into late endosomes and fusing with lysosomes. Etf-3 directly binds host-cell ferritin light chain and induces ferritinophagy, thus increasing cellular labile iron pool for *Ehrlichia* proliferation. We developed nanobodies (Nbs) targeting each of effectors (Etf-1, Etf-2, Etf-3) and found that intracellular delivery of the Nbs by using lipid nanoparticle (LNP)-encapsulated mRNA (mRNA-LNP) inhibits effector functions, and also *E. chaffeensis* infection of host cells and mice.

P78 Enabling Extrahepatic Delivery of siRNA Through Increased Potency, Advanced Chemistry and Bioconjugation

T. Einfalt[1]
[1]Novartis Pharma AG, Basel, Switzerland

siRNA therapeutics harness the RNA interference pathway to achieve sequence-specific silencing of disease-driving genes at the mRNA level. Recent advances in siRNA chemistry are enabling therapeutic expansion to extrahepatic tissues. Modern siRNA constructs potencies are relaxing delivery constraints that previously limited activity outside the liver. A central driver of this transition is the substantial increase in intrinsic siRNA potency enabled by optimized sequence design and advanced chemical stabilization. In synergy targeting bioconjugation strategies are expanding the accessible tissue space. Beyond GalNAc-mediated liver targeting, conjugation to fatty acids or antibodies enables modulation of circulation time, biodistribution and cellular uptake, opening paths to delivery in heart, muscle, central nervous system, lung and adipose tissues. Although key barriers including systemic clearance, vascular permeability, cellular uptake and endosomal escape remain, improved pharmacokinetic properties combined with higher potency are allowing us to achieve meaningful gene silencing in these extrahepatic environments. Together, the convergence of increased potency with advances in delivery chemistry is driving a paradigm shift from liver-centric applications toward broad extrahepatic gene silencing and enabling new therapeutic opportunities across multiple disease areas and tissues.

P79 Immune Induction Mechanisms of Artificial Nucleic Acids and Immune Evasion using Drug Delivery System based on Extracellular Vesicles

M. Hata,[1] D. Okamoto[2], Y. Mikame[1], O. Takeuchi[2], T. Uehata[2], A. Yamayoshi[1]
[1]Dept. of Life Sci. and Tech., Institute of Science Tokyo
[2]Grad. Sch. Med., Kyoto Univ.

Nucleic acid drugs have emerged as a promising therapeutic modality because they enable direct targeting of genes, but immunogenicity and intracellular delivery technology (DDS) remain key challenges. While the FDA-approved mipomersen incorporates chemical modifications to avoid immune recognition, it is reported to induce inflammatory reactions. To develop non-immunogenic nucleic acid drugs, we investigated the sensor molecules responsible for recognizing mipomersen. Transfection of mipomersen into various nucleic acid sensor-deficient mouse embryonic fibroblasts (MEFs) revealed that mipomersen-induced interferon (IFN) expression was markedly attenuated in cGAS-deficient cells. Given that cGAS is known to recognize double-stranded DNA, our findings suggest that intracellular transfection of the single-stranded oligonucleotide mipomersen activates the cGAS-STING pathway. Furthermore, we will discuss our novel exosome-hijacking DDS (ExHijack-Oligo) that may reduce immune activation during intracellular delivery.

P80 RNA-guided degradation of DDB2 by lincRNA-p21-derived oligonucleotides restores chemosensitivity in breast cancer.

Wei-Chien Huang[1], Yu-Hao He[1], Ya-Ling Wei[1], Yi-Lin Chen[1], Hsin-Chiao Chou[1], Li-Chi Kuan[1], Min-Han Chi[1], Shu-Wei Hu[1], Fang-Ju Cheng[1], Yi-Lun Yeh[1], and Mien-Chie Hung[1]
[1]China Medical University, Taichung, Taiwan

Therapeutic targeting of the DNA damage response in breast cancer has mainly focused on homologous recombination deficiency and replication stress, whereas other DNA repair pathways remain less explored. Here, we identify damaged DNA-binding protein 2 (DDB2), a nucleotide excision repair factor associated with aggressive tumor behavior and treatment resistance, as a therapeutically actionable vulnerability in breast cancer. We show that the p53-responsive long non-coding RNA lincRNA-p21 suppresses breast tumor growth and enhances the response to platinum agents and doxorubicin by promoting DDB2 degradation. Mechanistically, lincRNA-p21 associates with DDB2, DDB1, and Cul-4 to facilitate assembly of the DDB2/DDB1/Cul-4 E3 ligase complex, leading to DDB2 polyubiquitination and proteasome-dependent degradation. Guided by the identification of minimal functional elements within lincRNA-p21, we engineered lincRNA-p21-derived oligonucleotides that bind DDB2, reduce its protein abundance, and restore chemosensitivity. Delivery of these oligonucleotides using lipid nanoparticles or exosome-based formulations decreased DDB2 expression and inhibited tumor growth across multiple breast cancer models, including triple-negative breast cancer. In addition to impairing DNA repair capacity and sensitizing tumors to DNA-damaging agents, targeting DDB2 with lincRNA-p21-derived oligonucleotides may increase tumor immunogenicity by enhancing DNA damage-associated stress responses and promoting a more immune-responsive tumor microenvironment. Together, these findings identify DDB2 as a targetable nucleotide excision repair vulnerability and suggest that RNA-guided selective protein degradation may provide a therapeutic strategy that integrates chemotherapy sensitization with immune activation in breast cancer.

P81 Chronic Interferon Alpha Signalling Causes ZBP1-dependent Immunopathology

I.Jorgacevic[1], K.Kleemann[1], P.Müller[1], M.Öztürk[1], A.Roers[2] and R.Behrendt[1]
[1]University Bonn, University Hospital Bonn, Institute of Clinical Chemistry and Clinical Pharmacology, Venusberg-Campus 1, 53127 Bonn, Germany
[2] University Hospital Heidelberg, Institute for Immunology, Im Neuenheimer Feld 305, 69120 Heidelberg, Germany

Chronic interferon alpha (IFN) signaling causes systemic autoimmunity through the induction of interferon-responsive genes (ISGs). Z-DNA binding protein 1 (ZBP1) is an IFN-inducible Z nucleic acid sensor that regulates cell death and inflammation. We identified ZBP1 as an essential driver of IFN-induced immunopathology. Inactivation of ZBP1 doubles the life span of mice that chronically express IFNα4 from conventional dendritic cells. We identified splenic macrophages and pDCs as highly responsive to IFN-induced ZBP1 activation. Moreover, ZBP1-deficiency completely rescued embryonic lethality of mice that ubiquitously express IFN. Collectively, among the hundreds of ISGs, we identify ZBP1 as an essential effector of IFN-induced lethal immunopathology in mice.

P82 Sensing of nucleic acids in immune-mediated glomerulonephritis

Paula Verneuil Carnerero [1], Celia Kho [1], Sümeyye Ertugrul [1], Moritz Blankart [1], Patrick Droste [2], Tarek Elmzzahi [3], Peter Boor [2], Christian Kurts [1]* and Zeinab Abdullah [1]*
[1]Institute of Experimental Immunology, University Hospital Bonn
[2]Department of Pathology, University Hospital Aachen
[3]Immunogenomics & Neurodegeneration, German Centre for Neurodegenerative Diseases

Patients suffering from chronic viral infections such as Human Immunodeficiency Virus (HIV) or Hepatitis C Virus (HCV) often also develop a lupus-like autoimmune disease characterized by increased levels of anti-DNA, anti-Nucleosomes, anti-snRNP autoantibodies. These patients develop a RNA virus infection-associated glomerulonephritis (rVI-GN). To study the mechanisms underlying disease development, we established the neoLCMV model, which allowed us to determine that TLR7 and TLR2 signaling are crucial for the disease development. TLR7 activation drives the expansion of unconventional T cells and the maturation of autoreactive B cells in an extrafollicular manner. Using TLR7 KO mice, we found reduced numbers of unconventional T cells and extrafollicular B cells in the spleen, lower levels of autoantibodies together with fewer DNA- and Nucleosomes-specific B cells. On the other hand, TLR2 activation contributes to disease progression promoting neutrophil extracellular traps (NETs) in the liver. This TLR2-driven NETosis could be the source of self-antigen for the activation of autoreactive B cells. In TLR2 KO mice, NET formation was reduced, together with lower autoantibodies and disease development. However, the ligands activating these receptors still need to be identified.

P83 Gelsolin Protects Against Imiquimod-Induced Psoriatic Inflammation by Regulating the MAM-ER Stress Axis

Towa Tanaka,[1] Daisuke Ori,[1,2] Norisuke Kano,[1] and Taro Kawai[1,3]
[1] Laboratory of Molecular Immunobiology, Division of Biological Science, Graduate School of Science and Technology, Nara Institute of Science and Technology (NAIST)
[2] Department of Immunology, Faculty of Medicine, Kagawa University
[3] Life Science Collaboration Center (LiSCo))

Imiquimod (IMQ) is widely used as a mouse model of psoriasis, yet the mechanisms underlying its inflammatory activity remain incompletely understood. Here, we show that IMQ induces endoplasmic reticulum (ER) stress and NLRP3 inflammasome activation independently of Toll-like receptor 7 (TLR7) and identify gelsolin (GSN) as a novel IMQ-binding protein. IMQ promotes mitochondria-associated ER membrane (MAM) formation, activating the IRE1α-XBP1s pathway and inducing mitochondrial dysfunction and inflammatory responses. GSN-deficient mice showed increased IMQ-induced psoriasiform skin inflammation with enhanced ER stress and inflammatory cytokine expression, indicating that GSN suppresses MAM-mediated inflammatory signaling. Furthermore, reduced GSN expression in human psoriatic lesions correlated with elevated ER stress markers. These findings identify GSN as a critical negative regulator of the MAM-ER stress axis and a potential therapeutic target for psoriasis.

P84 Roles of Interleukin-24 in Proteasome-Associated Autoinflammatory Syndrome with Immunodeficiency Model Mice

I. Sasaki,[1] A. Kurara,[1] N. Wakaki-Nishiyama,[1] S. Okadome,[1] M. Tane,[1] T. Kato,[1] S. Kaji,[1] E Tosuji,[1] K. Isono,[2] H. Hemmi[1] and T. Kaisho[3]
[1] Dept. of Immunology, Wakayama Medical University (WMU), Wakayama 641-8509, Japan
[2] Laboratory Animal Center, School of Medicine, WMU
[3] Industry-Government-Academia Collaboration Promotion Headquarters, WMU

Type I interferonopathy can be triggered by accumulation of nucleic acids or unnecessary proteins. Analysis of cells from patients with proteasome-associated autoinflammatory syndrome (PRAAS) showed proteasome dysfunction, protein accumulation, and upregulation of interferon-stimulated genes expression. Seth et al. showed that accumulated cytosolic IL-24 can bind to PKR and function as a PKR ligand rather than as a cytokine by using PRAAS model cell lines (Sci Immunol 2022, 7:eabi6763). We proposed a new disease category, PRAAS with immunodeficiency (PRAAS-ID) by analyzing patient-derived cells and mutant mice with patient-derived variant (*Psmb9*$^{G156D/+}$ mice) (Nat Commun 2021, 12:6819). These mice recapitulate patient phenotypes, such as adaptive immunodeficiency or myeloid cell expansion. We here examined roles of IL-24 by crossing the PRAAS-ID model mice with IL-24 deficient mice. IL-24 deficiency failed to rescue immune abnormalities in *Psmb9*$^{G156D/+}$ mice, indicating that these defects are IL-24-independent. Our findings demonstrate that PRAAS-ID is pathologically distinct from PRAAS in terms of requirements for IL-24.

P85 Innate Immune Activation Induces Cryptic Self-Antigen Presentation to Drive Autoreactive T Cell Activation

S. Higuchi,[1] S. Mori[1] and H. Arase[1,2]
[1]Department of Immunochemistry, Research Institute for Microbial Diseases, The University of Osaka, Suita, Osaka, Japan.
[2]Laboratory of Immunochemistry, WPI Immunology Frontier Research Center, The University of Osaka, Suita, Osaka, Japan.

Innate immune responses play an important role in shaping adaptive immunity. However, excessive innate immune activation can induce aberrant adaptive immune responses to self-antigens, yet the mechanisms by which innate immune signaling directs antigen-specific adaptive immune responses remain unclear. TLR7 stimulation is known to affect MHC class II-mediated antigen presentation by antigen-presenting cells. We found that TLR7 stimulation alters the self-antigen repertoire presented by MHC class II. We also conducted TCR analysis of $CD4^+$ T cells that underwent clonal expansion in a TLR7 agonist-induced lupus mouse model. We found that these TCRs recognized self-antigens preferentially presented by MHC class II on TLR7-stimulated cells. Notably, clonally expanded T cells responding to TLR7-stimulated cells were also observed in patients with systemic lupus erythematosus (SLE). Together, these findings suggest that excessive innate immune activation can promote autoimmunity by reshaping antigen-specific adaptive immunity through altered MHC class II-mediated self-antigen presentation.

P86 Cell-free DNA as a Promoter of Bacterial Superinfection in Viral ARDS

Zhaorong Chen,[1] Susanne Schulz,[1] Mario Fox,[1] Lennart Wild,[1] Konrad Peukert[1] and Christian Bode[1]
[1] University of Bonn, University Hospital Bonn, Department of Anesthesiology and Intensive Care Medicine, Bonn, Germany

Superinfections are one of the main complications in patients suffering from viral ARDS corresponding with poor prognosis and high mortality. In particular, the immunosuppressive state after viral infections is a main driver for these superinfections. Cell-free (cf) DNA has been emerged as a driver of immunosuppression in various diseases. Here, we show that ARDS patients with secondary infections already possess significantly higher cfDNA in the lungs at time of ICU admission compared to ARDS patients without secondary infection. Similarly, Influenza A virus infection increases pulmonary cfDNA in mice. Secondary infection with *Streptococcus pneumoniae* increases mortality in PR8-infected mice, which is significantly reduced by DNase I (a cfDNA degrader), A151 (a DNA-sensing inhibitor), AIM2 deficiency but not by cGAS deficiency or STING inhibitors (H151 and C176). Interestingly, AIM2 deletion reduces bacterial burden and increases pulmonary IFNb levels without influencing viral load or lung damage. *In vitro* experiments further demonstrate that cfDNA impairs macrophage responses to secondary bacterial stimulation. Collectively, our findings suggest that pulmonary cfDNA accumulation facilitates bacterial superinfection in viral ARDS, possibly via immunosuppression. Targeting cfDNA clearance or DNA sensing provides a promising therapeutic strategy for ARDS.

P87 STING Proton Channel Controls T Cell Death and Tumour Immune Evasion

Cong Xing[1], Zhen Tang[1], Kun Song[1], Heng Lyu[1], Antonina Araszkiewicz[1], Wanwan Huai[1], Nicole Dobbs[1], Xuewu Zhang[2,3], and Nan Yan[1,4]
[1]Department of Immunology, [2]Department of Pharmacology, [3]Department of Biophysics, [4]Department of Microbiology, UT Southwestern Medical Center, Dallas, TX, USA.

The cGAS-STING pathway is a central mediator of immune responses to infection and cellular stress by inducing type I interferon signaling. Recent studies have uncovered that STING also functions as a proton channel, although studying the physiological role of this channel has been limited by a lack of genetic and pharmacological tools in mouse. Here, we identify a conserved mutation in the STING transmembrane domain. This mutant selectively abolishes STING proton channel activity while preserving ER-to-Golgi trafficking and immune signaling. Utilizing a newly generated *Sting1-mutant* knock-in mouse, we demonstrate that STING-induced T cell death is strictly dependent on the proton channel activity. Consequently, genetic ablation of the STING proton channel robustly restricts tumour growth in vivo by preventing T cell death in the tumour microenvironment. Transcriptomic profiling further reveals that the STING proton channel operates largely independently of the global activation transcriptome, specifically regulating only TFEB and autophagy-related gene networks. Collectively, our findings provide further support for STING as a bona fide proton channel and establish the STING proton channel as the basis for STING-induced T cell death. This also provides a powerful tool to investigate the interferon-independent dimensions of STING biology in health and disease.

P88 Combined ADAR1 Inhibitor and 5AZA-CdR Treatment Reveals Cellular State–Dependent Innate Immune Vulnerability in Acral Melanoma

Moeko Minakuchi,[1] Segundo Del Aguila,[1] Yulissa Anali Tirado Gonzalez, [1] Kazuko Nishikura, [1] and Jessie Villanueva.[1]
[1]The Wistar Institute, Molecular and Cellular Oncogenesis Program

Acral melanoma (AM) shows limited actionable mutations and reduced response to immune checkpoint inhibitors, likely due to a “cold” tumor microenvironment.
ADAR1 suppresses innate immune activation induced by endogenous dsRNA via A-to-I editing and is a potential therapeutic target, but determinants of response remain unclear.
We previously reported that ADAR1 inhibitor (ADAR1i-124) combined with 5AZA-CdR induces endogenous dsRNA stress and innate immune activation, though its dependence on cellular state in AM is unknown.
Here, AM cell lines were classified as ADAR1i-sensitive or -resistant. In both groups, RNA sensor activation (MDA5, PKR), interferon (IFN) signaling, interferon-stimulated genes (ISGs), and antigen presentation were induced, indicating a shared viral mimicry-like response.
RNA-seq analysis suggested that resistant cells exhibit a reduced capacity for IFN signal amplification. Combination treatment further enhanced ISGs induction, which was abolished by JAK inhibition.
These observations suggest that RNA sensing activation by ADAR1 inhibition is commonly induced in both sensitive and resistant cells, whereas differences in downstream IFN signal amplification distinguish sensitive from resistant phenotypes, with cellular stress responses such as cell cycle abnormalities and DNA damage occurring in parallel. Collectively, these observations suggest that vulnerability to RNA sensing responses is governed by cellular state.

P89 Integrin-Linked Kinase Facilitates Inflammasome Priming During Dengue Virus Infection Through the TBK1–Akt–NF-κB Axis

CP. Chang,[1,2] and YC. Li[1]
[1]Department of Microbiology & Immunology, College of Medicine, National Cheng Kung University, Taiwan
[2]Center of Infectious Disease and Signaling Research, National Cheng Kung University, Taiwan

Imbalanced innate immune responses in hosts infected with dengue virus (DENV) can lead to uncontrolled viral replication and increased disease severity. The interaction between DENV and host proteins is believed to play a crucial role in this process. Our previous research has shown that integrin-linked kinase (ILK) facilitates DENV replication by disrupting type I interferon signaling. Notably, ILK has recently been implicated in chemokine-mediated activation of the NLRP3 inflammasome; however, its role in DENV-induced inflammasome activation remains to be fully elucidated. Here, we show that DENV infection or TLR activation significantly increases mRNA expression of inflammasome-related genes in monocytes; however, these levels are reduced upon ILK silencing, suggesting that ILK plays a role in priming inflammasome activation pathways. Mechanistically, ILK-mediated inflammasome priming is regulated through the Akt–NF-κB pathway. Furthermore, our results indicate that ILK acts as a scaffold protein, forming the TBK1–Akt complex and promoting NF-κB phosphorylation and nuclear translocation, which in turn drive the transcription of genes related to the inflammasome. Overall, our study reveals a molecular link through which ILK participates in DENV infection and inflammasome activation.

P90 Innate Immune Memory by DNA and β-Glucan

Asuka Joy Tobuse[1], Kouji Kobiyama[1,2], and Ken J Ishii[1]
[1]Division of Vaccine Science, IMSUT, The University of Tokyo
[2]Division of Rheumatology, University of California San Diego, La Jolla
*Significantly shortened authors and affiliations

In the spread of unknown or emerging pathogens, vaccine design, development and distribution is a race against time. During this time, emergency strategies are crucial. Our study reveals that without antigen, intranasal administration of DNA incorporated into the triple helix structure of soluble β-glucan can protect mice against lethal flu and SARS-CoV-2 infection. Mechanistically, we identified that such protective effects could only be achieved by direct introduction of DNA + β-glucan into the lung tissue. Furthermore, we observed conventional innate activation pathways by macrophages, followed by simultaneous introduction of rare innate lymphoid cell (ILC) 1 and ILC3 in the lungs, which has not been reported before. Collectively, these findings presents the importance of cell-cell communication for memory maintenance and a novel strategy for the use of DNA molecules to induce broad, antigen-independent immune memory against respiratory viral infections.

P91 Targeting Cap1 RNA Maturation of SARS-CoV-2 and Influenza A Virus

Y. Tsukamoto[1], M. Igarashi[2], T. Wadenpohl[1], CE. Müller[3], and H. Kato[1]
[1]Institute of Cardiovascular Immunology, Medical Faculty, University Hospital Bonn, University of Bonn, Bonn, Germany
[2]Division of Global Epidemiology, International Institute for Zoonosis Control, Hokkaido University, Sapporo, Japan
[3]PharmaCenter Bonn and Pharmaceutical Institute, Pharmaceutical and Medicinal Chemistry, University of Bonn, Bonn, Germany

Methylation is one of the critical modifications that regulates numerous biological processes. Guanine capping and methylation at the seventh position (m^7G) have been shown to mature mRNA, increasing its stability and translational efficiency. However, the m^7G-capped cap0 RNA remains immature and requires further methylation of the first nucleotide (N1-2'-O-Me) to reach the full maturation, a process known as cap1. The N1-2'-O-Me group on cap1 RNA prevents its recognition by innate immune sensors such as RIG-I and IFIT1 as non-self.

Viruses have also evolved various strategies to produce self-like capped RNAs with the N1-2'-O-Me that can potentially evade the antiviral response and establish an efficient replication. Here, we present evidence showing how an N1-2'-O-Me defect affects the replication of SARS-CoV-2 and influenza A virus. These indicate that cap1 maturation is a promising process to target for antiviral intervention.

P92 Cell Cycle Checkpoint Inhibitors as Innate Immune Response Inducers to Enhance Anti-Tumour Immune Responses

R.B. Tjeerdsma,[1] F.J. Bakker[1] and M.A.T.M. van Vugt[1]
[1]Department of Medical Oncology, University of Groningen, University Medical Center Groningen, Hanzeplein 1, 9713 GZ, Groningen, the Netherlands.

Interferon signaling is an established determinant of immune checkpoint inhibitor (ICI) efficacy. Previous studies, including our own, have shown that transmission of unrepaired DNA damage into mitosis generates cytoplasmic DNA fragments that can be recognized by damage-associated molecular pattern (DAMP) receptors such as cGAS. cGAS-STING signaling subsequently triggers an innate immune response characterized by type I interferon signaling and downstream cytokine expression and release. Given the limited response to ICI treatment in cancer patients, this project aimed to investigate whether cell cycle checkpoint inhibitors, alone or combined with DNA-damaging chemotherapeutics, can induce an innate immune response to ultimately enhance immunotherapy efficacy. Using a triple-negative breast cancer cell line expressing a fluorescence-based interferon reporter, we found that inhibition of the cell cycle checkpoint regulator ATR synergistically induces a type I interferon response when combined with DNA-damaging agents. This response is largely dependent on cGAS. We will discuss ongoing efforts to dissect the mechanisms driving innate immune activation in this setting, determine why interferon induction is restricted to specific genotoxic agents, and assess whether combined treatment enhances immune cell recruitment and activation.

P93 Macrophage Immunomodulatory Activity and Anti-Proliferative Properties of an Amino Acid-Modified Thrombin Aptamer

Tatum Melati Andini[1], Hiroki Kanazawa[2], Jiro Kondo*[2], Soyoung Park*[3], Daron M. Standley[1]
[1]Research Institute for Microbial Diseases (RIMD), The University of Osaka, Suita, 565-0871, Japan
[2]Department of Materials and Life Sciences, Sophia University, Tokyo, 102-8554, Japan
[3]Earth-Life Science Institute (ELSI), Institute of Science Tokyo, Tokyo, 152-8550, Japan

Chemical modification of nucleic acids with functional moieties can enhance their biological properties and functional diversity. Previously, we developed T3F, an amino acid–nucleic acid hybrid (ANH) derivative of the thrombin-binding aptamer TBA15, by introducing phenylalanine at the thymine 3 (T3) position within the loop region. Compared with native TBA15, T3F showed enhanced thrombin-binding affinity and improved anticoagulant activity.
Given its partial structural similarity to AS1411, a G-rich oligonucleotide with established anti-cancer activity, we evaluated the effects of T3F on tumor cell growth and macrophage polarization, as thrombin contributes to tumor progression and inflammatory signaling. T3F showed anti-proliferative effects against tumor cells and promoted M1-associated responses, including increased NOS2 expression and TNF-α production. These findings suggest that ANH-modified thrombin aptamers may provide insight into the relationship between thrombin inhibition, tumor cell growth, and macrophage-associated immune responses.

P94 Selective oncolysis via oncomiR-driven DNA self assembly

T. Aiba, K. Morihiro and A. Okamoto
Department of Chemistry and Biotechnology, Graduate School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan

Artificial nucleic acids can activate innate immunity and are attractive candidates for cancer immunotherapy. However, their poor cancer selectivity often causes systemic immunotoxicity. To address this issue, we developed a hairpin DNA self-assembly system that selectively activates immune responses in cancer cells.

The system comprises two engineered hairpin DNAs (oHPs) that respond to the oncogenic microRNA miR-21, which is overexpressed in many cancers. Upon miR-21 recognition, the oHPs self-assemble into long double-stranded DNA aggregates that activate the intracellular DNA sensor cGAS. This triggers the cGAS–STING pathway, leading to IFN-β production and selective killing of miR-21-positive cancer cells.

In tumor-bearing mice, intratumoral administration of oHPs using an appropriate delivery system significantly suppressed tumor growth and promoted the accumulation of $CD8^+$ and $CD4^+$ T cells around tumors. Antitumor efficacy was further enhanced when oHPs were combined with anti-PD-1 antibody treatment.

These results establish intracellular DNA self-assembly as a novel cancer-selective immune amplification strategy and highlight its potential as an effective platform for targeted cancer immunotherapy.

P95 The IκB Protein Charon Is Essential for Relish Activation in the Drosophila STING Pathway

T. Lorentzen[1], W. Luo[1,2], J. Nguyen[1], G. Haas[1], H. Cai[2], C. Meignin[1] and JL. Imler[1,2].
[1]University of Strasbourg, M3i CNRS UPR9022, Strasbourg, France.
[2]Sino-French Hoffman institute, Guangzhou Medical University, Guangzhou, China.

The STING pathway is an evolutionarily conserved signaling module that plays a central role in antiviral immunity. In mammals, STING activates inflammatory and antiviral responses through IRF3 and NF-κB, but the mechanisms controlling NF-κB activation and transcriptional specificity remain poorly understood. In *Drosophila melanogaster*, viral infection activates STING through cyclic dinucleotides produced by cGAS-like receptors. STING signaling induces an antiviral transcriptional program via the kinase IKKβ and the p105-like NF-κB transcription factor Relish. Interestingly, Relish also mediates the antibacterial responses downstream of the IMD pathway, raising the question of how distinct transcriptional outputs are generated downstream of STING and IMD. Among the genes induced by STING activation in several drosophila species but also in honeybees, we identified Charon, a nuclear IκB-like protein harboring ankyrin repeats that interacts with Relish. Here, we show that Charon is specifically required for STING signaling, while being dispensable for the IMD pathway. Our data indicate that Charon promotes the recruitment of Relish to the promoters of STING-regulated genes, leading to gene expression and triggering antiviral immunity. These findings identify Charon as a novel regulator of STING-dependent NF-κB signaling in *Drosophila* and reveal intriguing similarities between the functions of Charon in insects and IκBζ in mammals.

P96 A 3‘-UTR Regulatory Element in the MDA5 Regulator Gene RIOK3 Controls the Hemoglobin Switch in Humans

Bjorg Gudmundsdottir[1], John F. Tisdale[1]
[1]Cellular and Molecular Therapeutics, National Heart, Lung, and Blood Institute, National Institutes of Health; Bethesda, MD, USA.

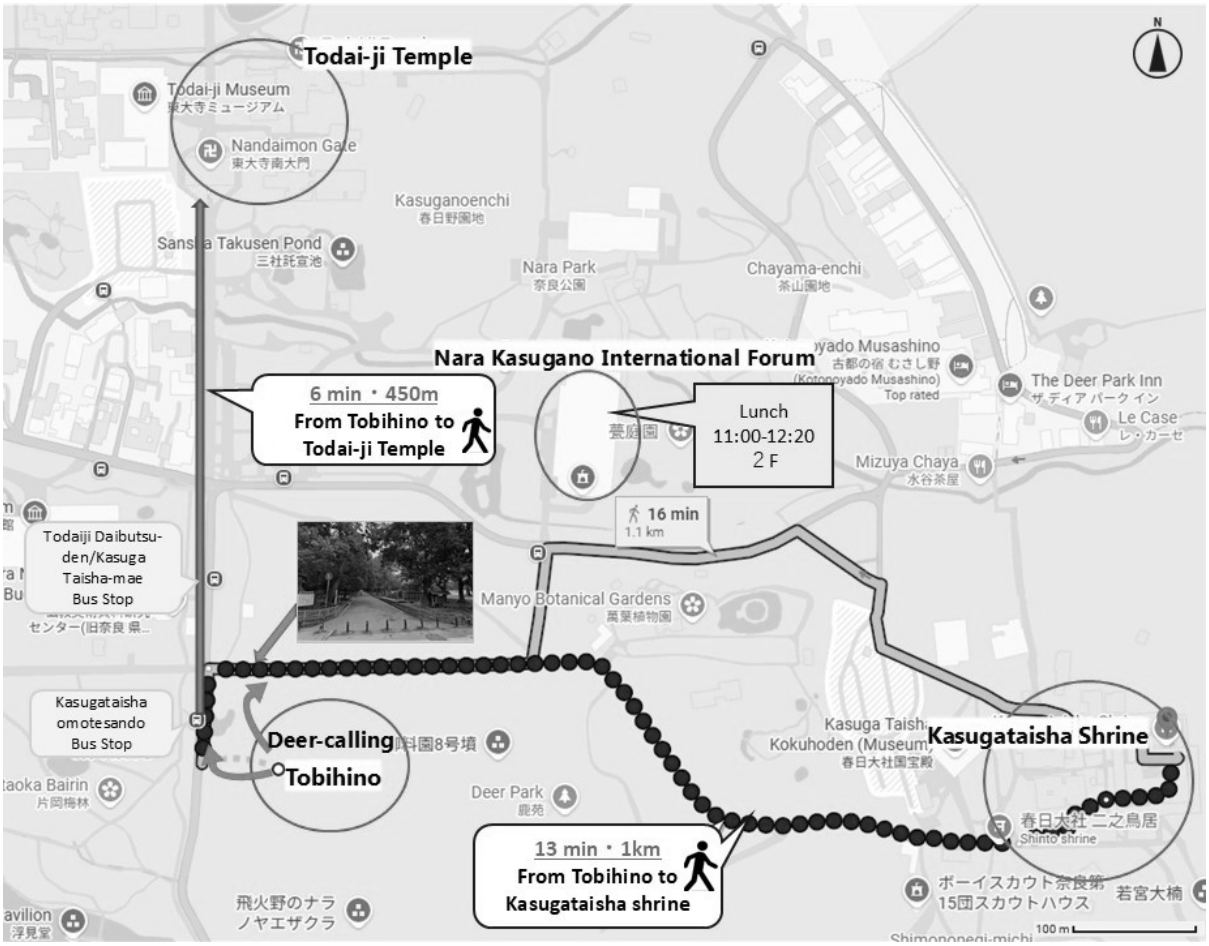
Sickle cell disease (SCD) is caused by a point mutation in the adult β-like globin gene, HBB, leading to hemoglobin polymerization in erythroid cells. Because reactivating fetal hemoglobin expression can alleviate SCD symptoms, significant effort has focused on elucidating the mechanisms of fetal hemoglobin repression in adults. We have found that the serine/threonine kinase RIO kinase 3 (RIOK3) plays a key role in the switch from fetal to adult beta globin expression. RIOK3 is a critical component of the innate immune response, phosphorylating and inhibiting the multimerization of the double-stranded RNA sensor MDA5. Knocking down a specific *RIOK3* transcript via the 3’-UTR in human erythroblasts completely switches hemoglobin production from adult to fetal hemoglobin, as measured by RT-qPCR and HPLC. Importantly, knocking down the transcript in erythroblasts from SCD patients completely prevents sickling in an in vitro sickling assay. RIOK3 immunoprecipitation and mass spectrometry in developing erythrocytes demonstrate that RIOK3 interacts with RNA-binding and innate immunity proteins. These data raise important questions about the roles of RNA-sensing and innate immunity-specific pathways in regulating hemoglobin expression in humans.

Excursion on September 17

Deer-Calling Experience
Meeting Time: 9:00AM
Meeting Point: Tobihino, Nara
Please make your own way to the meeting point and arrive by 9:00 AM.
The meeting point is approximately 550 m from the conference venue, about a 7-minute walk.
Alternatively, from JR Nara Station or Kintetsu Nara Station, take **Route 2 (City Loop, Clockwise), a local city bus**, and get off at **Kasuga Taisha Omotesando Bus Stop**. From there, it is approximately a 1-minute walk to the meeting point.

Nara is a unique city in Japan where wild deer freely roam the streets and parks as part of everyday life.
The “Shikayose” (Deer Calling) is a traditional Nara experience in which deer that have spent the night resting in the mountains are called down by the sound of a horn. This will be a truly special experience during your stay in Japan.
Participation is free of charge for those who wish to join.

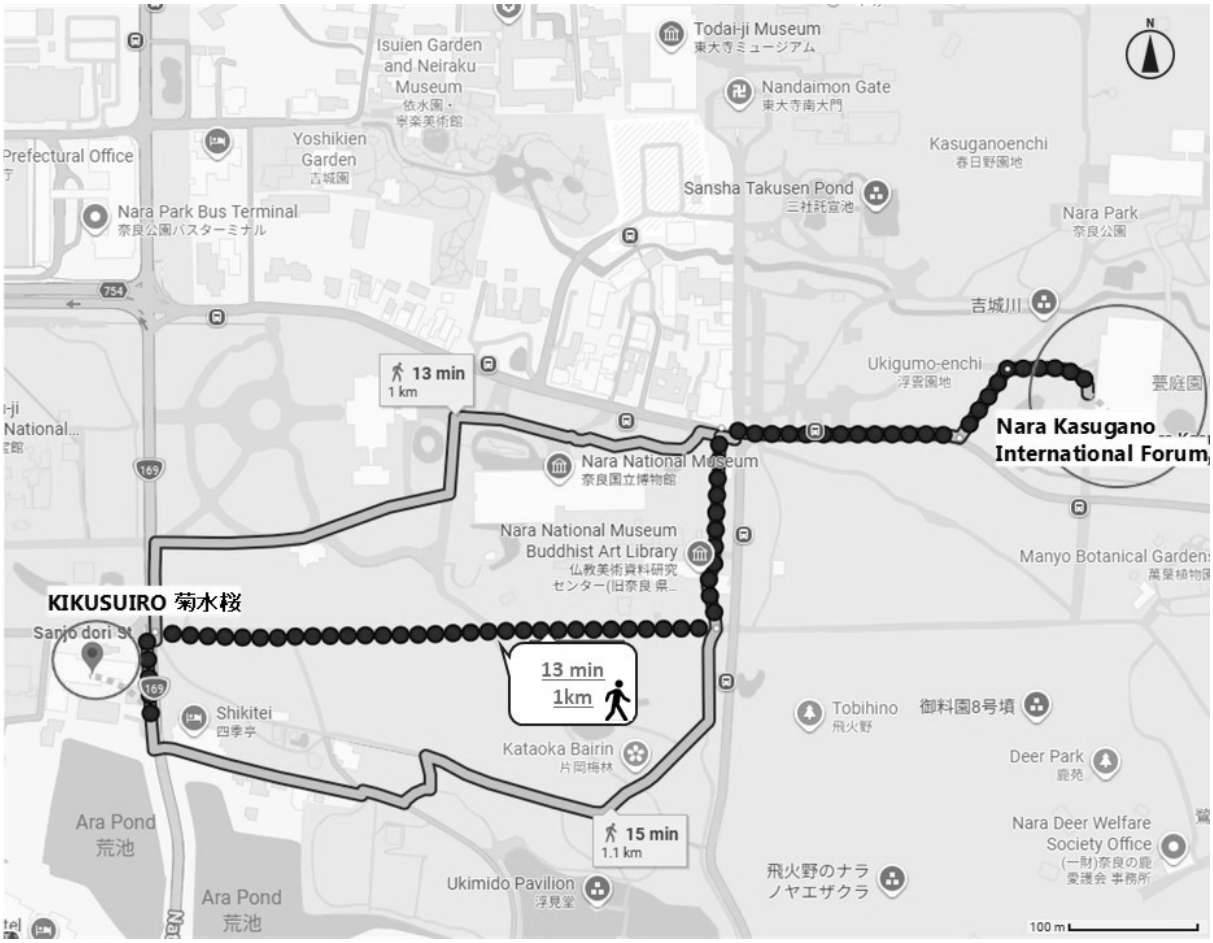
After the Deer Calling Experience (around 9:30), participants will have free time until the start of the afternoon sessions. During this time, they may visit nearby sites that represent Japan’s rich cultural heritage, such as **Todai-ji Temple** and **Kasuga Taisha Shrine**, both of which are part of the UNESCO World Heritage Site “Historic Monuments of Ancient Nara.



Meet the Experts on September 17

Date & Time: September 17, 2026, 18:00
Venue: Kikusuiro Japanese Restaurant

Participation is limited to those who have registered for the dinner in advance, as well as invited speakers and Committee Members.
The restaurant is approximately 1 km from the conference venue, about a 13-minute walk. A member of the NAIS26 team will accompany participants and guide them to the restaurant.
A seated dinner will be held in an intimate setting with guest speakers who are experts in their respective fields. Participants will have the opportunity to enjoy an authentic traditional Japanese dinner while interacting with the invited speakers and other participants.



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