WPI Immunology Frontier Research Center 2023-2024

Osaka University

Immunology Frontier Research Center

Annual Report of IFReC 2023-2024

Osaka University

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World Premier International Research Center Initiative

WPI Osaka University iFReC

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Message from the Director

As the Director of the Immunology Frontier Research Center (WPI-IFReC) at Osaka University, I am very pleased to present the IFReC annual report for fiscal year 2023. Since joining the WPI Academy in 2017, we at IFReC have pioneered a unique academic-industry partnership that unleashes new possibilities in collaborative research. IFReC goes beyond basic research and social engagement, and aims to significantly contribute to our university's educational system by developing graduate programs specifically for international students specializing in immunology, with details to be announced in 2024.

After the COVID-19 pandemic began to subside in late 2022, Japan officially downgraded COVID-19 in May 2023 to the same category that includes seasonal influenza. This led to the relaxation or complete removal of various restrictive measures, allowing many regions to resume their pre-COVID-19 activities. Universities, for example, have successfully transitioned back to in-person classes and events.

Building on the success of the inaugural event, IFReC and ImmunoSensation^2 (University of Bonn) again co-organized the second "International School on Advanced Immunology" in FY2023 leading to discussions about future collaborations. Additionally, IFReC co-organized two symposia: the "Finnish-Japanese Immunology Symposium" in Finland and the "IFReC-Doherty Institute and Partners Immunology Symposium" in Osaka. Moreover, we successfully hosted "The International Symposium on Microbiology and Immunology," our 13th international symposium. These events brought together international researchers and facilitated high-level discussions on the frontiers in immunology.

IFReC plays a central role in immunology and infectious disease research, fostering collaborations among various research departments and institutes such as the Research Institute for Microbial Diseases (RIMD), the Center for Infectious Diseases Education and Research (CiDER) and the Center for Advanced Modalities and DDS (CAMaD) at Osaka University. We are committed in our efforts to continue basic research in immunology and to seek ways to make meaningful contributions to society, and through research and education, we will drive the advancement of science and shape the future of immunology research worldwide.

Kiyoshi TAKEDA, MD / PhD
Director
WPI Immunology Frontier Research Center
Osaka University

Organization

Organization Chart

As of March 2024

Osaka University
International Advanced Research Institute (IARI)

MEXT
WPI Initiative Program Committee
JSPS

WPI Osaka University
iFReC

- International Scientific Advisory Board
- Director
- Research Groups
  - Immunology Groups
  - Imaging Groups
  - Informatics Groups
  - Unit for Combined Research Fields
- Management Committee
  - Board of Representatives
- Deputy Directors
- Administrative Director
  - Administrative Department
    - General Affairs Section
    - Accounting Section
- Research Planning and Management Office

Joint Research Chairs with the Pharmaceutical Companies

- Innovative Drug Discovery in Immunology (Chugai Pharma)
- Immune-Therapeutic Drug Discovery (Otsuka Pharma)
- Innate Immunity
- Innovative Drug Discovery in Host Defense

Common Facilities

- Core Instrumentation Facility
- Animal Resource Center for Infectious Diseases
- Network Administration Office

Administrative Office

General Affairs Section

- Employment and acceptance procedures for researchers and staff
- Social insurance (for part-time staff), employment insurance
- Various research support (MTA, patents, animal experiments, safety etc.)
- Support for international researchers and students
- IFReC Kishimoto Foundation Fellowship Program

Accounting Section

- Budget drafting / implementation / management
- Acceptance and implementation of third-party funding
- Purchasing and payment of payroll, travel expense and honorarium
- Health insurance procedures
- Management of facilities

Research Planning and Management Office

- Research promotion and support (consultation for grants and patents, etc.)
- Establishing research environments (facility and safety management, research agreement, etc.)
- Fostering young scientists (Winter School, Advanced Postdoc Program, orientation, etc.)
- Organizing scientific events (symposia, colloquia, seminars, etc.)
- Public relations (publishing, website, outreach to citizens, etc.)

Cooperative Institutions

- Institute for Frontier Life and Medical Sciences, Kyoto University, Japan
- RIKEN Center for Integrative Medical Sciences, Japan
- University College London, UK
- ImmunoSensation^2, Cluster of Excellence, the Rheinische Friedrich-Wilhelms-University of Bonn, Germany
- The Peter Doherty Institute for Infection and Immunity, the University of Melbourne, Australia

Committees & Advisory Board for IFReC

The World Premier International Research Center Initiative (WPI)

Program Director

As of November, 2023

Akira UKAWA	WPI Program Director and Academy Director

Program Committee Members

Michinari HAMAGUCHI (Chairperson)	Director General, Strategic Center Biomedical Advanced Vaccine Research and Development for Preparedness and Response(SCARDA), Japan Agency for Medical Research and Development (AMED) Councelor to the President, Japan Science and Technology Agency (JST)
Mariko HASEGAWA	President, Japan Arts Council
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Takaaki KAJITA	Professor, Institute for Cosmic Ray Research, The University of Tokyo Nobel laureate in Physics (2015)
Maki KAWAI	President, National Institutes of Natural Sciences
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Ryozo NAGAI	President, Jichi Medical University
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Richard DASHER	Director, US-Asia Technology Management Center, Stanford University, USA
Victor Joseph DZAU	President, National Academy of Medicine, USA
Pavel KABAT	Secretary-General, International Human Frontier Sciences Program Organization (HFSPO), France
Matthias KLEINER	University Professor, Technical University Dortmund, Germany
LIM Chuan Poh	Chairman, Singapore Food Agency (SFA)
Mona NEMER	Chief Science Advisor, Government of Canada
Jean ZINN-JUSTIN	Scientific adviser, Institute of Research into the Fundamental Laws of the Universe (IRFU/CEA), France

WPI Academy

In 2017, MEXT established the WPI Academy to be the vanguard in internationalizing and further renovating Japan's research environment. The WPI Academy is a much-anticipated upgrade of WPI institutes,and is expected to position Japan as a hub at the pinnacle of international researcher circulation. In the decade ahead, the research institutes of WPI and WPI Academy will work together to hold public relations and outreach activities.

Program Officer for IFReC

Kouji MATSUSHIMA	Professor, Research Institute for Biomedical Sciences, Tokyo University of Science

International Scientific Advisory Board for IFReC

As of March, 2024

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Anne O'GARRA	The Francis Crick Institute, UK
Jeffrey RAVETCH	Rockefeller University, USA
Art WEISS	University of California, San Francisco/Howard Hughes Medical Institute, USA
Joachim SCHULTZ	DZNE/LIMES Institute, University of Bonn, Germany
Kayo INABA	Japan Agency for Medical Research and Development, Japan
Kazuhiko YAMAMOTO	RIKEN Center for Integrative Medical Sciences, Japan
Osamu OHARA	Kazusa DNA Research Institute, Japan
Hiroshi KIYONO	Future Medicine Education and Research Organization at Chiba University, Japan

Laboratories

As of March 2024

Host Defense

Shizuo Akira, MD/PhD

Professor	Shizuo Akira
Associate Professor	Kazuhiko Maeda
Assistant Professor	Kiyoharu Fukushima
Postdoctoral Fellow	3
Research Assistant	4
Visiting Scientist	3
Support Staff	4

Our research is dedicated to unraveling the intricate mechanisms of host defense against a spectrum of pathogens, encompassing both innate and acquired immunity. Additionally, we are committed to developing efficacious therapeutic interventions for immune-related disorders. A focal point of our investigation lies in understanding the function of Regnase-1, an RNA-binding protein (RBP) implicated in autoimmune disease development. Specifically, we are exploring how Regnase-1 degrades a subset of inflammatory mRNAs, thus contributing to the maintenance of a finely tuned immune response. The insights gained from our recent research not only shed light on Regnase-1's role in natural killer (NK) cells but also unveil its self-regulatory mechanism and enzymatic activity in RNA degradation. This knowledge has the potential to pave the way for novel strategies aimed at preventing autoimmune diseases.

Role of Regnase-1 in NK cells

Recent studies have emphasized the critical role of Regnase-1/ZC3H12A in the function and metabolism of CD8+ T cells, as well as in maintaining B cell balance to prevent immune-related disorders. However, how Regnase-1 regulates NK cell signaling and their anti-tumor functions remains unclear. To investigate, we used mice with NK cell-specific *Regnase-1* knockout (*Reg1*$^{\Delta NK}$). We found that knocking out Regnase-1 enhances the ability of NK cells to fight tumors by increasing IFN-γ and cytolytic protein production. Analysis of NK cells from the spleen and tumor-infiltrating NK cells revealed an activated subpopulation in *Reg1*$^{\Delta NK}$-NK cells, with high expression of cytotoxic genes. Additionally, we also found increased expression of CXCR6 in *Reg1*$^{\Delta NK}$-NK cells, which typically remains low in healthy NK cells, promoting their infiltration into tumors. The enhanced anti-tumor activity and tumor infiltration of *Reg1*$^{\Delta NK}$-NK cells were linked to the IFN-γ signaling pathway. Blocking IFN-γ prevented the accumulation of CXCR6+ *Regnase-1*-deficient NK cells in tumors and CXCL16 expression in the tumor microenvironment. Furthermore, *Reg1*$^{\Delta NK}$-NK cells showed increased expression of octamer-binding protein 2 (OCT2) and IκBζ, which form a complex with NF-κB and bind to the *Ifng* promoter, enhancing its transcription. These findings suggest that inhibiting Regnase-1 in NK cells could enhance their anti-tumor activity and infiltration into tumors. This could lead to innovative strategies, such as *Regnase-1* deleted chimeric antigen receptor (CAR)-NK therapy, to improve NK cell-based immunotherapies against solid tumors.

Regulation of Regnase-1 3'UTR

Regnase-1 autoregulates its own expression by binding to stem-loop (SL) RNA structures in its 3'UTR and cleaving its mRNA. To study the importance of Regnase-1-mediated autoregulation in immune responses, we created mice with a 2-bp deletion in the target SL of the *Regnase-1* 3'UTR. This deletion prevented SL formation, reducing Regnase-1-mediated mRNA degradation. The mutant mice showed normal hematopoietic cell differentiation. Biochemically, the mutation increased Regnase-1 mRNA stability and levels of both Regnase-1 mRNA and protein in mouse embryonic fibroblasts (MEFs). *Il6*, a Regnase-1 target gene, was consistently suppressed in mutant MEFs. Additionally, Regnase-1 protein levels were higher in mutant MEFs compared to wild-type MEFs both at baseline and after proinflammatory cytokine stimulation. These findings suggest a negative feedback loop for Regnase-1 expression and offer a unique model for studying Regnase-1 overexpression *in vivo*. Further validation is currently underway in skin inflammation and lung inflammation models.

Role of Nuclease-null Regnase-1

Regnase-1 is an RNase that plays a crucial role in dampening immune responses by breaking down inflammatory mRNAs. We created mice with a single point mutation in the catalytic center of Regnase-1's RNase domain (D141N), rendering it unable to function as an endonuclease. The D141N mutant mice developed systemic inflammation, immune cell infiltration in multiple organs, and progressive lung granuloma formation. In D141N mutant mice, CD4+ T cells, which are mainly affected by this mutation, exhibited heightened activity in the mTORC1 pathway, leading to autoimmune characteristics. RNA-seq analysis revealed high expression of the serine-threonine kinase *Pim2*, which is involved in the inflammation of the lungs in these mice. Inhibition of Pim2 kinase activity improved granulomatous inflammation, reduced immune cell infiltration and proliferation in the lungs, and decreased adhesion molecule expression on CD4+ T cells. In this study, we verified that *Pim2* is indeed a target gene of Regnase-1, suggesting that Pim2 is involved in facilitating the adhesion and migration of immune cells to inflamed tissues. Our findings shed light on the importance of Regnase-1's RNase activity in immune regulation and highlight the potential of targeting Pim2 as a therapeutic strategy for modulating abnormal immune responses.

Exploration of Cellular Dynamism and Crosstalk in Lung Fibrosis

Fibrosis is a life-threatening disease with unknown causes. The activation of monocytes and macrophages is associated with fibrosis development, but the underlying pathogenesis remains poorly understood. Currently, we are trying to understand the complex spatial and intercellular dynamics of fibrotic lesions and clarify how fibroblast activation are maintained in fibrotic lungs in association with profibrotic immune cell populations.

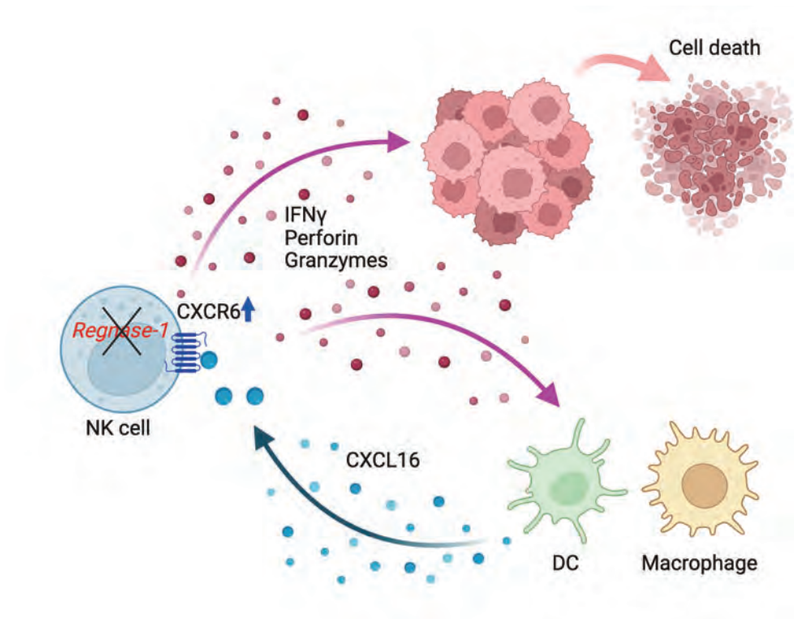


Figure.
The accumulation of NK cell and their heightened production of cytotoxic proteins within the tumor microenvironment bolster the anti-tumor activity of *Reg1*$^{\Delta NK}$.

Recent Publications

1. Sun X, Nagahama Y, et al. Deletion of the mRNA endonuclease Regnase-1 promotes NK cell anti-tumor activity via OCT2-dependent transcription of Ifng. Immunity. (2024).
2. Lu Y, et al. CGRP sensory neurons promote tissue healing via neutrophils and macrophages. Nature. 628:604-611 (2024).
3. Kawai T, et al. Decoding Toll-like receptors: Recent insights and perspectives in innate immunity. Immunity. 57:649-673 (2024).
4. Akira S and Maeda K. Control of RNA stability in immunity. Ann Rev Immunol. 39:481-509 (2021).
5. Fukushima K, et al. Dysregulated expression of the nuclear exosome targeting complex component Rbm7 in non-hematopoietic cells licenses the development of fibrosis. Immunity. 52:542-556 (2020).

Immunoglycobiology

Taroh Kinoshita, PhD
Yoshiko Murakami, MD/PhD (Co-PI)

Professor	Taroh Kinoshita
	Yoshiko Murakami
Research Assistant	1
Visiting Scientist	2
Support Staff	3

Our major interest has been biosynthesis and deficiencies of glycosylphosphatidylinositols (GPIs), which are a class of glycolipids that serve as membrane anchors for more than 150 different cell surface proteins in mammals, in addition to being present as free glycolipids. In FY2023, we made the following progress.

Role of ARV1 in GPI biosynthesis

ARV1 is an ER-resident membrane protein involved in the homeostasis of various lipids including GPI. However, the mechanism by which ARV1 regulates GPI biosynthesis has not yet been fully elucidated. We reported last year that ARV1 associates with PIGQ, a component of the initial enzyme complex in the GPI biosynthesis pathway, and is required for the upregulation of GPI biosynthesis (Liu Y-S et al, J. Cell Biol., 2023). We further extended our study on ARV1's role in the initial enzyme, GPI-N-acetylglucosamine transferase, and found that ARV1 is required for the efficient usage of phosphatidylinositol (PI) by this enzyme complex. In fact, the GPI-N-acetylglucosamine transferase complex containing ARV1, but not the complex lacking ARV1, had associated PI and used it to generate N-acetylglucosamine-PI upon the addition of UDP-N-acetylglucosamine (Lu T et al, manuscript in preparation).

Origin of alkyl-acyl glycerol in mammalian GPIs

PI moiety in GPIs is derived from cellular PI, however, the PI in mammalian GPI's is mainly in the alkyl-acyl form, whereas cellar PI is usually in the diacyl form. The mechanism by which alkyl-acyl PI moiety is generated has been unclear. We showed that mammalian cells contain small amounts of alkyl-acyl PIs and preferentially use them for GPI synthesis. Like other ether phospholipids, the generation of alkyl-acyl PI is dependent on the alkyl-phospholipid biosynthesis pathway present in the peroxisome, which starts from dihydroxyacetone phosphate (Li X et al, manuscript in preparation).

Gene therapy of inherited GPI deficiency model mice

To establish effective therapies for inherited GPI deficiency, we generated model mice by knocking out the *Piga* gene in the brain. These model mice exhibited more severe symptoms than those observed in humans with inherited GPI deficiency. Gene therapy using Adeno-associated virus PHP.eB (AAV-PHP.eB), aimed at systemically restoring normal *Piga* mRNA levels in the model mice, was effective in prolonging lifespan and ameliorating some symptoms (Murakami Y et al, Mol. Therapy — Methods Clin. Dev., 2023). These results, together with our previously reported gene therapy results with *Pigo* mutant model mice (Kuwayama R et al, Nat Commun., 2022), suggest that AAV-mediated gene replacement is a promising approach for the effective treatment of inherited GPI deficiency.

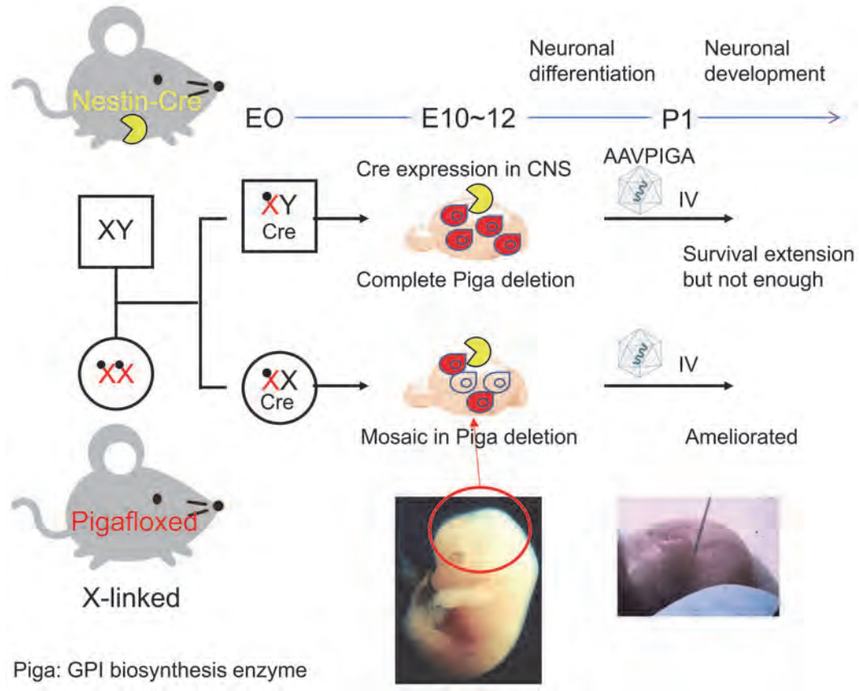


Figure.
Gene therapy in inherited GPI deficiency model mice. Mice with brain-specific KO of the *Piga* gene were intravenously injected on days 1-2 with AAV-PHP.eB carrying PIGA cDNA. Due to the X-linkage of *Piga*, heterozygous KO females become mosaic with GPI-expressing and -deficient cells in the brain. Survival was extended in both males and females, and some neurological phenotypes were ameliorated. (cited from Murakami Y et al, Mol. Ther. Methods Clin. Dev., 14:32(1):101176 (2023))

Recent Publications

1. Murakami Y, Umeshita S, Imanishi K, Yoshioka Y, Ninomiya A, Sunabori T, Likhite S, Koike M, Meyer KC and Kinoshita T. AAV-based gene therapy ameliorated central nervous system specific GPI defect in mouse models. Mol Ther Methods Clin Dev. 14:32(1):101176 (2023).
2. Liu IS, Wang Y, Zhou X, Zhang L, Gao XD, Murakami Y, Fujita M and Kinoshita T. Accumulated precursors of specific GPI-anchored proteins upregulate GPI biosynthesis with ARV1. J Cell Biol. 222: e202208159 (2023).
3. Kuwayama R, Suzuki K, Yoshioka Y, et al. Establishment of a mouse model of inherited PIGO deficiency and therapeutic potential of AAV-based gene therapy. Nat Commun. 13:3107 (2022).
4. Ishida M, Maki Y, Ninomiya A, et al. Ethanolamine-phosphate on the second mannose is a preferential bridge for some GPI-anchored proteins. EMBO Rep. e54352 (2022).
5. Wang Y, Menon AK, Maki Y, et al. Genome-wide CRISPR screen reveals CLPTM1L as a lipid scramblase required for efficient glycosylphosphatidylinositol biosynthesis. Proc Natl Acad Sci USA. 119(14): e2115083119 (2022).

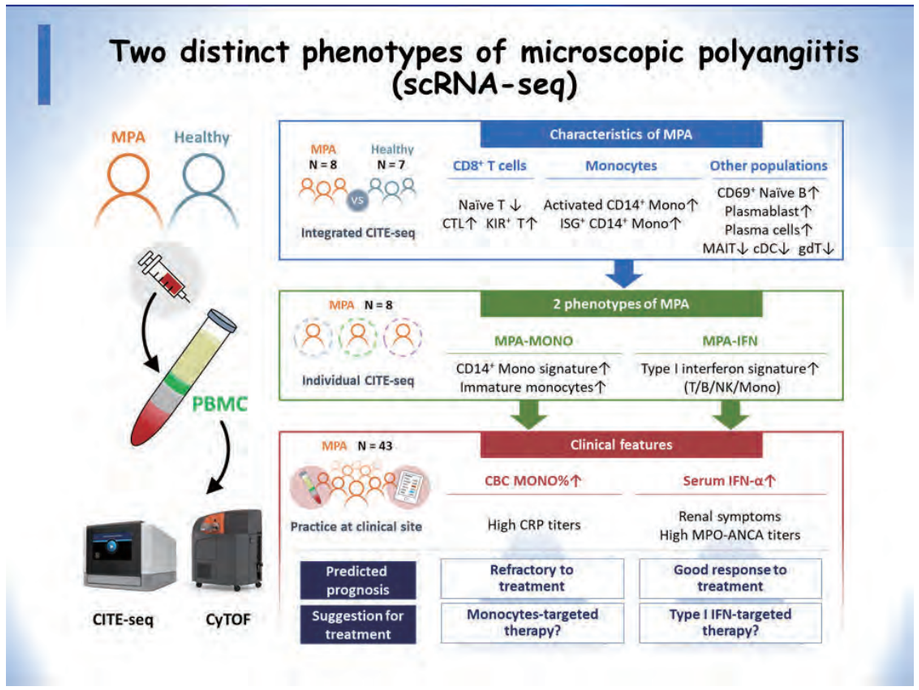
Immunopathology

Atsushi Kumanogoh, MD/PhD

Professor	Atsushi Kumanogoh
Assistant Professor	Takahiro Kawasaki
Research Assistant	5
Support Staff	5

Our research team is involved in two approaches, that is, basic and clinical immunology. As basic aspects of our projects, our proposed study is the regulation of immune cell motility and migratory behavior *in vivo* by soluble and membrane-bound 'immune guidance molecules' such as semaphorins and their receptors. Semaphorins were originally identified as axon-guidance molecules that function during neuronal development. However, cumulative evidence indicates that semaphorins also participate in immune responses, both physiological and pathological, and they are now considered to be potential diagnostic and/or therapeutic targets for a range of diseases. Beyond such basic implications, we are trying to apply the findings from this proposed study into the diagnosis/therapy for human immunological disorders, such as autoimmunity, allergy, immune deficiency, cancer/metastasis, and neurodegenerative diseases. We recently focus on how to apply the findings obtained by bench work into the bedside. Under such research background, we have performed single cell analysis on patients with ANCA-associated vasculitis.

The immunological basis of the clinical heterogeneity in autoimmune vasculitis remains poorly understood. In this study, we conduct single-cell transcriptome analyses on peripheral blood mononuclear cells (PBMCs) from newly-onset patients with microscopic polyangiitis (MPA). Increased proportions of activated $CD14^+$ monocytes and $CD14^+$ monocytes expressing interferon signature genes (ISGs) are distinctive features of MPA. Patient-specific analysis further classifies MPA into two groups. The MPA-MONO group is characterized by a high proportion of activated $CD14^+$ monocytes, which persist before and after immunosuppressive therapy. These patients are clinically defined by increased monocyte ratio in the total PBMC count and have a high relapse rate. The MPA-IFN group is characterized by a high proportion of ISG^+ $CD14^+$ monocytes. These patients are clinically defined by high serum interferon-alpha concentrations and show good response to immunosuppressive therapy. Our findings identify the immunological phenotypes of MPA and provide clinical insights for personalized treatment and accurate prognostic prediction.



Recent Publications

1. Nishide M, Shimagammi H and A Kumanogoh A. Single-cell analysis in rheumatic and allergic diseases: Beyond the sea of data, challenges for clinical application. Nat Rev Immunol. (in press).
2. Naito Y, et al. Tumor-derived semaphorin 4A improves PD-1-blocking antibody efficacy by enhancing CD8(+) T cell cytotoxicity and proliferation. Sci Adv. 9: eade0718 (2023).
3. Nishide M, et al. Single-cell multi-omics analysis identifies two distinct phenotypes of newly-onset microscopic polyangiitis. Nat Commun. 14:5789 (2023).
4. Edahiro R, et al. Single-cell analyses and host genetics highlight the role of innate immune cells in COVID-19 severity. Nat Gen. 55: 753-767 (2023).
5. Tsujimoto K, et al. The lysosomal Ragulator complex activates NLRP3 inflammasome in vivo via HDAC6. EMBO J. 42: e111389 (2023).

Immunochemistry

Hisashi Arase, MD/PhD

Professor	Hisashi Arase
Assistant Professor	Hui Jin Kazuki Kishida
Postdoctoral Fellow	2
Research Assistant	6
Visiting Scientist	1
Support Staff	4

A) Misfolded proteins complexed with MHC class II molecules are targets for autoimmune diseases.

MHC class II allelic polymorphisms are associated with susceptibility to many autoimmune diseases. However, the role of MHC class II molecules in autoimmune disease susceptibility has remained unclear. We have found that misfolded cellular autoantigens are rescued from protein degradation by MHC class II molecules (*Int. Immunol*. 2013). Moreover, we discovered that misfolded proteins complexed with MHC class II molecules serve as targets for autoantibodies in autoimmune diseases such as rheumatoid arthritis, antiphospholipid syndrome, and ANCA-associated vasculitis (*PNAS* 2014; *Blood*. 2015; *Br. J. Dermatol*. 2017; *Arthritis Rheumatol*. 2017; *Arthritis Rheumatol*. 2021). We also found a strong correlation between autoantibody binding to misfolded proteins transported to the cell surface by MHC class II molecules and susceptibility to autoimmune diseases. Further analyses revealed that self-antigens complexed with MHC II molecules can abrogate self-tolerance, thereby inducing an autoimmune response (*Science Advances* 2022). These findings demonstrate that misfolded proteins, which would normally not be exposed to the immune system, are involved in the pathogenicity of autoimmune diseases as 'neo-self' antigens (Figure 1).

B) Studies on host-pathogen interaction

The immune system has coevolved with infectious diseases, which suggests that studies on host-pathogen interactions are crucial for understanding the immune system. We have found that PILRα, a member of the inhibitory paired receptors, plays a crucial role in regulating the immune response (*Nat. Immunol*. 2012; *Int. Immunol*. 2015; *Eur. J. Immunol*. 2016), as well as in HSV-1 infection (*Cell* 2008; *J. Virol*. 2009). Similarly, Siglec-4 and Siglec-7, which are also paired receptors, are implicated in VZV infection (*PNAS* 2010; *BBRC* 2022). The LILR family represents another group of paired receptors. We discovered that the activation of LILRA2 is involved in detecting immunoglobulin abnormalities in microbial infection (*Nature Microbiology* 2016). Additionally, we found that RIFINs, which are products of the multigene family of Plasmodium falciparum, contribute to immune evasion by binding to the inhibitory receptors LILRB1 and LILRB2 (Figure 1. *Nature* 2017; *Nature* 2020; BBRC 2021). From our analysis of SARS-CoV-2 infection, we found that certain antibodies against the spike protein cause conformational changes in the spike protein, thereby enhancing its infectivity (*Cell* 2021, Figure 2). This finding suggests that SARS-CoV-2 may use antibodies to enhance its infectivity. These findings underscore the crucial role of host-pathogen interactions in regulating infectious diseases.

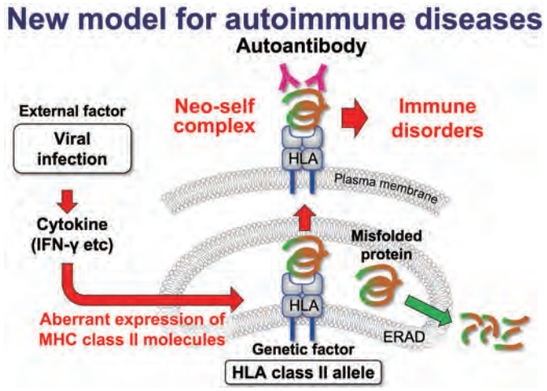


Figure 1.
Misfolded proteins transported to the cell surface by MHC class II molecules are targets for autoantibodies.
Misfolded cellular proteins are generally degraded in the cells and are not transported to outside the cells. Therefore, misfolded proteins transported to the cell surface by MHC class II molecules may be recognized as 'neo-self' antigens by the immune system, which initiates an aberrant immune response to self-antigens (*Int. Immunol.* 2013; *PNAS* 2014; *Blood* 2015, *Br. J. Dermatol*. 2017; *Arthritis Rheumatol*. 2017, *Arthritis Rheumatol*. 2020; *Arthritis Rheumatol*. 2022; *Science Advances* 2022).

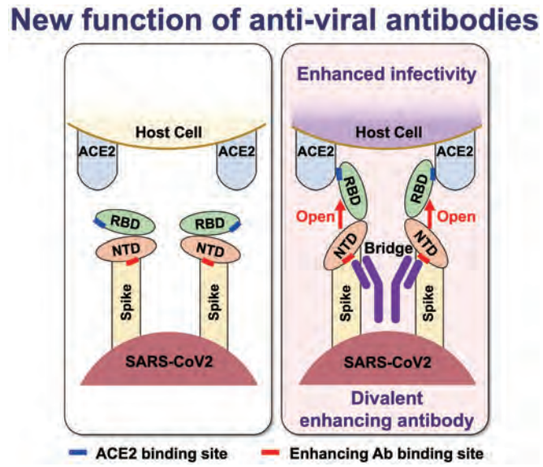


Figure 2.
SARS-CoV-2 infectivity enhancing antibodies.
Some antibodies against NTD of spike protein induces conformational change in spike protein to induce the open form of RBD and enhance the infectivity of SARS-CoV-2 (*Cell* 2021).

Recent Publications

1. Jin H, Kishida K, Arase N, Matsuoka S, Nakai W, Kohyama M, Suenaga T, Yamamoto K, Sasazuki T, Arase H. Abrogation of self-tolerance by misfolded self-antigens complexed with MHC class II molecules. Sci Adv. 8: eabj9867 (2022).
2. Liu Y, Soh WT, Tada A, Arakawa A, Matsuoka S, Nakayama EE, Li S, Ono C, Torii S, Kishida K, Jin H, Nakai W, Arase N, Nakagawa A, Shindo Y, Kohyama M, Nakagami H, Tomii K, Ohmura K, Ohshima S, Okada M, MatsuuraY, Standley DM, Shioda T, Arase H. An infectivity-enhancing site on the SARS-CoV-2 spike protein is targeted by COVID-19 patient antibodies. Cell. 184:3452-3466 (2021).
3. Saito F, et al. Immune evasion of Plasmodium falciparum by RIFIN via inhibitory receptors. Nature. 552:101-105 (2017).
4. Hiwa R, Ohmura K, Arase N, Jin H, Hirayasu K, Kohyama M, Suenaga T, Saito F, Terao C, Atsumi T, Iwatani H, Mimori T, Arase H. Myeloperoxidase/HLA Class II Complexes Recognized by Autoantibodies in Microscopic Polyangiitis. Arthritis Rheumatol. 69:2069-2080 (2017).
5. Hirayasu K, Saito F, Suenaga T, Shida K, Arase N, Oikawa K, Yamaoka T, Murota H, Chibana H, Nagai H, Nakamura Y, Katayama I, Colonna M, Arase H. LILRA2 is an innate immune sensor for microbially cleaved immunoglobulins. Nat Microbiol. 1:1-7 (2016).

Immune Regulation

Tadamitsu Kishimoto, MD/PhD
Sujin Kang, PhD

Professor	Tadamitsu Kishimoto
Associate Professor	Sujin Kang
Assistant Professor	Hozaifa Metwally
Research Assistant	1
Visiting Scientist	1
Support Staff	8

Gp130–HIF1α axis-induced vascular damage is prevented by the short-term inhibition of IL-6 receptor signaling

Endothelial integrity is important for maintaining immune homeostasis. Previously, we identified that interleukin (IL)-6 trans-signaling induced endothelial activation in the context of cytokine production and activation of the coagulation cascade. Treatment with an anti-IL-6 Receptor (IL-6R) antibody, tocilizumab inhibited endothelial activation and prevented the development of cytokine release syndrome (CRS). Protection against endothelial damage is recognized as a frontline approach to preventing the progression of CRS. Accumulating evidence has demonstrated that interleukin-6 (IL-6) promotes vascular endothelial damage during CRS, although the molecular mechanisms remain to be fully elucidated. Targeting IL-6 receptor signaling delays CRS progression; however, current options are limited by persistent inhibition of the immune system. Here, we show that endothelial IL-6 trans-signaling promoted vascular damage and inflammatory responses via hypoxia-inducible factor-1α (HIF1α)–induced glycolysis. Using pharmacological inhibitors targeting HIF1α activity or mice with the genetic ablation of gp130 in the endothelium, we found that inhibition of IL-6R–HIF1α signaling in endothelial cells protected against vascular injury caused by septic damage and provided survival benefit in a mouse model of sepsis. In addition, we developed a short half-life anti-IL-6R antibody (silent anti-IL-6R antibody) and found that it was highly effective at augmenting survival for sepsis and severe burn by strengthening the endothelial glycocalyx and reducing cytokine storm, and vascular leakage. Together, our data advance the role of endothelial IL-6 trans-signaling in the progression of CRS and indicate a potential therapeutic approach for burns and sepsis (Figure 1).

Threonine Phosphorylation of STAT1 Restricts Interferon Signaling and Promotes Innate Inflammatory Responses

The STAT1 working paradigm has focused on interferons (IFN)-mediated JAK-Tyr701 phosphorylation and antiviral defense. We have shown that lipopolysaccharide (LPS)-induced Thr^{749} (equivalent to mouse Thr^{748}) phosphorylation of STAT1, which alters its target DNA motif, and stimulates the expression of IL-12p40 and ARID5A, which increased the stability of *IL6* mRNA independently of the canonical IFN-JAK pathway in human macrophage. To investigate the physiological relevance of the Thr^{748} phosphorylation of STAT1 in regulating the host's defense and inflammatory responses, we generated genetically-engineered mice expressing phospho-deficient threonine748–to-alanine (T748A) mutant Stat1. T748A mice were resistant to LPS–induced lethality while exhibiting undisturbed IFN signaling and total expression of Stat1. Notably, the T748A point-mutation of Stat1 recapitulates the safeguard effect of the genetic ablation of *Stat1* following LPS-induced lethality, indicating that the Thr^{748} phosphorylation contributes inflammatory functionalities of Stat1. LPS-induced Toll-like receptor 4 endocytosis activates a cell-intrinsic IκB kinase (IKK)–mediated Thr^{748} phosphorylation of Stat1, which promotes macrophages inflammatory response while restricting the IFN and anti-inflammatory responses. Moreover, we found that the Thr^{748} phosphorylation of STAT1 is important for maintaining intestinal epithelial barrier and symbiotic gut microbiome composition. Following dextran sodium sulfate (DSS)-induced colitis, T748A mice phenocopy the detrimental pathology of the complete genetic ablation of *Stat1* as evaluated by weight loss, colon lengths and histopathological analysis. Together with the canonical tyrosine and serine, our identification of the Thr^{748} completes the phosphorylation catalog of the transactivation domain of STAT1, and more generally other STAT proteins. Our findings show that Stat1 displays a phosphorylation-dependent modular functionality in innate immune responses: IFN phospho-tyrosine dependent, and inflammatory phospho-threonine dependent, which provides deeper insight on the combinatorial signaling logic of Stat1 in shaping cell-specific and context-dependent responses (Figure 2).

Arid5a controls intestinal homeostasis by regulation of the cell cycle of plasmablasts

AT-rich interactive domain 5a (Arid5a) plays a crucial role in the immune system through transcriptional and post-transcriptional processes. Arid5a was abundantly expressed in B cells, but the role of Arid5a in B cells remains unclear. To investigate the role of Arid5a in B cells, we generated B cell-specific Arid5a knock-out mice (Arid5a^{BKO}). Immunoglobulin isotypes in the serum were comparable between the control and Arid5a^{BKO} mice, whereas the concentration of IgA in feces was decreased in Arid5a^{BKO} mice. In addition, the percentage of IgA-secreting cells was significantly reduced in the large intestine of Arid5a^{BKO} mice, whereas no significant differences in the small intestine and peyer`s patches. By comparison of the frequency of antibody-secreting cells (ASCs) in the large intestine, we found that the frequency of ASCs was decreased in Arid5a^{BKO} mice. Deficiency of Arid5a in B cells was confirmed to lead to a reduced frequency of plasmablast. Mechanistically, Arid5a modulated the cell cycle of plasmablasts by regulating the expression of Erdr1 upon stimulation with differentiation factors. Moreover, the deficiency of Arid5a affected the IgA-microbiota axis and enhanced the susceptibility to colitis. Collectively, the findings revealed that Arid5a played a crucial role in maintaining intestinal homeostasis by controlling the cell cycle of plasmablasts.

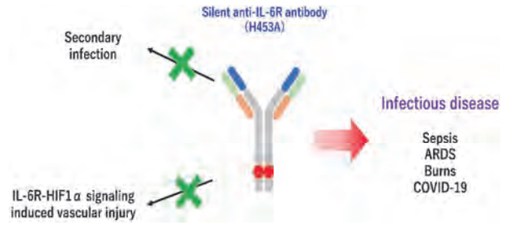


Figure 1.
Giving medication a short half-life anti-IL-6 to patients of Sepsis, ARDS, Burns, etc. is expected to suppress vascular damage or secondary infection.

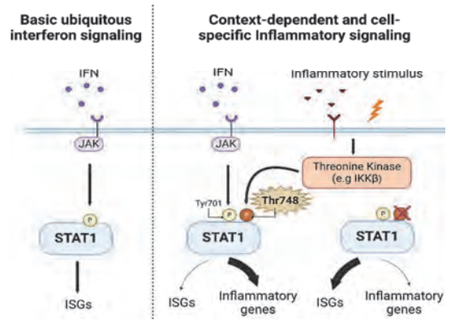


Figure 2.
Threonine phosphorylation of STAT1 regulates the host's inflammatory responses.

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1. Kang S, Onishi S, Ling Z, Inoue H, Zhang Y, Chang H, Zhao H, Wang T, Okuzaki D, Matsuura H, Takamatsu H, Oda J, Kishimoto T. Gp130-HIF1α axis-induced vascular damage is prevented by the short-term inhibition of IL-6 receptor signaling, PNAS. 121(2):e2315898120 (2024).
2. Metwally H, Elbrashy MM, Ozawa T, Okuyama K, White JT, Tulyeu J, Søndergaard JN, Wing JB, Muratsu A, Matsumoto H, Ikawa M, Kishi H, Taniuchi I and Kishimoto T. Threonine Phosphorylation of STAT1 Restricts Interferon Signaling and Promotes Innate Inflammatory Responses. PNAS. 121(17):e2402226121 (2024).
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4. Kishimoto T and Kang S. IL-6 Revisited: From Rheumatoid Arthritis to CAR T Cell Therapy and COVID-19. Annu Rev Immunol. 40:323-348 (2022).

Mucosal Immunology

Kiyoshi Takeda, MD/PhD

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Ulcerative colitis (UC) and Crohn's disease (CD), clinical phenotypes of inflammatory bowel disease (IBD), are chronic disorders of the gastrointestinal tract involving inflammation and ulceration. Genome-wide association studies have identified more than 200 susceptible loci that are either shared by UC and CD or related to one disease. Among them, the gene encoding OTU deubiquitinase 3 (OTUD3) is located in the UC-associated 1p36 locus (*RNF186-OTUD3-PLA2G2E*). In addition, the intronic variant rs10799593 and the missense variant rs758098056 in *OTUD3* were identified as UC risk single nucleotide polymorphisms. In patients with UC, dysbiosis is linked to alterations in the epigenome, transcriptional profile, and metabolism of host cells as well as changes in the composition of host and microbial metabolites. However, it remains poorly understood how UC risk genes interact with the dysbiotic microbiota to influence the onset and/or progression of the disease through modulating host cell physiology.

OTUD3 prevents the aggravation of colitis through modulation of the microbial cGAMP-STING-IFN-β pathway in colonic fibroblasts.

In the murine and human colons, OTUD3 protein was detectable in PDGFRα^{+} fibroblasts, whereas it was not detected in epithelial cells and CD45^{+} lamina propria cells. Accordingly, *Pdgfra-cre*; *Otud3*$^{f/f}$ mice, but not *LysM-cre*; *Otud3*$^{f/f}$, *Villin-cre; Otud3*$^{f/f}$, and *Cd4-cre*; *Otud3*$^{f/f}$ mice, suffered from more severe colitis accompanied by increase in the numbers of neutrophils, eosinophils, CD64^{+} macrophages, and Ly6C^{int} CD11b^{+} dendritic cells compared with *Otud3*$^{f/f}$ mice during administration of dextran sodium sulfate (DSS). Ingenuity pathway analysis based on the results from RNA-seq analysis using colonic fibroblasts exhibited significantly enriched upstream regulators of the genes upregulated in the cells from *Otud3*$^{-/-}$ mice, which included molecules related to the STING-IFN-β signaling pathway. *Otud3*-deficient colonic fibroblasts stimulated with poly(dA:dT) showed enhanced phosphorylation of TBK1 and IRF3 compared with those in wild-type cells. Additionally, IFN-β production in response to poly(dA:dT) was increased in *Otud3*-deficient fibroblasts relative to wild-type cells. During DSS-induced colitis, the introduction of a *Sting* deficiency into *Otud3*$^{-/-}$ mice lessened the clinical parameters and ameliorated large intestinal pathology, indicating that OTUD3 negatively regulates the STING-IFN-β signaling pathway in fibroblasts and thereby prevents the aggravation of colitis.

Promoted transcription of interferon-stimulated genes (ISGs) in fibroblasts from the colon of *Otud3*$^{-/-}$ mice was markedly reduced by antibiotic treatment, implying that OTUD3 downregulates the microbiota-dependent activation of the STING-IFN-β pathway in colonic fibroblasts. Bacteria trigger STING activation without direct interaction with host cells through secreting 3′3′-cGAMP. Elevated intracellular levels of 3′3′-cGAMP in colonic fibroblasts cultured in the presence of 3′3′-cGAMP became undetectable following treatment with the transporter VRAC inhibitor DCPIB. 3′3′-cGAMP-induced phosphorylation of TBK1 and IRF3 was accelerated in colonic fibroblasts from *Otud3*$^{-/-}$ mice compared with that in cells from wild-type mice. UC risk variant rs758098056 in the second exon of *OTUD3* (human OTUD3 (p. R90Q) corresponding to murine OTUD3 (p. R89Q)), is located in the OTU domain of OTUD3. We found that this missense variant links to reduced catalytic activity of OTUD3 against K27-linked polyubiquitination on STING. Compared with wild-type mice, *Otud3*$^{R89Q/R89Q}$ mice showed more severe disease manifestation with worsened large intestinal pathology during DSS-induced colitis. In this context, peritoneal injection of DCPIB reduced severe clinical parameters along with the amelioration of large intestinal pathology in *Otud3*$^{R89Q/R89Q}$ mice. Among patients with UC, carriers that were heterozygous for the rs10799593 in the first intron of *OTUD3* displayed low expression of *OTUD3* mRNA as well as OTUD3 protein in fibroblasts from the non-inflamed site of the colon compared with non-carriers, whereas K27-linked STING polyubiquitination was enhanced in colonic fibroblasts from rs10799593 heterozygous carriers. We found that 3′3′-cGAMP-induced transcription of ISGs was upregulated in colonic fibroblasts from rs10799593 heterozygous carriers. These findings indicate that OTUD3 contributes to prevention of colitis progression by inhibiting excessive activation of the cGAMP-STING-IFN-β pathway in intestinal fibroblasts.

Promotion of 3′3′-cGAMP production in the UC-associated dysbiotic microbiota.

Because a similar allele frequency of rs10799593 was shown between Japanese patients with UC and healthy controls (HCs), we hypothesized that augmented 3′3′-cGAMP production in the microbiota influences intestinal pathology in carriers of *OTUD3* risk variants. We found that the enhanced colonization of cGAMP-producing bacteria species in UC patients. Accordingly, the fecal concentration of 3′3′-cGAMP was high in patients with UC compared with that in HCs. In addition, *Otud3*$^{R89Q/R89Q}$ mice showed pathological features of UC in the colon after transplantation of a fecal microbiome with the potential to produce excessive cGAMP from UC patients. Collectively, these results demonstrate the critical role of OTUD3 in the maintenance of intestinal homeostasis and highlight one mechanism of interaction between the host (OTUD3 in fibroblasts) and microbiota (STING agonist cGAMP) in UC development.

The evidence suggests that UC is an idiopathic, multifactorial, and progressive disorder linked to impaired quality-of-life and an increased risk of fibrosis and colorectal cancer. Therefore, the development of a personalized and optimized approach for facilitating mucosal healing and long-lasting remission is desirable. Given our data in mice and humans, cGAMP transporter VRAC and cGAMP-producing bacteria may be therapeutic targets for patients with UC risk variants in *OTUD3*.

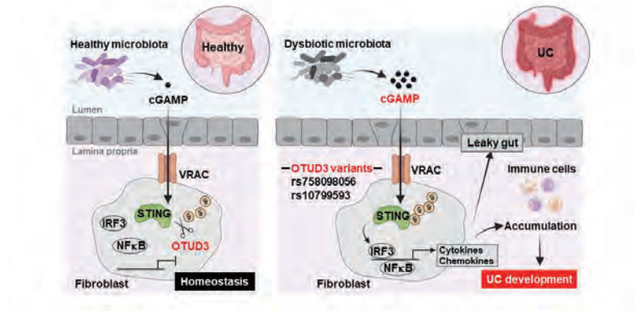

Figure.
OTUD3 prevents ulcerative colitis by modulating microbiota-mediated STING activation.
Left: OTUD3 contributes to maintenance of the large intestinal homeostasis by finely tunning bacterial cGAMP-STING signaling in fibroblasts. Right: Ulcerative colitis (UC) risk variants in *OTUD3* cooperate with dysbiotic microbiota-derived cGAMP to trigger disease progression.

Recent Publications

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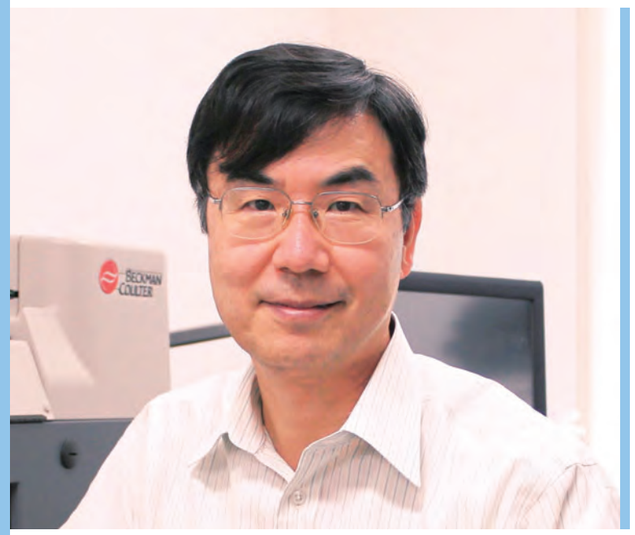
3. Otake-Kasamoto Y, Kayama H, Kishikawa T, Shinzaki S, Tashiro T, Amano T, Tani M, Yoshihara T, Li B, Tani H, Liu L, Hayashi A, Okuzaki D, Motooka D, Nakamura S, Okada Y, Iijima H, Takeda K, Takehara T. Lysophosphatidylserines derived from microbiota in Crohn's disease elicit pathological Th1 response. J Exp Med. 219: e20211291 (2022).

4. Tani H, Li B, Kusu T, Okumura R, Nishimura J, Okuzaki D, Motooka D, Arakawa S, Yoshihara T, Ogino T, Tsai SH, Furuta Y, Muneta M, Nakamura S, Fukusaki E, Yamamoto K, Yagita H, Kayama H and Takeda K: The ATP-hydrolyzing ectoenzyme E-NTPD8 attenuates colitis through modulation of P2X4 receptor-dependent metabolism in myeloid cells. Proc Natl Acad Sci USA. 118: e2100594118, (2021).

5. Morita N, Umemoto E, Fujita S, Hayashi A, Kikuta J, Kimura I, Haneda T, Imai T, Inoue A, Mimuro H, Maeda Y, Kayama H, Okumura R, Aoki J, Okada N, Kida T, Ishii M, Nabeshima R, Takeda K. GPR31-dependent dendrite protrusion of intestinal CX$_3$CR1^{+} cells by bacterial metabolites. Nature 566:110-114 (2019).

Experimental Immunology



Shimon Sakaguchi, MD/PhD

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	Kelvin Chen Yigene
	Tay Jian Wei Christopher
Postdoctoral Fellow	3
Research Assistant	3
Visiting Scientist	16
Support Staff	4

This laboratory studies: (i) the cellular and molecular basis of immunologic self-tolerance, in particular the roles of regulatory T cells (Tregs); (ii) the strategy for eliciting effective immune responses to autologous tumor cells, or inducing immunologic tolerance to organ transplants, by targeting Tregs; and (iii) the cause and pathogenetic mechanism of autoimmune diseases by analyzing the balance between Tregs and autoimmune T cells.

T cells mediate a variety of common autoimmune diseases such as rheumatoid arthritis (RA) and type I diabetes. Both the production of autoreactive conventional T (Tconv) cells through T cell selection in the thymus and their pathogenic activation in the periphery critically depend on T-cell receptor (TCR) signaling upon recognition of self-peptide-bound major histocompatibility complexes (self-pMHCs). In addition, depending on the strength of the interaction between TCRs and self-pMHCs, some developing T cells differentiate into Treg cells, which specifically express the transcription factor Foxp3 and suppress the activation of pathogenic self-reactive T cells that have escaped thymic negative selection. It remains to be determined, however, how qualitative or quantitative alteration of TCR signaling itself should affect thymic generation of Treg and autoreactive Tconv cells and their peripheral functions to cause autoimmune diseases. There is accumulating evidence in humans and rodents that genetic anomalies or variations in TCR-proximal signaling molecules, such as ZAP-70 and LAT, and also in the molecules interacting with them, such as PTPN22 and CBL family proteins, are causative of and predisposing to a variety of autoimmune diseases. Among them, ZAP-70 mutations are unique in that they produce a wide spectrum of immunological disorders encompassing immunodeficiency, autoimmunity, immunopathology, and allergy in humans and rodents, by strictly affecting the T cell compartment (Sakaguchi et al., *Nature* 2003, Ito et al., *Science* 2014). Modulation of the structure of ZAP-70 or the amount of its expression can therefore be instrumental in deciphering how quantitative or qualitative alteration of TCR signaling impacts on thymic production and peripheral activation of self-reactive Tconv as well as Treg cells, consequently the balance between the two populations, to cause a plethora of autoimmune/inflammatory diseases.

This year, we expressed in normal mice mutated ZAP-70 molecules with different affinities for the CD3 chains, or wild-type ZAP-70 at graded expression levels under tetracycline-inducible control (Tanaka A, Maeda S, et al., *J. Exp. Med*., 2023). Both manipulations reduced TCR signaling intensity to various extents and thereby rendered those normally deleted self-reactive thymocytes to become positively selected and form a highly autoimmune TCR repertoire. The signal reduction more profoundly affected Treg development and function because their TCR signaling was further attenuated by Foxp3 that physiologically repressed the expression of TCR-proximal signaling molecules, including ZAP-70, upon TCR stimulation. Consequently, the TCR signaling intensity reduced to a critical range generated pathogenic autoimmune Tconv cells and concurrently impaired Treg development/function, leading to spontaneous occurrence of autoimmune/inflammatory diseases, such as autoimmune arthritis and inflammatory bowel disease.

The results provide a general model of how attenuation of TCR signaling intensity via ZAP-70 and other TCR-proximal signaling molecules, whether due to genetically-induced structural anomalies or reduced expression of structurally intact forms, can be causative of autoimmune and other immunological disorders by affecting thymic development and peripheral function of Tconv and Treg cells.

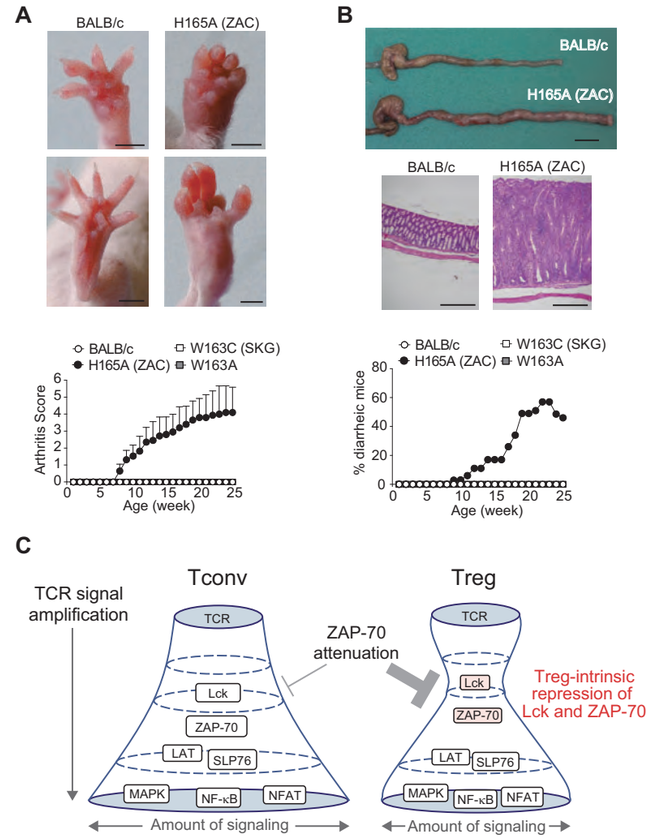

Figure.
Spontaneous development of autoimmune diseases in ZAP-70 mutated mice.
(A and B) Among ZAP-70 mutant strains (H165A (ZAC), W163C (SKG), W163A), only H165A (ZAC) mice spontaneously develop autoimmune arthritis (A) and colitis with severe diarrhea (B).
(C) Key molecules of the TCR signaling pathway, such as Lck and ZAP-70, are specifically down-regulated in Treg cells. TCR signal attenuation through ZAP-70 to a critical autoimmune range differentially affects the development and function of Treg and Tconv cells, leading to spontaneous development of autoimmune diseases.

Recent Publications

1. Ichiyama K, Long J, Kobayashi Y, Horita Y, Kinoshita T, Nakamura Y, Kominami C, Georgopoulos K, Sakaguchi S. Ikzf1 association with Foxp3 for gene repression in Treg cells: disruption of the association causes autoimmunity and promotes tumor immunity. Immunity. (2024).

2. Tanaka A, Maeda S, Nomura T, Llamas-Covarrubias MA, Tanaka S, Jin L, Lim EL, Morikawa H, Kitagawa Y, Akizuki S, Ito Y, Fujimori C, Hirota K, Murase T, Hashimoto M, Higo J, Zamoyska R, Ueda R, Standley DM, Sakaguchi N, Sakaguchi S. Construction of a T-cell receptor signaling range for spontaneous development of autoimmune disease. J Exp Med. 220(2):e20220386 (2023).

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Lymphocyte Differentiation

Tomohiro Kurosaki, MD/PhD

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Support Staff	4

Antibodies (Abs) are produced by terminally differentiated plasma cells (PCs) and have distinct effector functions depending on their isotype. While the majority of PCs generated in the secondary lymphoid organs die within days after their formation (termed short-lived PCs; SLPCs), a small majority persists for months, years, or even decades. These long-lived PCs (LLPCs) are found in locations distinct from their generation site, often in the bone marrow. Hence, in order to distinguish between SLPCs and LLPCs, previous studies determined the number of PCs residing in the bone marrow, considering them as LLPCs. However, since it is known that this bone marrow population also includes recently generated SLPCs, determining the exact number of LLPCs is not possible using this approach. To overcome the limitation of the current assay system, we developed an *in vivo* time-stamping method for PCs, thereby allowing us to directly measure the decay of the labeled bone marrow PCs (Figure).

Using this method, we first demonstrated that the homeostatic PC pool in the bone marrow was continuously replenished by recently arriving B220^{hi}MHC-II^{hi} cells, a small fraction of which then matured into a B220^{lo}MHC-II^{lo} LLPC pool. Second, in the NP-CGG immune response, contrary to expectation, we could not find significant survival differences between GC-independent and -dependent NP+ PCs in the bone marrow. Third, in the bone marrow, about 10-20% of the newly arrived SLPCs are maturated to LLPCs and their longevity in the case of mouse is more than 1 year. Fourth, although it has been thought that IgM type LLPCs do not exist, they exist, probably recognizing auto-antigens and microbiota. Finally, in contrast to NP^{+}B220^{hi}MHC-II^{hi} SLPCs in the bone marrow, the NP^{+}B220^{lo}MHC-II^{lo} LLPCs with greater survival potential were more likely to be immobilized in the niches, suggesting an association between adhesion strength and provision of survival signals to PCs.

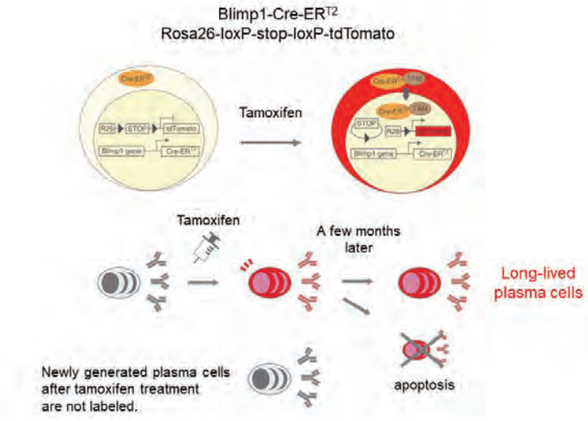

Figure 1.
To track long-term survival of plasma cells, we sought to generate an experimental system that could irreversibly label plasma cells in an inducible manner. To this end, we established a new mouse line in which plasma cells were labeled by a fluorescent reporter in response to a specific drug treatment.

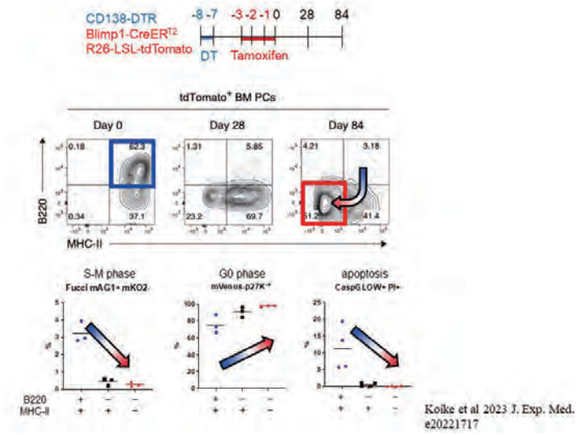

Figure 2.
Plasma cells right after arriving at bone marrow are B220^{hi} MHC-II^{hi}, whereas those survived for long period of time in bone marrow become B220^{lo} MHC-II^{lo}. Bone marrow plasma cells exit cell cycle and acquire enhanced survival potential during progressive differentiation.

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1. Inoue T, Matsumoto Y, Kawai C, Ito M, Nada S, Okada M, Kurosaki T. Csk restrains BCR-mediated ROS production and contributes to germinal center selection and affinity maturation. J Exp Med. (2024).
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3. Ise W, Kurosaki T. Tissues of origin matter to plasma cell longevity. Nat Immunol. 25(2):194-195 (2024).
4. Inoue T, Kurosaki T. Memory B cells. Nat Rev Immunol. 24(1):5-17 (2024).
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Malaria Immunology

Cevayir Coban, MD

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Research Assistant	3

Our laboratory focuses on the elucidation of host-pathogen interactions. We mainly work on malaria, but also cover several other infectious diseases such as leishmaniasis and respiratory infections. We study both innate and adaptive immune responses against these diverse pathogens in order to develop successful vaccines against them.

Adjuvant discovery and development platform

Adjuvants are considered essential vaccine components for enhancing vaccine responses. Recently, we have been involved in the discovery of novel adjuvants as part of the AMED SCARDA project led by Prof. Jun Kunisawa. We have systematically screened innate and adaptive immune signaling molecules involved in the mode of action (MOA) of adjuvants and vaccines. One of the recent findings is to understand how the combination of TLR9 and STING agonists synergistically induce innate and adaptive responses to generate robust anti-tumor responses (*Temizoz et al., International Immunology, 2022*). Our recent projects have focused on investigating B cell development and the pathways involved in germinal center (GC) formation for the generation of potent antibody responses against infections and during vaccination. We found that TBK1, the well-known innate immune signaling kinase that controls antiviral immune responses and nucleic acid-mediated type I interferon responses, is very important for the generation of GCs that confer sterile immunity to reinfection (*Lee et al., J Exp Medicine, 2022*).

Elucidation of host-pathogen interactions

Our laboratory has been engaged in several aspects of immunopathology caused by Plasmodium parasites. Recently, our team has been investigating the possible bone marrow niches responsible for malaria-induced loss of memory. It is becoming increasingly clear that perturbation of the bone marrow microenvironment by infection and inflammation affects hematopoiesis and may affect immune cell development. Our recent findings demonstrate that the mesenchymal stromal CXCL12-abundant reticular (CAR) cell population is reduced during acute malaria infection (*Lee et al., Int. Immunology, 2024*). The reduction of CXCL12 and IL-7 signals in the bone marrow affects the lymphopoietic niche, which leads to the depletion of common lymphoid progenitors, B cell progenitors, and mature B cells, including plasma cells in the bone marrow. We found that IFNγ is responsible for the upregulation of Sca1 on CAR cells, yet the decline in CAR cell and B cell populations in the bone marrow is IFNγ-independent. Interestingly, while B cell populations declined, HSCs and multipotent progenitors increased with the expansion of myelopoiesis and erythropoiesis. This suggests that malaria may affect host immunity by modulating the bone marrow niche (*Lee et al., Int. Immunology, 2024*).

Infection and Cancer

Previously, we investigated the role of Lipocalin 2 (LCN2, also known as siderocalin or neutrophil gelatinase-associated lipocalin (NGAL)) in malaria infection that bolsters innate and adaptive immune responses to malaria infection through modulation of iron metabolism (*Zhao et al., Cell Host Microbe, 2012*). LCN2 expression is also increased in cancer. In carcinogenesis, in addition to the accumulation of somatic mutations, stroma-associated immunity is an important regulator of tumor growth. Tumor cells create a microenvironment by releasing various mediators to maintain their presence and spread. Due to the infiltration of monocytes and leukocytes against the tumor, it is hypothesized that the iron balance is disrupted by excessive iron consumption, possibly leading to increased expression of LCN2 as an intracellular iron transporter. We recently investigated the expressions of programmed cell death ligand-1 (PD-L1) and LCN2 in breast cancers with various molecular subtypes, along with their correlations with other prognostic indicators, including Ki-67, lymph node metastasis, histological grade, tumor-infiltrating lymphocyte (TILs) accumulation, and necrosis. We found that there is an association of LCN2 with known prognostic factors and molecular subtypes. Moreover, significant elevations of LCN2 and PD-L1 expressions were observed in triple-negative and HER2-positive breast cancers. The findings from this research may contribute to the immunotherapeutic application of LCN2 and its prognostic significance in breast cancer management (*Ekemen et al., Breast Cancer: Targets and Therapy, 2024*).

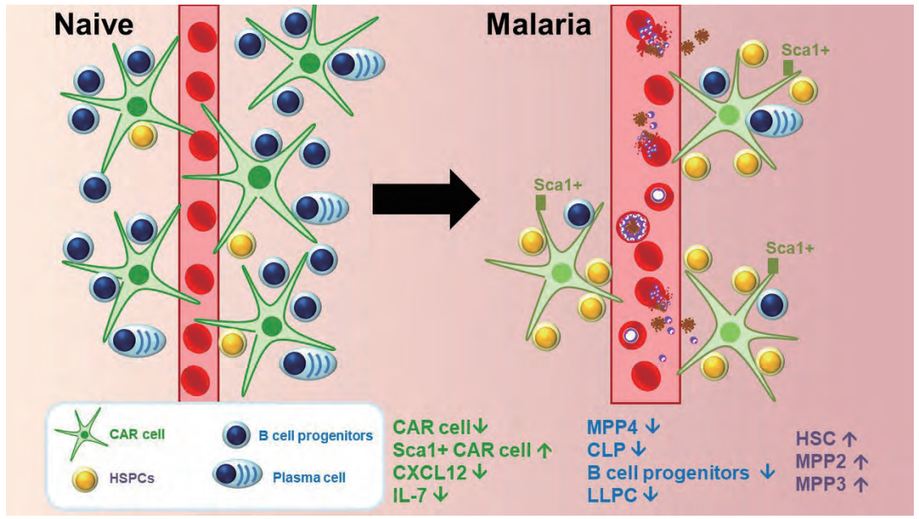

Figure.
Acute malaria suppresses the B lymphocytic niche in the bone marrow through the alteration of CXCL12-abundant reticular cells (*Lee et al., International Immunology, 2024*).

Recent Publications

1. Lee MSJ, Matsuo Dapaah J, Del Rosario Zorrilla C, Omatsu Y, Nagasawa T, Uemura S, Iwama A, Ishii KJ, Coban C. Acute malaria suppresses the B lymphocytic niche in the bone marrow through the alteration of CXCL12-abundant reticular cells. Int Immunol. dxae012 (2024).
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4. Lee MSJ, Inoue T, Ise W, et al. B cell intrinsic TBK1 is essential for germinal center formation during infection and vaccination in mice. J Exp Med. 219(2):e20211336 (2022).
5. Coban C. The host targeting effect of chloroquine in malaria. Curr Opin Immunol. 66:98-107 (2020).

Vaccine Science

Ken J. Ishii, MD/PhD

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Our laboratory aims to understand the immunological mechanisms involved in intra- and inter-cellular signaling pathways that mediate the immunogenicity of successful vaccines, as well as biological responses to adjuvants. This understanding will aid in the development of new concepts, modalities, and next-generation immuno-preventive and/or therapeutic agents against infectious diseases, cancer, allergy, and other non-communicable diseases.

DMXAA is a partial STING agonist competing for human STING activation.

DMXAA is a mouse-specific stimulator of interferon gene (STING) agonist that has shown STING-dependent anti-tumor activity. Despite its inability to fully activate human STING, DMXAA has reached phase III in lung cancer clinical trials. The mechanism by which DMXAA is effective against human lung cancer is currently unknown. In this study, we demonstrated that DMXAA is a partial STING agonist that may interfere with agonistic STING activation, which could explain its partial anti-tumor effect observed in humans because STING was reported to be pro-tumorigenic for lung cancer cells with low antigenicity. Nonetheless, we developed a novel DMXAA derivative, 3-hydroxy-5-(4-hydroxybenzyl)-4-methyl-9H-xhanthen-9one (HHMX), which has the potential to strongly antagonize STING pathway activation both in humans and mice. HHMX was found to suppress abnormal responses caused by STING gain-of-function mutations that lead to STING-associated vasculopathy with onset in infancy (SAVI) in vitro. Furthermore, HHMX treatment was observed to reduce abnormal STING pathway activity in peripheral blood mononuclear cells from SAVI patients. Finally, in a SAVI mouse model, HHMX demonstrated a strong therapeutic effect by slowing disease progression (Figure 1). These findings suggest that HHMX may have therapeutic potential for STING-associated autoinflammatory diseases.

Challenges in developing personalized neoantigen cancer vaccines

Promising results from the clinical data related to cancer immunotherapy emphasized the importance of utilization of the immune system for fighting against cancer. Vaccines have a proven track record of promoting protective immune responses against pathogens. As a result, vaccines targeting cancer neoantigens are being advocated to direct and enhance immune responses against tumors while minimizing the collateral damage to the healthy tissue. Indeed, over one hundred clinical trials and extensive preclinical research to test various strategies for discovering neoantigens and formulating vaccines have been performed up to now. However, it is important to acknowledge that despite the efforts to develop neoantigen vaccines, most clinical trials have not yet provided strong evidence of efficacy. This review discusses the challenges that must be addressed to maximize the potential of neoantigen vaccines in cancer therapy by focusing primarily on the vaccine design and the tumor microenvironment.

TLR9 and STING agonist combinations are robust cancer immunotherapeutics and neopeptide cancer vaccine adjuvants.

Agonists for TLR9 and stimulator of IFN genes (STING) have therapeutic applications as anti-tumor agents and vaccine adjuvants. However, their clinical use is limited due to the weak IFN induction of the clinically available TLR9 agonist and the undesired type 2 immunity induced by STING agonists. We have previously shown that combining TLR9 and STING agonists overcame limitations by synergistically inducing innate and adaptive IFNγ, making the combination an advantageous type 1 adjuvant, suppressing STING-mediated type 2 immunity and culminating in robust anti-tumor activities when used as a therapeutic agent for cancer immunotherapy in B16 F10 and EG-7 mouse tumor models. Furthermore, we also found a potent anti-tumor effect of the combination adjuvant in a Pan02 peritoneal dissemination model of pancreatic cancer only when agonists for TLR9 and STING were administered locally. The mechanisms of action involve both CD4 and CD8 T cells. Mechanistically, IL-12 is crucial and synergistically coordinates with type I IFNs to regulate the anti-tumor effect of the combination. Synergism operates within individual antigen-presenting cells and is regulated at the level of promotor activation, transcription and translation of IL-12. Such intracellular transcriptional synergy may hold a key in successful cancer immunotherapy and provide further insights into dual agonism of innate immune sensors during host homeostasis and diseases.

In collaboration with Erasmus Medical Center, we have further extended the applications of our combination adjuvant to neoantigen peptide cancer vaccines and found that K3 CpG+c-di-AMP adjuvanted vaccine is able to induce potent Ag-specific Th1-type and CD8+ T cell immune responses against synthetic long peptides and neopeptide pools derived from melanoma and mesothelioma tumors. Indeed, K3 CpG+c-di-AMP adjuvant formulation induced 10 times higher T cell responses against neopeptides than the TLR3 agonist, which is currently the leading adjuvant in clinical trials of neoantigen peptide vaccines. Moreover, therapeutic vaccination with 20-mer peptide combined with K3 CpG+c-di-AMP adjuvant synergizes in vivo with α-PD-1 treatment to better control an ICB-resistant tumor. Thus, our findings show that the TLR9+STING-adjuvanted vaccine formulation generates robust T cell immunity against synthetic long peptides and is a promising candidate to further improve neoantigen vaccine platforms.

The 100 Days Mission

During the response to COVID-19, it became clear that developing safe, effective, and affordable vaccines, therapeutics, and diagnostics for infectious diseases is crucial to saving lives. Ken J. Ishii, as a scientific advisory member for CEPI, IPPS, and STEG for G7, is committed to contributing to the 100 Days Mission. The 100 Days Mission aims to prepare for a pandemic threat by ensuring that approved, accurate, and rapid diagnostic tests, initial therapeutic regimens and vaccines that have been produced at large scale and ready for global deployment are available within the first 100 days of emergence of a new pathogen.

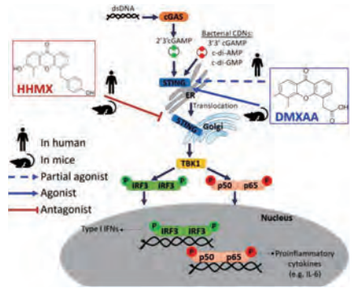

Figure.
Mode of action of DMXAA and its derivative HHMX in human and mice.
DMXAA is a mouse-selective STING agonist, but only a partial agonist in humans, interfering with agonistic STING activation. Based on this data, a novel DMXAA derivative, 3-hydroxy-5-(4-hydroxybenzyl)-4-methyl-9H-xhanthen-9one (HHMX), was developed. HHMX has the potential to become a therapeutic agent for STING-associated autoinflammatory diseases, including SAVI, as it can antagonize the STING signaling pathway in both humans and mice.

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1. Temizoz B, Shibahara T, Hioki K, Hayashi T, Kobiyama K, Lee MSJ, Surucu N, Sag E, Kumanogoh A, Yamamoto M, Gursel M, Ozen S, Kuroda E, Coban C, Ishii KJ. 5,6-dimethylxanthenone-4-acetic acid (DMXAA), a Partial STING Agonist, Competes for Human STING Activation. Front Immunol. 15:1353336 (2024).
2. Katsikis PD, Ishii KJ, Schliehe C. Challenges in developing personalized neoantigen cancer vaccines. Nat Rev Immunol. 24(3):213-227 (2024).
3. Kaku Y, Okumura K, Padilla-Blanco M, Kosugi Y, Uriu K, Hinay AA Jr, Chen L, Plianchaisuk A, Kobiyama K, Ishii KJ. Genotype to Phenotype Japan (G2P-Japan) Consortium; Zahradnik J, Ito J, Sato K. Virological characteristics of the SARS-CoV-2 JN.1 variant. Lancet Infect Dis. 24(2):e82 (2024).
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Immunoparasitology

Masahiro Yamamoto, PhD

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Regulatory T (Treg) cells expressing the transcription factor (TF) Foxp3 also express other TFs shared by Th subsets under certain conditions. In 2023, to determine the roles of T-bet-expressing Treg cells, we have reported generation of a mouse strain, called VeDTR mice, in which T-bet/Foxp3 double-positive cells were engineered to be specifically labelled and depleted by a combination of Cre- and Flp-recombinase-dependent gene expression control. Characterization of T-bet$^+$Foxp3$^+$ cells using the VeDTR mice revealed the high resistance under oxidative stress, which was involved in T-bet$^+$Foxp3$^+$ cells to accumulate in tumor tissues. Moreover, short-term depletion of T-bet$^+$Foxp3$^+$ cells only led to anti-tumor immunity but not autoimmunity, whereas that of whole Treg cells did both.

To date, several methods to inducibly delete immune cell populations have been developed using cytotoxic antibodies and chemicals such as clodronate liposome, or by herpesvirus thymidine kinases or DTR that are directly expressed under the cell-specific gene promoters and are able to render the cells sensitive to ganciclovir or DT, respectively. DTR can also be expressed indirectly using Cre recombinase-inducible mice which allow their expression in various cell types upon lineage-specific Cre-mediated excision of a loxP-flanked stop cassette under the *Rosa26* promoter. However, these conventional methods exhibit limited specificity toward the intended target immune cells because most of immune cell types rarely defined by single gene but rather by intersectional expression of multiple genes. To improve the specificity of Cre-mediated depletion of macrophage/monocyte subpopulations, transgenic mouse systems utlizing sequential promoter activities were developed. Alternatively, intersectional genetic methods using the Cre/loxP and Flp/FRT double recombination systems or binary split Cre recombinases have been introduced to dissect various cell subpopulations in brain. However, these intersectional genetic strategies have not been developed for conditional and selective depletion of immune cells. In the present study, we have created the VeDTR mouse line that can intersectionally label and conditionally deplete a cell-type-of-interest by YFP and DTR, respectively.

T-bet$^+$Foxp3$^+$ cells showed higher suppressive activity than T-bet$^-$Foxp3$^+$ cells. Moreover, TCR-stimulated T-bet$^+$Foxp3$^+$ cells preferentially expressed perforin and anti-oxidant proteins. Consistent with high concentrations of T-bet$^+$Foxp3$^+$ cells in tumor tissues compared with spleens, it has been shown that perforin expression in Treg cells is important for Treg cell-mediated suppression of anti-tumor immunity. Preferential expression of anti-oxidant proteins such as MT3 was also required for Treg cell localization at tumor tissues that are shown to contain increased concentrations of ROS, since MT3-deficient Treg cells were sensitive to oxidative stress and failed to accumulate in tumor tissues.

It was of note that whole Treg cell depletion in Foxp3-Cre/VeDTR(ΔFRT) mice induced histopathology and morbidity, whereas inducible T-bet$^+$Foxp3$^+$ cell depletion in Foxp3-Cre/Tbx21-Flp/VeDTR(LF) mice did not lead to autoimmunity. Although some intratumor CD45$^+$ cell subsets such as NK cells and CD11b$^+$ cells were somewhat differentially affected by depletion of T-bet$^+$Foxp3$^+$ cells or whole Treg cells, the resultant slow tumor growth was comparable, suggesting that the difference might seldomly influence anti-tumor immunity. Indeed, antibody-mediated deletion of NK1.1$^+$ cells alone did not recover tumor growth in T-bet$^+$Foxp3$^+$ cell-depleted mice. From a cancer immunotherapy perspective, considering the comparable slowed tumor growth but less autoimmunity, targeting T-bet$^+$Foxp3$^+$ cells appears to be safer than that targeting whole Treg cells.

Collectively, the intersectional genetic labeling/depletion of T-bet$^+$Foxp3$^+$ cells revealed their specific roles in suppressing tumor immunity.

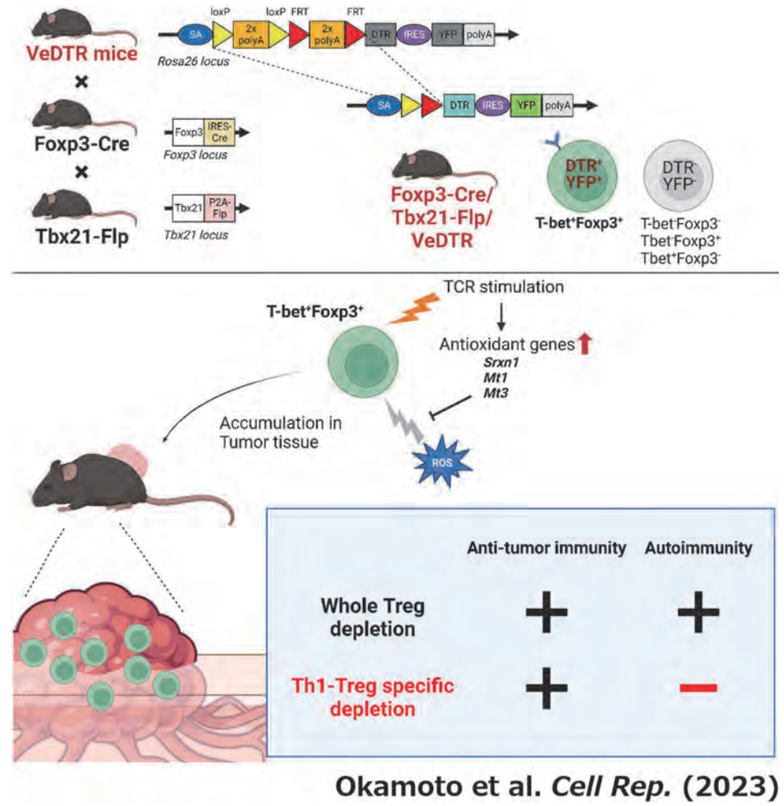

Figure.
The novel VeDTR mouse system specifically targets Th1-Treg cells.

Recent Publications

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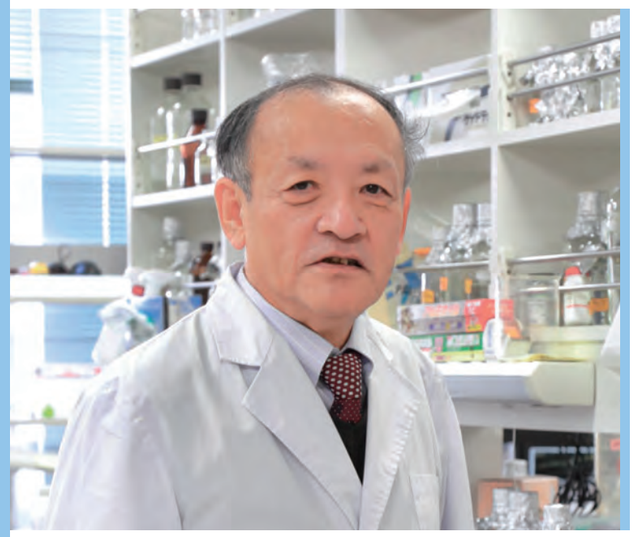
Biochemistry & Immunology



Shigekazu Nagata, PhD

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The phospholipid composition of plasma membranes exhibits an asymmetrical distribution across their inner and outer leaflets, with phosphatidylserine (PtdSer) predominantly localized in the inner leaflet. This asymmetry is upheld by ATP-dependent flippases, which facilitate the translocation of PtdSer from the outer to the inner leaflet. However, during apoptosis, cell fusion, and cell activation, the asymmetrical distribution of phospholipids is disrupted by scramblases that non-specifically scramble phospholipids between leaflets, leading to the exposure of PtdSer on the cell surface.

Our previous studies (Segawa et al. Science 2014; Suzuki et al. Nature 2010, Science 2013) have identified the key players in this process: ATP11A, ATP11C, and CDC50A as flippases, TMEM16F as a Ca^{2+}-dependent scramblase, XKR8 as a caspase-dependent scramblase. We found a mutation of the TMEM16F gene in human Scott syndrome suffering hemophilia, which underscores its significance in exposing PtdSer in the activated platelets, crucial for activating blood clotting factors. Moreover, we've uncovered XKR8, forming complexes with Basigin or Neuroplastin, crucial for efficient clearance of dead cells and prevention of autoimmunity (Suzuki et al. PNAS 2016; Kohno et al. PNAS 2019; Yamashita et al. MCB 2019).

A high concentration of ATP (~ 4 mM) is present inside the cells, while its extracellular concentration is low (less than 30 nM). However, its extracellular concentration increases by several hundred μM in the inflamed tissues or tumor environment, where a large amount of ATP is released from the cells undergoing necrosis. ATP rapidly induces PtdSer-exposure in $CD25^{+}CD4^{+}$ T cells and macrophages by binding to its receptor, P2X7, followed by necrosis and release of inflammatory cytokines. In the CRISPR/Cas9 screening for the molecules involved in ATP-induced PtdSer-exposure, we found that XK, a paralogue of XKR8, and the VPS13A cytosolic lipid transporter, is required for the ATP-induced PtdSer-exposure downstream of P2X7. An unidentified signal from the ATP-engaged P2X7 receptor seems to activate the XK-VPS13A complex to scramble phospholipids in the plasma membranes (Figure 1). Patients of neuroacanthocytosis, a disorder that affects erythrocytes and the central and peripheral nervous system, carry a defect in *Xk* or *Vps13a*, indicating that the XK- and VPS13A-mediated scrambling of phospholipids plays an inevitable role in maintaining the homeostasis in hematopoietic and nervous systems.

How phospholipids are translocated between lipid bilayers by flippases and scramblases? We established the method to produce a large amount of human XKR8-Basigin complex and succeeded in purifying it to homogeneity. In 2021, we determined its tertiary structure at a resolution of 3.8 Å (Sakuragi et al. Nat. Struct. Mol. Biol. 2021). Its membrane-spanning region adopts a cuboid-like structure. At least seven charged residues essential for scramblase activity are placed from top to bottom as a staircase inside the molecule, providing a pathway for the translocation of phospholipids. The structure was recently refined with the complex embedded in lipid nanodisc (Sakuragi et al. J. Biol. Chem. 2024)(Figure 2). This structure showed that the C-terminal tail of XKR8 is engaged in intricate polar and van der Waals interactions with a groove at its cytoplasmic surface of XKR8. Point mutations to disrupt these interactions strongly enhanced the scrambling activity of XKR8, indicating that the cytoplasmic tail region of XKR8 functions as a plug to prevent the scrambling of phospholipids.

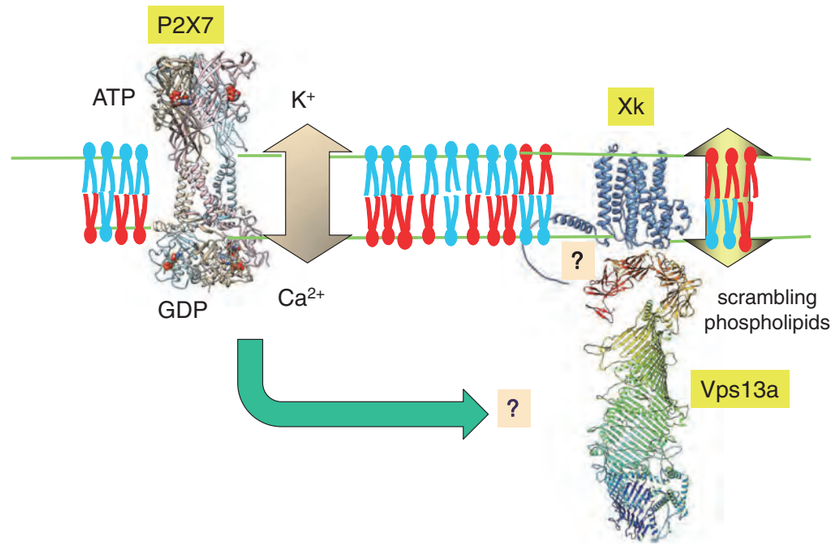

Figure 1.
Association of XK with VPS13A at the plasma membrane to scramble phospholipids.
VPS13A, a giant cytoplasmic protein (human VPS13A: 3174 aa), is associated with XK in the plasma membrane. P2X7 is a receptor for extracellular ATP and serves as a cation transporter. The binding of ATP to P2X7 activates the XK-VPS13A complex to scramble phospholipids by an unknown mechanism(s), leading to PtdSer-exposure and cell lysis.

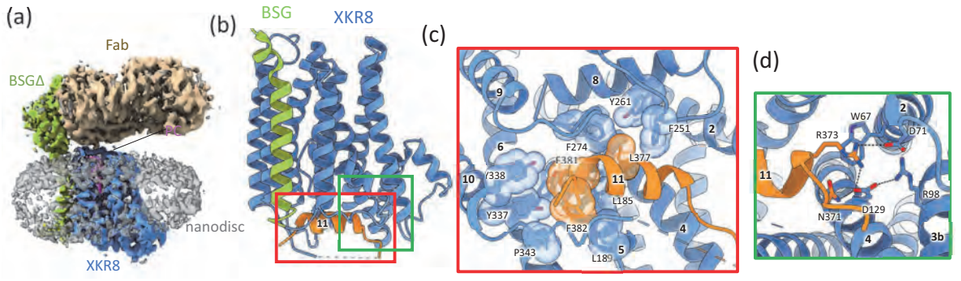

Figure 2.
The structure of the XKR8–basigin (BSG) complex. (a) a cryo-EM map of XKR8–BSG-anti BSG Fab complex in lipid nanodiscs. (b) A side view of the structure of the XKR8-Basigin complex. The helix 11 of XKR8 is shown in orange. The areas enclosed by the red (c) or green (d) box are viewed. Residues involved in the interaction are shown as a stick model or a sphere model. Likely, hydrogen bonds are shown as dotted lines.

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Molecular Neuroscience

Toshihide Yamashita, MD/PhD

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Research Assistant	2
Visiting Scientist	6
Support Staff	2

Disorders of the central nervous system, such as cerebrovascular diseases, cerebrospinal trauma, and encephalomyelitis, often cause spatiotemporal changes in the nervous system and in various biological systems, such as the immune system and vascular system. We have analyzed disorders of the neural networks in the central nervous system and the subsequent restoration process from the perspective of the functional network of biological systems (Fig. 1). Further, we have analyzed the mechanism by which the spatiotemporal dynamics in those biological systems control a series of processes (Fig. 2). Particularly, the ultimate goal of this study is to elucidate the manner in which the control mechanism is affected by the associations among the nervous system, immune system, and vascular system. Additionally, we aim to elucidate the processes involved in the functioning of living organisms with neural network disorders within the central nervous system by observing such disorders and their functional recovery process with respect to the dynamics of the entire biological system and by conducting a comprehensive analysis of the association between each system.

We observe the central nervous system as a single organ within a biological system. Further, studies from the perspective of how the entire biological system is involved in disorders and recovery of neural networks are scarce. By observing disorders in neural networks and the biological reactions during the subsequent recovery process as a "scrap-and-build" strategy, we aim to elucidate the mechanisms behind a series of reactions as well as their significance that may potentially lead to a new and original trend in Life Sciences.

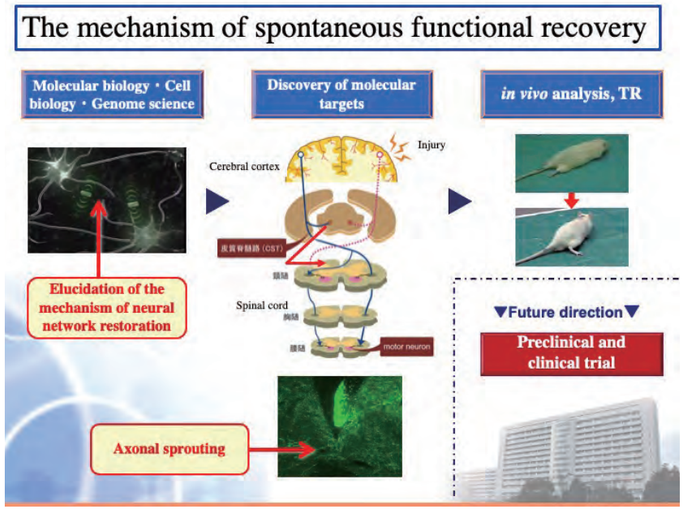

Figure 1.
The mechanism of spontaneous functional recovery.

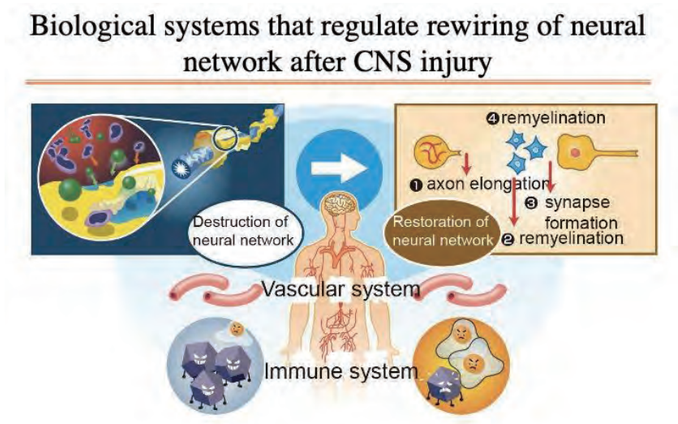

Figure 2.
Biological systems that regulate rewiring of neural network after CNS injury.

Recent Publications

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Molecular Immunology

Sho Yamasaki, PhD

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	Eri Ishikawa
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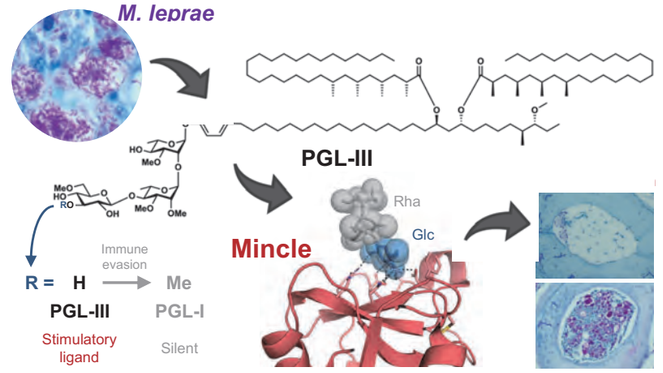
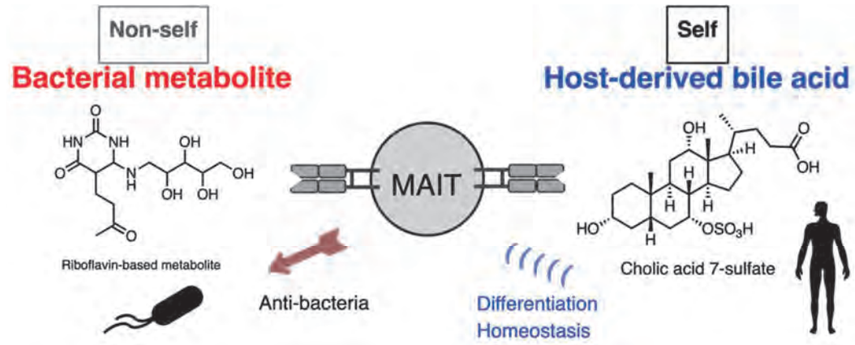
Research Assistant	6
Visiting Scientist	8
Support Staff	5





Although leprosy (Hansen's disease) is one of the oldest known diseases, the pathogenicity of *Mycobacterium leprae* (*M. leprae*) remains enigmatic. Indeed, the cell wall components responsible for the immune response against *M. leprae* are as yet largely unidentified. We reveal here phenolic glycolipid-III (PGL-III) as an *M. leprae*-specific ligand for the immune receptor Mincle. PGL-III is a scarcely present trisaccharide intermediate in the biosynthetic pathway to PGL-I, an abundant and characteristic *M. leprae* glycolipid. Using activity-based purification, we identified PGL-III as a Mincle ligand that is more potent than the well-known *M. tuberculosis* trehalose dimycolate. The cocrystal structure of Mincle and a synthetic PGL-III analogue revealed a unique recognition mode, implying that it can engage multiple Mincle molecules. In Mincle-deficient mice infected with *M. leprae*, increased bacterial burden with gross pathologies were observed. These results show that PGL-III is a noncanonical ligand recognized by Mincle, triggering protective immunity.

https://pubs.acs.org/doi/full/10.1021/acscentsci.3c00040

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1. Ito E, Inuki S, Izumi Y, Takahashi M, Dambayashi Y, Ciacchi L, Awad W, Takeyama A, Shibata K, Mori S, Mak JYW, Fairlie DP, Bamba T, Ishikawa E, Nagae M, Rossjohn J, Yamasaki S. Sulfated bile acid is a host-derived ligand for MAIT cells. Sci. Immunol. 9:eade6924 (2024).
2. Oka S, Watanabe M, Ito E, Takeyama A, Matsuoka T, Takahashi M, Izumi Y, Arichi N, Ohno H, Yamasaki S, Inuki S. Archaeal Glycerolipids Are Recognized by C-Type Lectin Receptor Mincle. J Am Chem Soc. 145:18538-18548 (2023).
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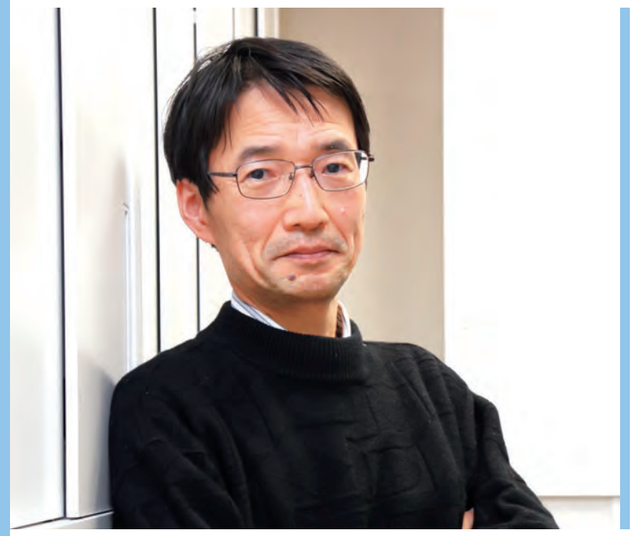
Stem Cell Biology and Developmental Immunology



Takashi Nagasawa, MD/PhD

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Special microenvironments known as niches are essential for the maintenance of hematopoietic stem cells (HSCs), which give rise to blood cells, and lympho-hematopoiesis within bone marrow (BM). We isolated a chemokine, CXCL12 (SDF-1/PBSF) as a molecule that stimulates the growth of B cell precursors (Nagasawa et al., *PNAS* 1994) and found that CXCL12 and its receptor CXCR4 are essential for the colonization of BM by HSCs during embryogenesis (Nagasawa et al., *Nature* 1996; Ara et al., *Immunity* 2003), the maintenance of a pool of HSCs (Sugiyama et al., *Immunity* 2006), and the development of immune cells, including B cells, plasmacytoid dendritic cells (pDCs), and NK cells as well as vascular formation and cardiogenesis (Tachibana et al., *Nature* 1998). Based on a key role of CXCL12 in HSC maintenance, we identified a population of fibroblastic cells expressing CXCL12 at high levels, termed CXCL12-abundant reticular (CAR) cells within BM (Sugiyama et al., *Immunity* 2006) and found that these BM-CAR cells are the major producer of CXCL12 and SCF (Omatsu et al., *Immunity* 2010), and the major cellular components of niches for HSCs and immune cells, including B cells and plasma cells (Tokoyoda et al., *Immunity* 2004; Sugiyama et al., *Immunity* 2006; Omatsu at al., *Immunity* 2010). Ding et al. showed that leptin receptor-expressing (LepR$^+$) cells overlap strongly with CAR cells in BM (Ding et al., *Nature* 2012). Inconsistent with the classical concept, we showed that numerous HSC niches remain empty and that all BM-CAR cells create facultative niches for HSCs (Shimoto et al., *Blood* 2017).

In addition, we showed that BM-CAR cells are mesenchymal stem cells, which give rise to adipocytes and osteoblasts, and that transcription factors, Foxc1 and Ebf3 are preferentially expressed in BM-CAR cells and play a critical role in the formation and maintenance of niches for HSCs and immune cells, inhibiting differentiation of BM-CAR cells into adipocytes and osteoblasts, respectively (Omatsu et al., *Nature* 2014; Seike et al., *Genes Dev*. 2018). Furthermore, we showed that BM-CAR cells require Runx1 or Runx2 to prevent their fibrotic conversion and maintain HSCs and hematopoiesis in adults (Omatsu et al., *Nat Commun* 2022).

In addition to the mouse, we revealed that the human counterpart of CAR cells, which specifically expressed CXCL12, Foxc1, and Ebf3, was the major component of nonhematopoietic cells in human BM. This enabled the evaluation of their alterations in various hematological disorders by flow cytometric and histological analyses (Aoki et al., *Br J Haematol* 2021).

Ding et al. suggest that CAR/LepR$^+$ cell-derived CXCL12 is not essential for the maintenance of HSCs since the number of HSCs was unaltered in BM when CXCL12 was conditionally deleted from LepR$^+$ cells using LepR-Cre;CXCL12$^{f/-}$ mice (Ding et al., *Nature* 2013). CXCL12 was deleted from only 70% of the CAR cells in the mutants, probably due to lower LepR expression in early postnatal bone marrow. Thus, we examined the role of CAR cell-derived CXCL12 in the behavior and maintenance of HSCs.

CXCL12 attracts HSCs to BM-CAR cells within bone marrow.

To visualize HSCs in the bone marrow sections, we generated HSC-reporter mice, Evi1-GFP mice, and CXCL12-tdTomato$^{f/f}$ mice, which enabled us to visualize CXCL12-intact and CXCL12-deficient CAR cells. Control and Ebf3-CreERT2;CXCL12-tdTomato$^{f/f}$;CXCL12$^{f/-}$ mice were transplanted with BM cells from Evi1-GFP mice and injected with tamoxifen three times. In these chimeric mice, CXCL12-intact CAR cells were reduced by about 2-fold, but the HSC numbers were unaltered. Histological analyses revealed that the frequencies of HSCs in contact with CXCL12-deficient CAR cells were markedly reduced compared to those in contact with CXCL12-intact CAR cells. These results suggest that HSCs detached from CXCL12-deficient CAR cells and attached to CXCL12-intact CAR cells in the mutants, demonstrating that CXCL12 attracts HSCs to CAR cells within BM.

BM-CAR cell-derived CXCL12 is essential for the maintenance of HSCs and hematopoietic progenitors.

To examine the role of CXCL12 produced by BM-CAR cells, Ebf3-CreERT2;CXCL12$^{f/-}$ mice were injected with tamoxifen eight times. qRT-PCR analysis revealed that CXCL12 was deleted from more than 99.5% of the CAR cells (CXCL12$^{\Delta CAR}$ mice). Flow cytometric analysis revealed that the numbers of phenotypic HSCs, common lymphoid progenitors (CLPs), pro-B cells, pre-B cells, mature B cells, pDCs, NK cells, granulocyte/macrophage progenitors (GMPs), granulocytes, megakaryocyte/erythrocyte progenitors (MEPs), and proerythroblasts were reduced in the BM of the mutants compared with control animals. The magnitude of the reduction was greater in B cell progenitors compared with HSCs, GMPs, and MEPs in CXCL12$^{\Delta CAR}$ mice. Analyses based on limiting-dilution, competitive repopulation assays revealed that the numbers of functional HSCs were markedly reduced in the BM of CXCL12$^{\Delta CAR}$ mice. These results indicate that CAR cell-derived CXCL12 is essential for the maintenance of HSCs and hematopoietic progenitors.

The ability of HSCs to generate B cell progenitors was markedly reduced in mice lacking CXCL12 in CAR cells.

Distinct subsets of HSCs, termed lymphoid-biased and myeloid-biased HSCs, that are stably biased towards the generation of lymphoid or myeloid cells exist in the BM (Müller-Sieburg et al., *Blood* 2002). A recent study showed that depletion of myeloid-biased HSCs in aged mice improved immune responses to viral infection (Ross et al., *Nature* 2024). We transplanted HSCs from CXCL12$^{\Delta CAR}$ or control mice with competitor BM cells into wild-type mice and found that donor contribution into B cell progenitors, i.e., % donor B cell progenitors divided by % donor myeloid progenitors (donor pro-B/GMP and pre-B/GMP reconstitution ratios), in recipients of HSCs from CXCL12$^{\Delta CAR}$ mice was markedly lower than that in recipients of HSCs from control animals, indicating a stable myeloid bias of HSCs from CXCL12$^{\Delta CAR}$ mice. Thus, the ability of HSCs to generate B cell progenitors was markedly reduced in CXCL12$^{\Delta CAR}$ mice.

Together, our findings indicate that CAR cell-derived CXCL12 supports the localization of HSCs and the maintenance of HSCs, especially lymphoid-biased HSCs, to produce the required number of B cell progenitors.

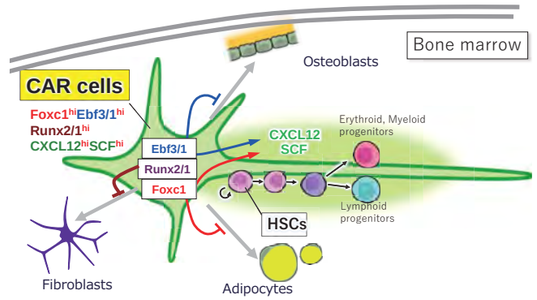

Figure 1.
The functions of BM-CAR cells.
CAR cells are the major cellular component of non-hematopoietic cells in bone marrow (BM) characterized by several salient features in both mouse and human. The transcription factors Foxc1, Ebf1/Ebf3, and Runx1/2 and cytokines, CXCL12 and SCF, are preferentially expressed in CAR cells and critical for the formation and maintenance of niches for HSCs and immune cells within the BM.

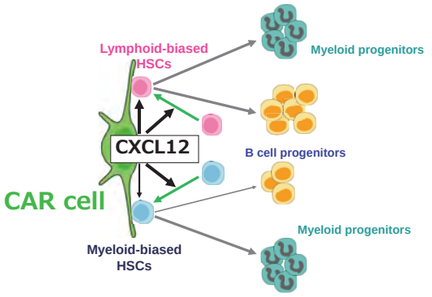

Figure 2.
Working model for how CXCL12 regulates HSCs to produce B cell progenitors.
CAR cell-derived CXCL12 plays a critical role in the localization of HSCs near their niches and the maintenance of HSCs, especially lymphoid-biased HSCs, in the BM.

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5. Sugiyama T, Kohara H, Noda M, Nagasawa T. Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. Immunity 25:977-988 (2006).

Aging Biology

Eiji Hara, PhD

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We all experience aging, a phenomenon in which our biological functions deteriorate. We all want to avoid aging because it leads to a decline in quality of life and the development of severe age-related diseases such as cancer and dementia. Therefore, research has been conducted in many countries with aging populations, including Japan, to extend a healthy life span by slowing the aging process. Recently, it has become clear that one of the causes of aging is the increase of senescent cells, which accumulate in the body with age and secrete various inflammatory substances, thereby accelerating the aging process. However, it has yet to be understood why senescent cells accumulate in the body with aging.

The gut microbiota in our intestinal tract, composed of an enormous number and variety of gut bacteria, plays an essential role in maintaining our health. It has become clear that unfavorable changes in the composition of the gut microbiota -dysbiosis- with aging are closely related to the development of various age-related diseases. However, little is known about why the dysbiosis of gut microbiota occurs with aging. We used genetically engineered mice in which senescent cells could be visualized alive *in vivo* and kept them in a typical environment with bacteria in the gut or a sterile environment with no bacteria in the body for an extended period to observe changes in the accumulation of senescent cells over time. As a result, we noticed that mice bred in a normal environment showed an increase in senescent cells in the abdomen with aging, while mice bred in a sterile environment showed no increase in senescent cells. Furthermore, when the organs isolated from these mice were examined, significant differences were observed mainly in the ileal part of the gut. We then used single-cell RNA sequencing analysis and other techniques to determine which cells in the ileum are inducing cellular senescence. We found that a part of germinal center B cells, which are responsible for immunoglobulin A (IgA) production in the gut, causes cellular senescence. IgA regulates the composition of the gut microbiota by binding to gut bacteria. Therefore, we hypothesized that cellular senescence of germinal center B cells in the ileum with aging might lead to decreased IgA production and abnormal intestinal microflora. We thus followed the same individual mice for two years and analyzed in detail the age-related changes in IgA production and gut microbiota. The results showed that the production of IgA decreases with age, and the binding of IgA to gut bacteria changes, leading to a dysbiosis of gut microbiota. Furthermore, the age-related decline in IgA production and diversity observed in wild-type mice was suppressed in mice where cellular senescence was prevented. In addition, it was found that B cells isolated from wild-type mice show a decrease in IgA production capacity and a marked reduction in the ability to control the gut microbiota as they age, while B cells isolated from mice with reduced susceptibility to cellular senescence show no decrease in IgA production capacity and maintain some ability to control the gut microbiota even as they age.

In conclusion, it is clear that in young mice, gut homeostasis is maintained by controlling the composition of the gut microbiota through IgA (Fig. 1 left), but when stimulated by gut bacteria over a long period during aging, the germinal center B cells in the ileum cause cellular senescence, resulting in a decrease in IgA quantity and diversity, which in turn provokes dysbiosis in the gut microbiota (Fig. 1 right). This study revealed that the gut microbiota, which is supposed to have a symbiotic relationship with the host, becomes stressful for the host in the long term, inducing cellular senescence of germinal center B cells in the ileum, which in turn causes dysbiosis of the gut microbiota. This discovery is expected to elucidate the causes of age-related abnormalities in gut microbiota, which have long been unknown, and to develop new methods to prevent abnormalities in gut microbiota, thereby contributing to the extension of healthy life expectancy.

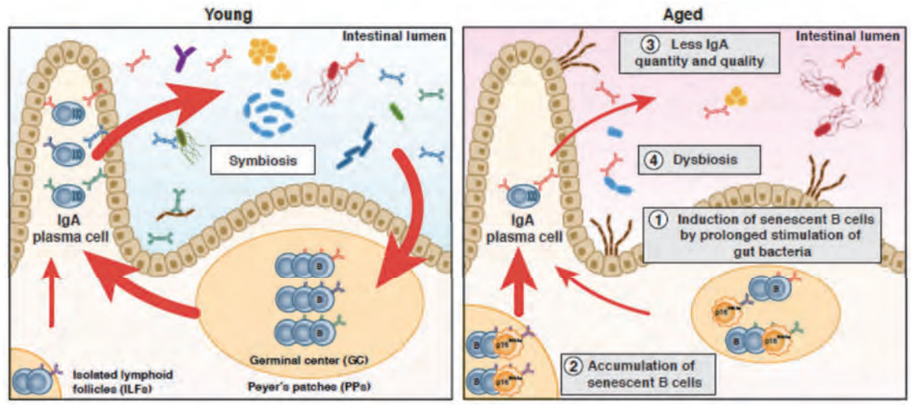

Figure.
A model of IgA-mediated crosstalk between the gut microbiota and cellular senescence. In young mice, GC B cells induced by the gut microbiota are appropriately selected in the PPs and differentiate into IgA plasma cells, secrete sufficient amounts of gut bacteria-specific IgA into the gut lumen to regulate the gut microbiota and maintain symbiosis (left). However, during the ageing process, cellular senescence is induced in the GC B cells of PPs and ILFs by continuous stimulation of the gut microbiota, leading to a decrease in the quantity and quality of IgA produced. This provokes changes in the gut microbiota known as dysbiosis (right).

Recent Publications

1. Kawamoto S, Uemura K, Hori N, Takayasu L, et al. Bacterial induction of B-cell senescence promotes age-related changes in the gut microbiota. Nat Cell Biol. 25:865-876 (2023).

2. Tsuji S, Minami S, Hashimoto R, et al. ARS-CoV-2 infection triggers paracrine senescence and leads to a sustained senescence-associated inflammatory response. Nat Aging. 2:115-124 (2022).

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Signal Transduction

Nobuyuki Takakura, MD/PhD

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Blood vessel formation is essential for organogenesis and organ integrity. Process of blood vessel is divided into two mechanisms. One is vasculogenesis, a do novo blood vessel formation usually observed in embryos. In this process, mesodermal cells differentiate into endothelial cells (ECs) and ECs form tube. Mural cells are recruited near ECs and adhere to ECs for the structural stabilization. After birth, new blood vessels are usually developed from preexisting blood vessels by the sprouting angiogenesis. There are more than three types of ECs for sprouting angiogenesis. Initially, tip ECs emerge for the guidance of migration direction of new branch. This cell type does not have ability to proliferate. Beneath tip ECs, stalk ECs adhere and they have ability to generate high amount of ECs for elongation of new branch. Finally, phalanx ECs emerge to induce maturation of new blood vessels by cell-cell adhesion through VE-cadherin. Moreover, ECs from arteries, veins and capillaries are genetically different. These suggest that ECs have heterogeneity; however, how such heterogeneity of ECs is induced has not been elucidated.

Tissue-resident stem cells play essential roles in tissue homeostasis and regeneration. We have previously reported that CD157-positive ECs in mice possess a vascular endothelial stem cell potential, contributing to physiological turnover and regeneration of the vasculature in the liver. Recently, we also found that ECs in the brain were divided into CD157-positive or -negative cells. We found that CD157-positive ECs showed higher proliferation and were beneficial compared to CD157-negative ECs upon inoculation in a chronic cerebral hypoperfusion model. We propose novel methods to improve the symptoms of chronic cerebral hypoperfusion using CD157-positive ECs (Matuis et al. *Commun biol* 2024). In this study, transplantation of multiple organ-derived ECs into a chronic cerebral hypoperfusion model in mice revealed the superior efficacy of brain-derived ECs. We found that brain-derived vascular ECs express more Unc 5B than other organs and angiogenesis was activated by netrin-1, suggesting that the netrin-1 and Unc 5B systems may be involved in brain-specific vascular system formation.

Moreover, we investigated transcription factors involved in the development or maintenance of vascular endothelial stem cells. We found that six transcription factors (ATF3, Bhlhe40, Egr1, Egr2, Elf3 and Klf4) increased CD157 in ECs and induced proliferation, angiogenesis, and drug resistance in these cells. These findings indicated that such transcription factors are useful clues for regenerative medicine relating to blood vessels and for understanding behavior of ECs (Konishi et al. *Mol Cell Biol* 2024).

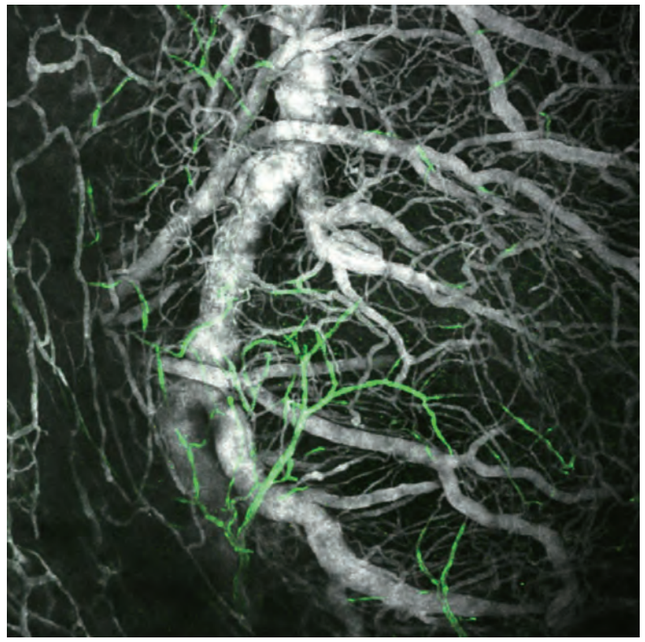

Figure.
Fluorescence images of vasculature transplanted with GFP-positive ECs derived from brains of green mice. We transplanted ECs into brains directly in a mouse model of chronic cerebral hypoperfusion. Blood flow was overlapped by labeling with AngioSPARK 680. Such trafficking was poor for liver-derived ECs and moderate for adipose-derived ECs. In addition, we injected tetramethylrhodamine-labeled dextran intravenously into each transplanted mouse and detected vascular permeability. Leakage was not observed from vessels generated by the transplantation of brain ECs. However, leakage occurred from vessels generated by the transplantation of fat or liver-derived ECs. Taken together, we concluded that brain-derived ECs generated adequate brain-specific blood vessels upon transplantation and that ECs from organs other than the brain may not fully function as cerebral vessels when transplanted ((Matuis et al. *Commun biol* 2024)).

Recent Publications

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Cutaneous Immunology

Manabu Fujimoto, MD/PhD

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Support Staff	1

Increased anti-oxidative action compensates for collagen tissue degeneration in vitiligo dermis

Vitiligo, a common disorder causing loss of skin pigment, shows enhanced skin tightness in affected areas compared to normal skin. Our study reveals that despite high oxidative stress in vitiligo, collagen production is maintained, with increased expression of collagen-related genes and antioxidants in vitiligo-derived fibroblasts. We observed more collagen fibers in vitiligo lesions, decreased collagen degradation due to reduced matrix metalloproteinases activity, and lower oxidative stress markers. The NRF2 pathway, crucial for combatting oxidative stress, was also upregulated. These findings suggest that both anti-oxidative actions and collagen integrity are preserved in vitiligo, offering insights into its biochemical resilience.

IL-10–Producing Potency from Blood B Cells Correlates with the Prognosis of Alopecia Areata

We investigated the role of B cells in the pathogenesis of alopecia areata (AA), an autoimmune disorder that causes hair loss. While cytotoxic T cells have been identified as a major factor, the involvement of B cells had not been studied. We found that B cells from patients with AA produced more of the anti-inflammatory cytokine IL-10 than B cells from healthy individuals. When AA patients were divided into good prognosis and poor prognosis groups based on hair regrowth, B cells from the good prognosis group produced more IL-10 than those from the poor prognosis group. CD8 T cells of patients with poor prognosis also showed reduced responsiveness to B cell-induced upregulation of IFN-g–producing activity.

CD69 Is Indispensable for Development of Functional Local Immune Memory in Murine Contact Hypersensitivity

This study explores the role of cell-surface molecules CD69 and CD103 in skin immune memory using a contact hypersensitivity (CHS) model in mice. By employing CD69-deficient (CD69KO) and CD103-deficient mice, the research observed the effects of these molecules on resident memory T (TRM) cells upon antigen re-exposure. Findings indicate that while CD103 deficiency does not significantly impair CHS, the absence of CD69 leads to a weakened CHS response upon repeated challenges. Despite the development of TRM cell-like CD8 T cells at the antigen exposure sites in CD69KO mice, these cells fail to function as effective TRM cells, as evidenced by diminished CHS responses mitigated by CD8 neutralization or FTY720, implicating the involvement of circulating CD8 T cells. Additionally, macrophage infiltration and chemokine levels (Cxcl1, Cxcl2) were reduced in CD69KO rechallenged sites, highlighting CD69's crucial role in facilitating effective immune recall through TRM cell functionality and macrophage recruitment.

Anti-α6β4 integrin autoantibodies inhibit the binding of laminins to α6β4 integrin in patients with pemphigoid and affect the gastrointestinal tract

This study investigates the role of anti-α6β4 integrin extracellular domain autoantibodies in patients with pemphigoid, exploring their impact on laminin-α6β4 integrin interactions and systemic effects. It involved analyzing sera from 20 pemphigoid patients to identify these autoantibodies and assess their ability to inhibit laminin binding to α6β4 integrin, expressed in its active conformation. The results confirmed the presence of IgG autoantibodies against the α6β4 integrin's extracellular domain in some patients, and these autoantibodies were capable of inhibiting the binding of laminins 511 and 332 to α6β4 integrin. Clinically, high antibody titres correlated with epithelial exfoliation in both skin and small intestine, with reductions in antibody levels corresponding to improvements in skin and gastrointestinal symptoms. This study highlights the significant inhibitory effect of anti-α6β4 integrin autoantibodies on integrin-laminin binding and their broader systemic impact, affecting multiple epithelial tissues.

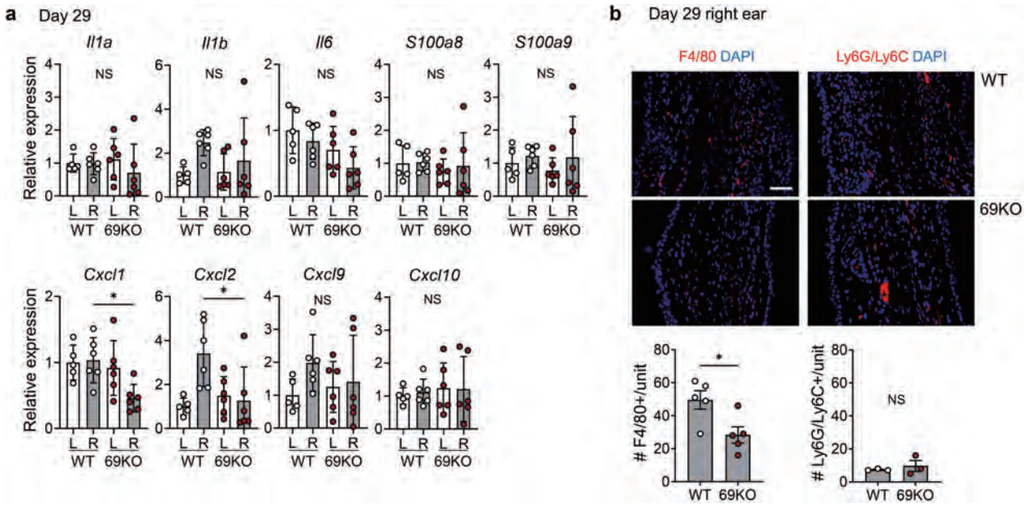

Figure.
The infiltration of macrophages is impaired after the second CHS in CD69KO mice. (a) The relative expression levels of the indicated genes evaluated by qPCR from day 29 ears of first CHS (denoted as L, white bars) and second CHS (denoted as R, gray bars) in WT (white dots) and 69KO (red dots) mice, normalized to the housekeeping gene. n = 5–6 per group. (b) Top: representative immunofluorescence results showing F4/80+ (left) and Ly6G/Ly6C+ (right) cells in day 29 right ears of WT and 69KO mice. Bar = 50 μm. Bottom: graphs showing the numbers of F4/80+ (left) and Ly6G/Ly6C+ (right) cells per unit area in day 29 right ears of WT and 69KO mice. n = 3–5 per group. Each dot represents the average from 4-unit areas of 3 specimens. CHS, contact hypersensitivity; NS, nonsignificant; WT, wild type.

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1. Connolly CM, et al. Idiopathic inflammatory myopathies: current insights and future frontiers. The Lancet Rheumatology. Feb 6(2). doi: 10.1016/S.2665-9913(23)00322-3 (2024).
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3. Wang WN, et al. Distinct Transcriptional Profiles in the Different Phenotypes of Neurofibroma from the Same Subject with Neurofibromatosis 1. J Invest Dermatol. 144(1):133-141 (2024).
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5. Matsumura Y, et al. IL-10‐Producing Potency from Blood B Cells Correlates with the Prognosis of Alopecia Areata. J Invest Dermatol. 143(5):871-874.e5 (2023).

Innate Immune Systems

Kazuyo Moro, DDS/PhD

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Clinically, interstitial lung diseases (ILDs) are classified into two main categories: idiopathic interstitial pneumonias (IIPs) and secondary ILDs. IIPs can be further subdivided into seven diseases. Pulmonary fibrosis is a specific type of ILD characterized by extracellular matrix deposition (such as collagen) in the alveolar interstitium, causing dyspnea. Among the IIPs, idiopathic pulmonary fibrosis (IPF), a progressive disease of unknown cause, has poor prognosis, with no effective treatment. Although the pathomechanism of IPF is not yet fully elucidated, tissue repair in response to repeated inflammation is thought to be involved in its development.

This year, we reported a novel mouse model for fibrosis capable of elucidating the pathogenesis of pulmonary fibrosis induced by internal factors. Fibrosis is a complex disease triggered by both actors (such as smoking and environmental agents) and internal factors (such as aging and autoimmune diseases) (Figure 1). Previous studies predominantly utilized bleomycin-induced mouse models to investigate fibrosis. Bleomycin induce DNA damage in epithelial cells, leading to the activation of T cells, neutrophils, and macrophages, ultimately resulting in TGFβ-dependent fibrosis (Figure 2 left). However, it has been recognized that this model is not ideal for comprehending fibrosis induced by internal factors.

We discovered that *Ifngr1*$^{-/-}$*Rag2*$^{-/-}$ mice, lacking the mechanisms to suppress group 2 innate lymphoid cells (ILC2s), spontaneously developed severe pulmonary fibrosis in an age-dependent manner. The lungs of young *Ifngr1*$^{-/-}$*Rag2*$^{-/-}$ mice showed normal structures but displayed cellular infiltration with fibrin accumulation at 15 weeks of age and obvious fibrosis was observed after 20 weeks of age. We expected that a detailed comparison of the inflammatory and fibrotic phases of *Ifngr1*$^{-/-}$*Rag2*$^{-/-}$ mice, in which fibrosis develops without external stimuli, would elucidate the mechanism of fibrosis caused by endogenous factors.

Levels of surfactant protein D (SP-D), a common clinical biomarker reflecting disease activity, increased and the saturation of percutaneous oxygen (SpO2) levels significantly decreased in aged mice. In these mice, the IL-33^{hi}IL-13^{hi} ILC2 subpopulation increased during the disease-onset phase before collagen production commenced. Although ILC2s are normally localized near bronchioles and blood vessels, ILC2s were increased in fibrotic areas along with IL-33 positive fibroblasts during fibrosis. Defect in ILCs and IL-33 in *Ifngr1*$^{-/-}$*Rag2*$^{-/-}$ mice prevented the development of fibrosis, indicating that IL-33-mediated activation of ILC2s is critical for fibrosis. Because *Ifngr1*$^{-/-}$*Il2rg*$^{-/-}$*Rag2*$^{-/-}$ mice congenitally lack ILC2s, we also confirmed that the acquired elimination of ILC2s by neutralizing antibodies (anti-Thy-1 antibody) prevent fibrosis. ILC2s were found to directly induce collagen production by fibroblasts *in vitro*, and pathogenic fibroblasts began producing IL-33 in the chronic phase, presumably establishing a positive feedback loop between fibroblasts and ILC2s leading to irreversible fibrosis (Figure 2 right).

Finally, the increased expression levels of *IL1RL1* (IL-33R) and *IL13*, along with decreased expression of *IFNGR1* were confirmed in ILC2s from idiopathic pulmonary fibrosis patients, suggesting that dysregulation of ILC2s may also cause endogenous fibrosis in humans. These results highlighting the applicability of *Ifngr1*$^{-/-}$*Rag2*$^{-/-}$ mice as a novel mouse model for fibrosis research.

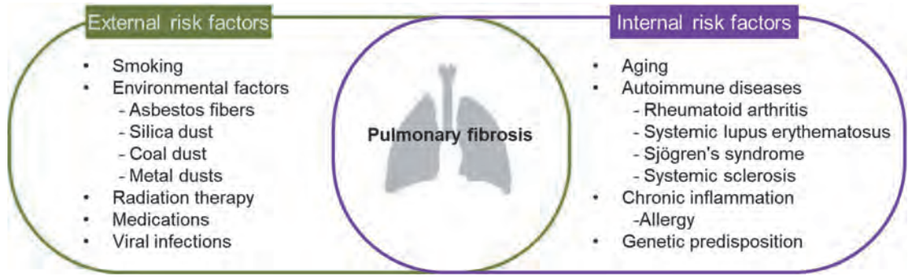


Figure 1.
External and internal risk factors for pulmonary fibrosis.

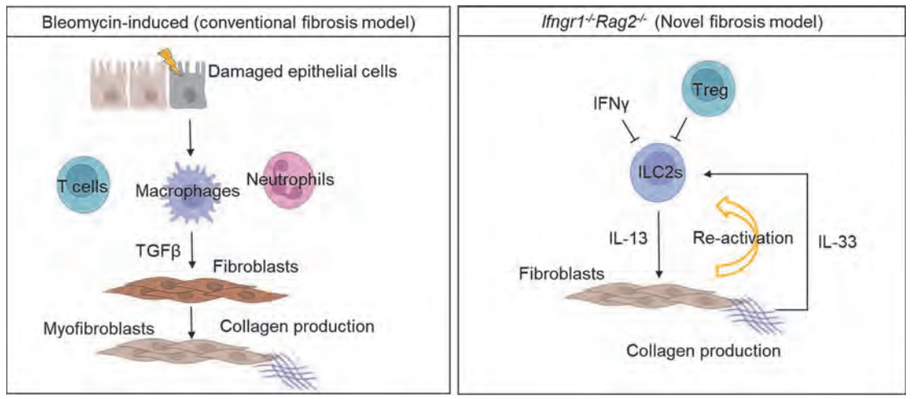


Figure 2.
The novel fibrosis model we present in this study consists of a mouse model in which fibrosis is induced by the deletion of two genes, *Ifngr1* and *Rag2*. In *Ifngr1*$^{-/-}$*Rag2*$^{-/-}$ mice, ILC2s directly influence fibroblasts, leading to the induction of collagen production. In addition, in the late stages of the disease, fibroblasts show increased IL-33 expression, suggesting that a feedback loop between ILC2s and IL-33 contributes to fibrosis exacerbations.

Recent Publications

1. Otaki N, Motomura Y, Terooatea T, Thomas Kelly S, Mochizuki M, Takeno N, Koyasu S, Tamamitsu M, Sugihara F, Kikuta J, Kitamura H, Shiraishi Y, Miyanohara J, Nagano Y, Saita Y, Ogura T, Asano K, Minoda A, Moro K. Activation of ILC2s through constitutive IFNγ signaling reduction leads to spontaneous pulmonary fibrosis. *Nat Commun* 14:8120 (2023).

2. Irie M, Kabata H, Sasahara K, Kurihara M, Shirasaki Y, Kamatani T, Baba R, Matsusaka M, Koga S, Masaki K, Miyata J, Araki Y, Kikawada T, Kabe Y, Suematsu M, Yamagishi M, Uemura S, Moro K, Fukunaga K. Annexin A1 is a cell-intrinsic metalloregulator of zinc in human ILC2s. *Cell rep* 42:112610 (2023).

3. Kobayashi T, Moro K. Tissue-Specific Diversity of Group 2 Innate Lymphoid Cells in the Skin. *Front Immunol* 13:885642 (2022).

Human Single Cell Immunology

James Wing, PhD

Associate Professor	James Wing
Assistant Professor	Jonas N. Søndergaard
Postdoctoral Fellow	3
Research Assistant	2
Visiting Scientist	1
Support Staff	1

The human immune system is highly complex, comprising of many interacting cell types. While approaches such as single cell sequencing (scRNAseq) have proven a critical advance in our ability to resolve this complexity, they are limited by the number of cells they can utilise making it difficult to see rare cell types or examine multiple samples over time. In contrast mass cytometry (CyTOF) can examine larger numbers of samples but is limited to only 40 or so markers, which is insufficient to examine multiple immune compartments in sufficient detail. To address this, we made a novel workflow for mass cytometry "Mass phenotyping" using this approach we can apply multiple specialised antibody panels to individual samples to resolve over a hundred immune markers in hundreds of human samples (Figure 1).

Using this approach, we examined large longitudinal cohorts of COVID-19 or Bacterial sepsis patients and mRNA vaccine recipients. This allowed us a wide overview of how the human immune system changes over time in these settings and a fine contrast of their similarities and differences using "Mass phenotyping" to screen hundreds of samples and single cell RNA sequencing to further investigate key differences. We primarily focused on the specialised CD4 helper cells known as Tfh and Tph and their interaction with antibody producing B-cells. During infection and vaccination, B-cells play a crucial role in producing antibodies and providing protection. However, our understanding of which B-cell types are most important remains incomplete. Previously, "classical memory" B-cells interacting with Tfh helper cells have been considered the primary responders to vaccines and infections, but this notion has been limited by technical challenges that have hindered in-depth analysis and the ability to examine large sample numbers over extended periods. By applying our new methods to on large cohorts of COVID-19 patients and mRNA vaccine recipients, we discovered that several forms of non-classical memory B-cells constituted the majority of SARS-CoV2-specific B-cells at different time points. During the acute phase, we identified a novel subset termed "Activated atypical" B-cells, which dominated the SARS-CoV2-specific B-cell response in both COVID-19 infection and mRNA vaccination. These cells were expanded at the same time as interferon producing Tfh subgroup known as Tfh1. In contrast to the acute phase, we observed a distinct pattern during the resting phase of the immune response. Our analysis revealed that a separate group of CD23+IL4R+ non-classical B-cells contained the majority of SARS-CoV2-specific memory (Figure 2). This finding highlights the heterogeneity of B-cell subsets involved in maintaining long-term immunity against SARS-CoV-2 and suggests that non-classical memory B-cells may play a more significant role than previously appreciated. The identification of these CD23+IL4R+ non-classical B-cells as key contributors to SARS-CoV2-specific memory provides valuable insights into the mechanisms underlying long-term protection against the virus and may have implications for the development of more effective vaccination strategies. Since this is the first description of a role for both Activated atypical memory and CD23+IL4R+ memory cells in vaccination and infection this is a major finding that changes our understanding of what types of cells respond to mRNA vaccination and may prove critical to the development of more effective vaccinations in the future.

This work is currently available as a preprint (Priest et al, Research Square, 2024).

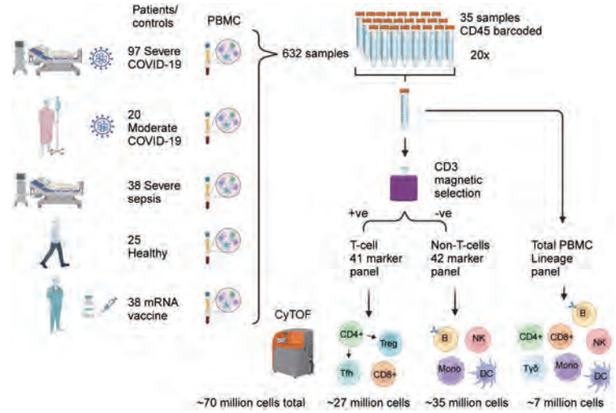


Figure 1.
Schematic of "mass phenotyping" workflow, a novel system allowing hundreds of samples to be run through a tri-panel design for detailed assessment of CD3+ (T-cells), CD3- (B, NK monocyte and DC) and a unmanipulated linage panel. This system allows hundreds of samples to be captured in high resolution.

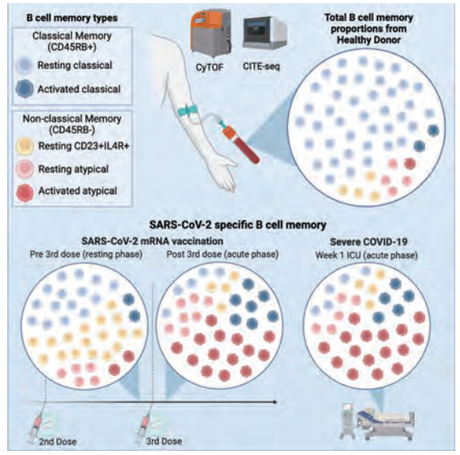


Figure 2.
Total and SARS-CoV2 specific B-cells following vaccination or SARS-CoV2 infection (COVID-19). While "classical" memory B-cells are the majority of B-cell memory we find that non-classical and atypical B-cells dominate the SARS-CoV2 response.

Recent Publications

1. Okamoto M, Sasai M, Kuratani A, Okuzaki D, Arai M, Wing JB, Sakaguchi S, Yamamoto M. A genetic method specifically delineates Th1-type Treg cells and their roles in tumour immunity. Cell Rep. 42:7 (2023).
2. Morita R, Kubota-Koketsu R, Lu X, Sasaki T, Nakayama EE, Liu Y-C, Okuzaki D, Motooka D, Wing JB, et al. COVID-19 relapse associated with SARS-CoV-2 evasion from CD4+ T-cell recognition in an agammaglobulinemia patient. iScience. 26:5 (2023).
3. Edahiro R, Shirai Y, Takeshima Y, et al. Single-cell analyses and host genetics highlight the role of innate immune cells in COVID-19 severity. Nat Gen. 55:753–767 (2023).
4. Shirai T, Nakai A, Ando E, et al Celastrol suppresses humoral immune responses and autoimmunity by targeting the COMMD3/8 complex. Sci Immunol. 8 (81) eadc9324. (2023).
5. Søndergaard JN, Tulyeu J, Edahiro R, …, Wing JB. A sex-biased imbalance between Tfr, Tph and atypical B cells, determines antibody responses in COVID-19 patients. Proc Natl Acad Sci USA. 120 (4) e2217902120 (2023).

Human Immunology (Single Cell Genomics)

Daisuke Okuzaki, PhD

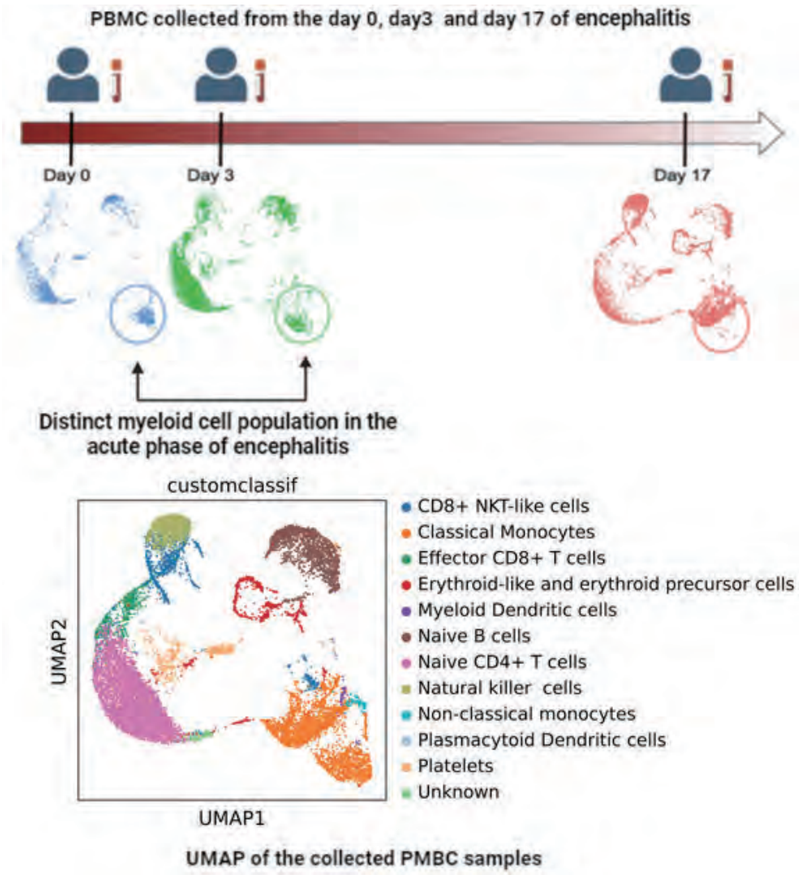
Associate Professor	Daisuke Okuzaki
Assistant Professor	Yuchen Liu
Postdoctoral Fellow	2
Visiting Scientist	2
Support Staff	1

We discovered a novel public antibody clonotype, namely PA-N-CoV1804, that reacts with both the SARS-CoV-2 N protein and several self-antigens. We found that the PA-N-CoV1804 clonotype underwent robust clonal expansion in a subset of COVID-19 patients. PA-N-CoV1804 was expressed exclusively in plasmablasts from COVID-19 patients. PA-N-CoV1804 harbors numerous somatic mutations, similar to antiviral antibodies derived from pre-existing memory cells for seasonal human coronaviruses. However, this clonotype was strongly reactive to the N protein of SARS-CoV-2 but not to that of seasonal coronaviruses. Therefore, these clones appear to originate from naive B cells and develop through de novo clonal expansion after SARS-CoV-2 infection. A manuscript describing this discovery is currently under peer review process.

In a joint research project conducted with Critical Care Medicine at Osaka University, a platform was established to serve as a guide for determining the necessity and duration of mechanical ventilation for severe COVID-19 patients admitted to the ICU at Osaka University Hospital. In a latent class analysis of whole blood RNA collected and sequenced from 40 patients, 45 genes were identified as effective markers for classifying the patients into three classes. Interaction between microRNA and mRNAs was also studied through simultaneous measurement of the mRNAs and microRNAs in the whole blood of severe COVID-19 patients. The integrated analysis revealed that, compared to interferon signaling in healthy subjects, interferon signaling was more activated in the COVID-19 patients.

By the end of the pandemic, our research focus shifted to the immune response following vaccination. In case of encephalitis temporally associated with COVID-19 vaccination, single-cell RNA sequencing (scRNA-seq) analysis was applied to elucidate the distinct immune signature in the peripheral immune system. Peripheral blood mononuclear cells (PBMCs) were analyzed using scRNA-seq to clarify the cellular components of the patients in both obtained data were compared to those acquired from a healthy cohort. The scRNA-seq analysis identified a distinct myeloid cell population in PBMCs during the acute phase of encephalitis. This specific myeloid population was neither detected in the remission phase of the disease nor in the healthy cohort. Our findings illustrate the induction of a unique myeloid subset in encephalitis temporally associated with COVID-19 vaccination. Further research into the dysregulated immune signature of COVID-19 vaccination-associated autoimmunity, including the cerebrospinal fluid (CSF) cells of central nervous system (CNS), is required to clarify the pathogenic role of this myeloid subset. The discovery was published in Frontiers in Immunology Feb. 23, 2023.

During the pandemic, we conducted non-COVID-related research that studied the transcriptome of *Vibrio parahaemolyticus* using a new long-read sequencing method, known as direct RNA sequencing. We developed a pipeline to process the data, revealing a more complex transcriptome landscape than previously understood, not only for *Vibrio* but for bacteria in general. Additionally, our original studies on developing a method for simultaneous detection of bacteria and eukaryotic cells at the single-cell level, and for the detection of circRNA using long-read sequencers, have the potential to advance research in many fields.



UMAP of the collected PMBC samples

Recent Publications

1. Kurosu T, Okuzaki D, Sakai Y, et al. Dengue virus infection induces selective expansion of Vγ4 and Vγ6TCR γδ T cells in the small intestine and a cytokine storm driving vascular leakage in mice. PLoS Negl Trop Dis. 17(11): e0011743 (2023).
2. Edahiro R, Shirai Y, Takeshima Y, et al. Single-cell analyses and host genetics highlight the role of innate immune cells in COVID-19 severity. Nat Genet. 55(5):753-767 (2023).
3. Namkoong H, Edahiro R, Takano T, et al. DOCK2 is involved in the host genetics and biology of severe COVID-19. Nature. 609(7928):754-760 (2022).
4. Ishikawa M, Shimada Y, Ozono T, et al. Single-cell RNA-seq analysis identifi es distinct myeloid cells in a case with encephalitis temporally associated with COVID-19 vaccination. Front Immunol. 14:998233 (2023).
5. Yamaguchi Y, Kato Y, Edahiro R, et al. Consecutive BNT162b2 mRNA vaccination induces short-term epigenetic memory in innate immune cells. JCI Insight. 7(22):e163347 (2022).

Immune Homeostasis

Yasutaka Okabe, PhD

Associate Professor	Yasutaka Okabe
Postdoctoral Fellow	1
Research Assistant	2
Support Staff	1

Intra-abdominal infection is the second most common cause of life-threatening sepsis, and it requires prompt medical treatment. Infection in the abdominal cavity (also reffered as the peritoneal cavity) can be caused by a pathological or traumatic loss of intestinal wall integrity, cirrhosis, pancreatitis, abdominal surgery, or peritoneal dialysis.

Macrophages in the peritoneal cavity are crucial component of abdominal immunity. We found transcription factor GATA6 is selectively expressed in macrophages in the peritoneal cavity. We reported that GATA6 acts as a master transcriptional regulator for functional specialization of peritoneal macrophages, as the deletion of GATA6 gene in peritoneal macrophages resulted in the defect of peritoneal specific gene expression program. Thus, GATA6-mediating program control macrophage positioning within peritoneal cavity, local proliferation, and peritoneal specific immune responses. In addition, we reported that retinoic acid, which is a lipophilic molecule derived from vitamin A, play essential role in the induction of GATA6 gene in peritoneal macrophages. We found retinoic acid nuclear receptors (RARs) bind to the promoter of GATA6 gene in retinoic acid dependent manner. In addition, the treatment of RAR inverse agonist (BMS493) abolished the expression of GATA6 gene in peritoneal macrophages. Lastly, vitamin A deficiency of mice caused to reduce GATA6 gene expression.

In addition to macrophages, the milky spots in omentum are atypical lymphoid tissues that play a pivotal role in regulating immune responses in the peritoneal cavity. The milky spots serve as a central gathering places for collecting antigens and particles from the peritoneal cavity, regulating the movement of lymphocytes, promoting the differentiation and self-renewal of immune cells, and supporting the local germinal center reaction. In addition, the milky spots exhibit unique developmental characteristics that combine the features of secondary and tertiary lymphoid tissues. These structures are innately programmed to form during fetal development; however, they can also be formed postnatally in response to peritoneal irritation such as inflammation, infection, obesity, or tumor metastasis. However, the mechanism underlying milky spot formation is poorly understood. We identified a subset of omental fibroblastic reticular cells (FRCs) that are characterized by the expression of retinoic acid converting enzyme. We found these FRCs are uniquely present in milky spots but not in lymph nodes. Furthermore, these FRCs are essential for the recruitment of circulating lymphocytes to milky spots, which is mediated by the induction of chemokine CXCL12 in a manner dependent on retinoic acid. Thus, our study demonstrates the stromal-immune cell interaction in the formation of nonclassical lymphoid tissues.

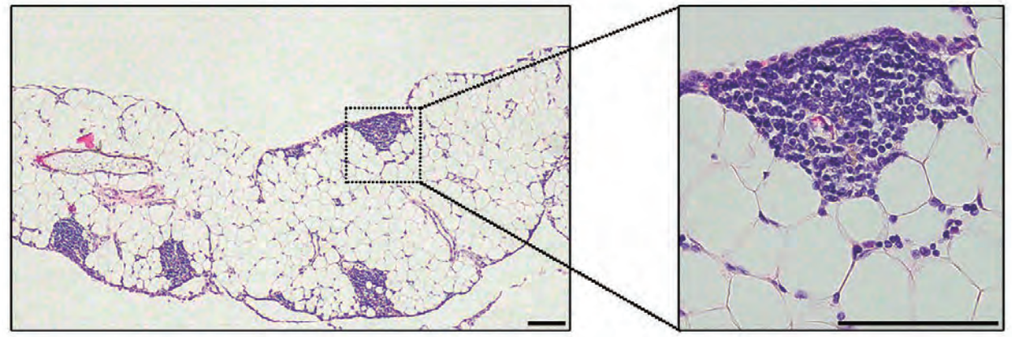


Figure.
Paraffin sections ofomentum fromWT mouse were stained with hematoxylin and eosin. Scale bar: 100 μm.

Recent Publications

1. Okabe Y. Development and organization of omental milky spots. Immunological Reviews, in press.
2. Yoshihara T. & Okabe Y. Aldh1a2+ fibroblastic reticular cells regulate lymphocyte recruitment in omental milky spots. J Exp Med. 220(5): e20221813 (2023).
3. Okabe Y. Immune Niche Within the Peritoneal Cavity. Curr Topic Microbiol Immunol. 434:123-134 (2021).

Cellular Immunotherapy

Naoki Hosen, MD/PhD

Professor	Naoki Hosen
Associate Professor	Hisashi Kato
	Kentaro Fukushima
	Jiro Fujita
Assistant Professor	Michiko Ichii
	Yasutaka Ueda
	Tomoaki Ueda
	Shinsuke Kusakabe
	Akihisa Hino
Visiting Scientist	1
Support Staff	3

We are focusing on cellular immunotherapy, especially chimeric antigen receptor (CAR)-T cell therapy for cancers. cells specifically recognize and activate cancer cells using the cancer-specific antigen recognition domain derived from mAbs. Activated CAR-T cells kill tumor cells and also proliferate extensively. CD19 CAR-T cells showed surprisingly high efficacy against acute lymphoblastic leukemia and malignant lymphoma. We discovered that the active conformer of an integrin β7 could serve as a specific therapeutic target for multiple myeloma (MM), an incurable hematologic cancer characterized by the accumulation of neoplastic plasma cells in the bone marrow (BM). The clinical trial of the CAR T-cell targeting the active conformation of the integrin β7 for MM is now underway. We screened more than 10,000 mAb clones raised against MM cells and identified R8H283 as an mAb that bound to MM cells but not to normal hematopoietic or non-hematopoietic cells. R8H283 specifically recognized CD98hc. R8H283 did not react with CD98hc monomer, but bound to CD98hc forming heterodimers with the light chains, which are amino acid transporters. MM cells expressed abundant CD98 heterodimers to take up amino acids for constitutive immunoglobulin production. Although CD98 heterodimers were also expressed in normal leukocytes, R8H283 did not react with them. Normal leukocytes expressed CD98hc glycoforms different from those expressed in MM cells, which may account for the lack of R8H283 reactivity in normal leukocytes. R8H283 exerted significant anti-MM effects without harming normal hematopoietic cells.

Development of CAR-T cell therapy targeting antigen structures formed as a result of post-translational events in various cancers

We have applied the same strategy to different types of cancer. For hematological cancers such as acute myeloid leukemia (AML), the only hurdle to developing CAR-T cell therapy is the lack of a suitable cancer-specific cell surface antigen. We have already generated a large number of mAbs that react with AML cells and have identified those that specifically react with AML cells. We have also applied the same strategy to various types of solid tumors in collaboration with various cancer treatment departments at Osaka University Hospital. We recently demonstrated that our screening method also works in glioblastoma.

Development of CAR NK cells

A major problem with CAR T cells is the high cost of producing them from autologous T cells for each patient. Since NK cells do not cause GVHD even when derived from allogeneic donors, CAR NK cells derived from a single donor can be used for multiple allogeneic recipients. The efficacy of cord blood-derived CD19 CAR NK cells has recently been demonstrated. We generate cord blood CAR-NK cells using our original CARs.

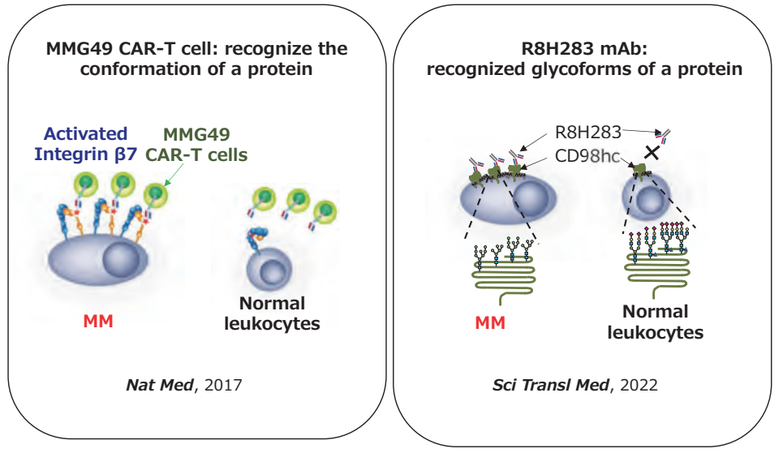


Figure 1.
Novel target antigen structures that we have identified for CAR-T cell therapy against multiple myeloma.

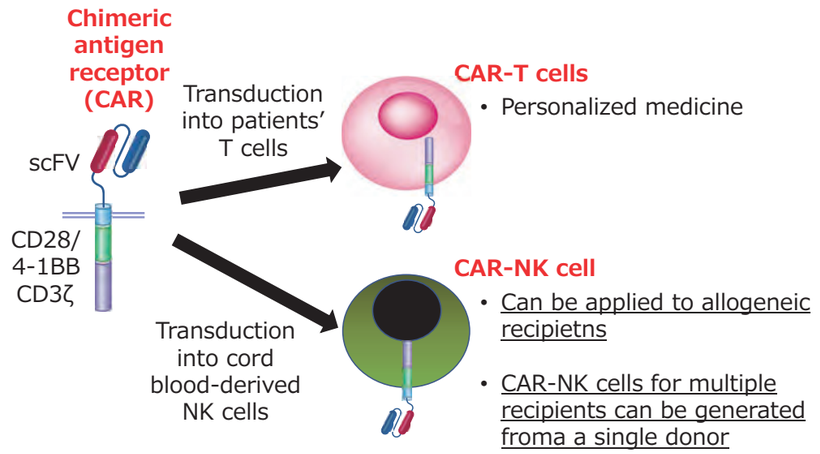


Figure 2.
CAR-NK cells can be used for allogenic recipients.

Recent Publications

1. Fukushima K, et al. Clostridium butyricum MIYAIRI 588 contributes to the maintenance of intestinal microbiota diversity early after haematopoietic cell transplantation. Bone Marrow Transplant. (2024).
2. Nakagawa T, et al. Identification of glioblastoma-specific antigens expressed in patient-derived tumor cells as candidate targets for chimeric antigen receptor T cell therapy. Neurooncol Adv. 5:vdac177 (2023).
3. Uchihara Y, et al. DNA damage promotes HLA class I presentation by stimulating a pioneer round of translation-associated antigen production. Mol Cell. 82:2557-2570 e2557 (2022).
4. Hino A, et al. Prolonged gut microbial alterations in post-transplant survivors of allogeneic haematopoietic stem cell transplantation. Br J Haematol. (2022).
5. Hasegawa K, et al. Selective targeting of multiple myeloma cells with a monoclonal antibody recognizing the ubiquitous protein CD98 heavy chain. Sci Trans Med. 14:eaax7706 (2022).

Microbiology and Immunology

Nobuhiko Kamada, PhD

Professor	Nobuhiko Kamada
Associate Professor	Sho Kitamoto
	Hiroko Kitamoto
Assistant Professor	Kohei Sugihara
Postdoctoral Fellow	2
Research Assistant	1
Support Staff	1

Our team has been studying the role of microbiota in the pathogenesis of gastrointestinal diseases, such as inflammatory bowel disease (IBD) and colorectal cancer (CRC). It has been reported that certain pathogenic members of commensal bacteria (namely 'pathobionts') are enriched under disease conditions and contribute to disease pathogenesis. However, the precise mechanisms by which such pathobionts thrive in disease conditions and trigger and/or exacerbate disease remain incompletely understood.

We demonstrated that L-serine metabolism plays a vital role in the survival of some pathobionts, including adherent–invasive *Escherichia coli*, a pathobiont associated with IBD. AIEC utilizes L-serine supplied by dietary protein intake in the gut lumen. Therefore, deprivation of dietary L-serine can suppress the expansion of AIEC in the gut. However, certain commensal bacteria can help AIEC overcome the dietary nutrient limitation. The presence of mucolytic bacteria allows AIEC to grow even under the restriction of dietary L-serine. Mucolytic bacteria, such as *Akkermansia muciniphila*, degrade the mucus layer and facilitate the encroachment of AIEC to the epithelial niche. In the epithelial niche, AIEC acquires L-serine from the amino acid pool of the colonic epithelium, allowing them to grow even under diet-derived L-serine restriction. Thus, the interaction between pathobionts and mucolytic commensal bacteria helps pathobionts switch their source of essential nutrients from the diet to host cells. In addition to AIEC for IBD, we are identifying pathobionts associated with various gastrointestinal diseases, such as CRC and intestinal fibrosis. We aim to elucidate the mechanisms by which these pathobionts adapt to disease-specific environments. Moreover, we are identifying partner bacteria that cooperate with pathobionts to promote disease progression and suppressors that inhibit the expansion of pathobionts. Also, we focus on the microbial and immune connections between the oral and gut mucosae in the pathogenesis of gastrointestinal diseases. We have discovered that inflammation in the oral mucosa results in the outgrowth of inflammatory oral pathobionts. Amassed oral pathobionts then naturally translocate to the gut and contribute to the pathogenesis of gut diseases. In addition, inflammatory immune cells arising during oral inflammation can also migrate to the gut. Gut-migrated inflammatory T cells of oral origin are activated by ectopically colonized oral pathobionts and contribute to inflammation in the gut mucosa. *Klebsiella aerogenes* is the most predominant bacteria species found in the inflamed oral cavity of periodontitis mice. We found that *K. aerogenes* employs chaperon usher pili (CUP) 1 for colonization in the inflamed gut but not in the oral mucosa. Thus, CUP1 serves as a key adhesion molecule for site-specific adaptation of the oral pathobiont.

Note: The PI is jointly appointed at IFReC and the University of Michigan (USA). These research projects were conducted at either IFReC or the University of Michigan.

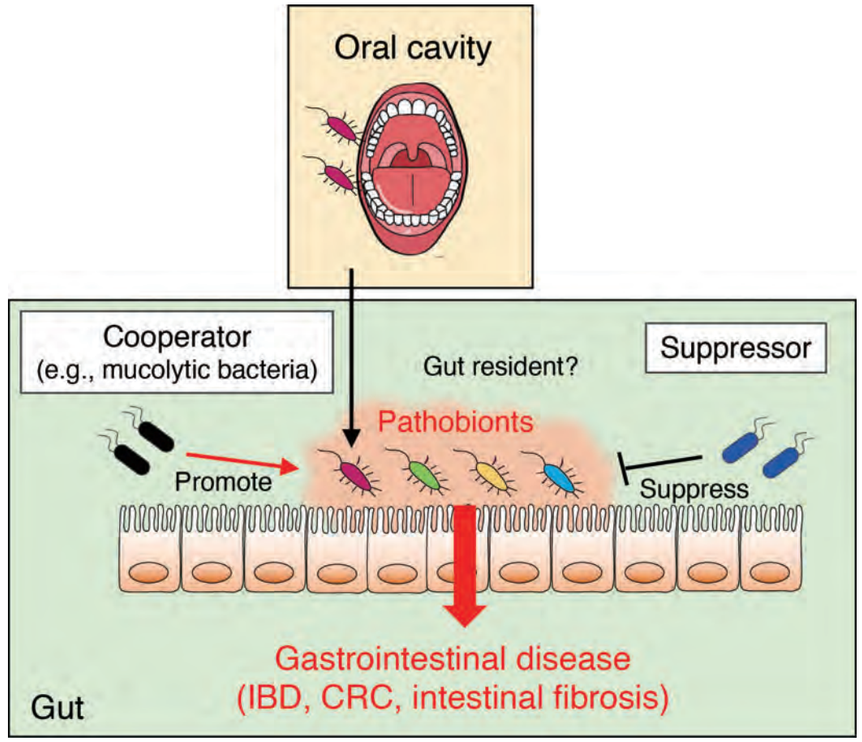


Figure.
Pathobionts promote various gastrointestinal diseases, including IBD, CRC, and intestinal fibrosis. Some pathobionts are oral origins. Certain symbiotic bacteria, such as mucolytic bacteria, act as 'cooperator' and help pathobionts acquire required nutrients. Some symbiotic bacteria may serve as 'suppressors.' These suppressors may directly and indirectly inhibit the expansion of pathobionts and subsequent disease progression.

Recent Publications

1. Guo Y, Kitamoto S, Caballero-Flores G, Kim Y, Watanabe D, Sugihara K, Núñez G, Alteri CJ, Inohara N, Kamada N. Oral pathobiont Klebsiella chaperon usher pili provide site-specific adaptation for the inflamed gut mucosa. Gut Microbes. 16(1):2333463 (2024).

2. Sugihara K, Kitamoto S, Saraithong P, Nagao-Kitamoto H, Hppstal M, McCarthy C, Rosevelt A, Muraleedharan CK, Gillilland III MG, Imai J, Omi M, Bishu S, Kao JY, Alteri CJ, Barnich N, Schmidt TM, Nusrat A, Inohara N, Golob JL, Kamada N. Mucolytic bacteria license pathobionts to acquire host-derived nutrients during dietary nutrient restriction. Cell Reports. 40(3):111093 (2022).

3. Kitamoto S, Nagao-Kitamoto H, Jiao Y, Gillilland III MG, Hayashi A, Imai J, Sugihara K, Miyoshi M, Brazil JC, Kuffa P, Hill BD, Rizvi SM, Wen F, Bishu S, Inohara N, Eaton KA, Nusrat A, Lei YL, Giannobile WV, and Kamada N. The intermucosal connection between the mouth and gut in commensal pathobiont-driven colitis. Cell. 182(2):447-462 (2020).

4. Nagao-Kitamoto H, Leslie JL, Kitamoto S, Jin C, Thomsson KA, Gillilland MG 3rd, Kuffa P, Goto Y, Jenq RR, Ishii C, Hirayama A, Seekatz AM, Martens EC, Eaton KA, Kao JY, Fukuda S, Higgins PDR, Karlsson NG, Young VB, Kamada N. Interleukin-22-mediated host glycosylation prevents Clostridioides difficile infection by modulating the metabolic activity of the gut microbiota. Nat Med. 26(4):608-617 (2020).

5. Kitamoto S, Alteri CJ, Rodrigues M, Nagao-Kitamoto H, Sugihara K, Himpsl SD, Bazzi M, Miyoshi M, Nishioka T, Hayashi A, Morhardt TL, Kuffa P, Grasberger H, El-Zaatari M, Bishu S, Ishii C, Hirayama A, Eaton KA, Dogan B, Simpson KW, Inohara N, Mobley HLT, Kao JY, Fukuda S, Barnich N, Kamada N. Dietary L-serine confers a competitive fitness advantage to Enterobacteriaceae in the inflamed gut. Nat Microbiol. 5(1):116-125 (2020).

Cutaneous Allergy and Host Defense

Yumi Matsuoka-Nakamura, MD/PhD

Professor	Yumi Matsuoka-Nakamura
Assistant Professor	Yuriko Yamazaki
Postdoctoral Fellow	1
Support Staff	3

Our laboratory, established in November 2022, is on a mission to unravel the complexities of inflammatory skin diseases such as atopic dermatitis (AD) and acne vulgaris. We are also deeply immersed in understanding how Methicillin-resistant *Staphylococcus aureus* (MRSA) evolves multi-drug resistance and perpetuates persistent infections within hospital environments. One of our pivotal research focus lies in exploring the dynamics between the accessory gene regulator (Agr) quorum sensing (QS) system of *S. aureus* and the host's innate immune response, both at the level of the skin and systemic infections. QS, a mechanism allowing bacteria to sense and respond to their population density, plays a crucial role in their survival strategy by modulating gene expression to optimize nutrient acquisition and compete with other bacteria for resources. Our investigations underscore the significance of QS in understanding the role of pathogenic bacteria in diseases, positioning it as a promising target for innovative therapeutic approaches.

In an earlier study, we leveraged bacterial whole-genome sequencing to examine *S. aureus* strains from the skin of Japanese infants. Our findings indicated a higher propensity for AD development in infants with *S. aureus* colonization on their cheeks. Notably, infants hosting S. aureus strains with spontaneous mutations in the agr QS system were more likely to remain healthy. This discovery points to a potential link between *S. aureus*, QS functionality, and the early onset of AD.

This year, we've made significant strides by reporting the impact of the skin microbiome and skincare interventions on AD in a Japanese birth cohort study. Analyzing 177 infants, all receiving skincare, we aimed to decipher the role of dysbiosis in AD's infancy onset. By the age of one, we observed AD development in 13 infants (7.3%), while three (1.7%) were diagnosed with a food allergy (FA), specifically to egg white (EW), with an overlap of one case also presenting AD. Furthermore, 61 infants (34.5%) without AD or FA showed sensitization to EW. Interestingly, the EW-sensitized group was associated with a higher use of moisturizer (TAM) and elevated serum total IgE levels compared to their healthy counterparts. Conversely, the AD group utilized less TAM yet exhibited higher IgE levels than healthy infants. Our microbiome analysis did not reveal significant diversity differences between healthy and AD groups, but it linked early life bacterial population shifts to subsequent AD development. Additionally, a negative correlation between TAM use and *Streptococcus sp.* abundance, alongside a positive correlation with *Cutibacterium acnes*, suggests that skincare practices could beneficially alter microbial compositions, potentially reducing AD risk. These insights advocate for the preventive potential of skincare interventions, including moisturizer use during infancy, against AD and FA.

Currently, we are delving into how Agr-QS influences MRSA's adaptation in hospital settings and its evolution towards multidrug resistance. Given MRSA's global health challenge, understanding its adaptive mechanisms in causing recurrent disease is crucial. Our ongoing project investigates MRSA strains from a neonatal intensive care unit (NICU) outbreak in a Japanese hospital, aiming to decode the bacterial attributes that facilitate *S. aureus*'s survival and persistence in hospital contexts.

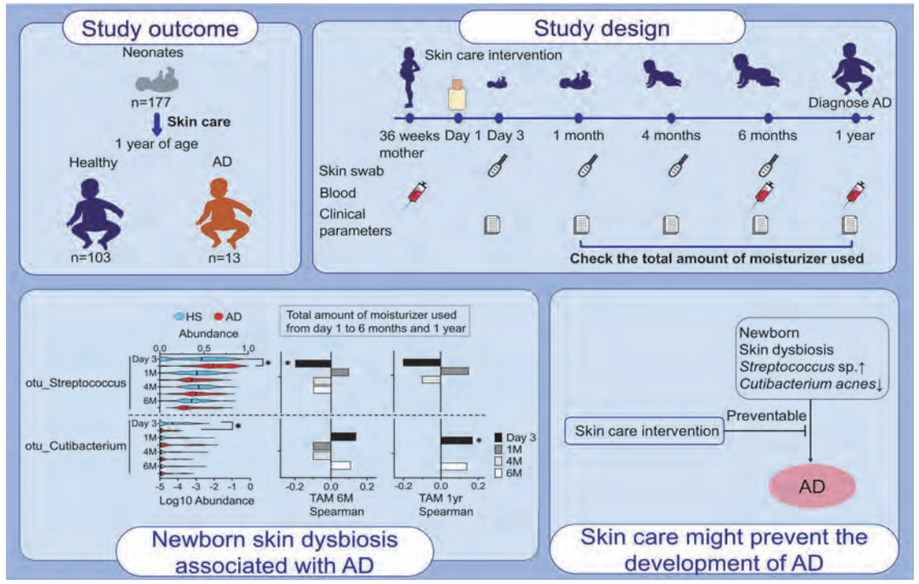


Figure.
Overview of skin care intervention cohort study.
AD, Atopic dermatitis; HS, Healthy subject; TAM, Total amount of moisturizer used.

Recent Publications

1. Aoyama R, Nakagawa S, Ichikawa Y, Inohara N, Yamazaki Y, Ito T, Sugihira T, Kono M, Akiyama M, Takahashi H, Takaya A, Ichikawa F, Nakano T, Tanaka S, Koyano Y, Fujimoto M, Núñez G, Shimojo N, Nakamura Y. Neonatal skin dysbiosis to infantile atopic dermatitis: Mitigating effects of skin care. *Allergy*. (2024).
2. Yamazaki Y, Ito T, Tamai M, Nakagawa S, Nakamura Y. The role of Staphylococcus aureus quorum sensing in cutaneous and systemic infections. *Inflamm Regen*. 44(1):9 (2024).
3. Matsumoto M, Nakagawa S, Zhang L, Nakamura Y, Villaruz AE, Otto M, Wolz C, Inohara N, Núñez G. Interaction between Staphylococcus Agr virulence and neutrophils regulates pathogen expansion in the skin. *Cell Host Microbe*. 29(6):930-940 (2021).
4. Miyachi H, Wakabayashi S, Sugihira T, Aoyama R, Saijo S, Koguchi-Yoshioka H, Fujimoto M, Núñez G, Matsue H, Nakamura Y. Keratinocyte IL-36 Receptor/MyD88 signaling mediates Malassezia-induced IL-17-dependent skin inflammation. *J Infect Dis*. 223(10):1753-1765 (2021).
5. Nakamura Y, Takahashi H, Takaya A, Inoue Y, Katayama Y, Kusuya Y, Shoji T, Takada S, Nakagawa S, Oguma R, Saito N, Ozawa N, Nakano T, Yamaide F, Dissanayake E, Suzuki S, Villaruz A, Varadarajan S, Matsumoto M, Kobayashi T, Kono M, Sato Y, Akiyama M, Otto M, Matsue H, Núñez G, Shimojo N. Staphylococcus Agr virulence is critical for epidermal colonization and associates with atopic dermatitis development. *Sci Transl Med*. 12:551 (2020).

Single Molecule Imaging

Toshio Yanagida, PhD
Ben Seymour, MD/PhD

Professor — Toshio Yanagida

Ben Seymour

How do humans and animals protect themselves so effectively against injury and illness for their entire lives? The brain is critical part of the systems that guide defensive action and physiology. This physiology involves two distinct components: behaviour that allows you to avoid injury/infection in the first place: a key part of which is the pain system that directs nocifensive actions (and also the closely related itch system). The second component is the behaviour that allows you to recover from injury or infection in a way that minimizes further harm. This system is best considered a multi-system system that incorporates brain, immune and endocrine axes, that work together to optimize body defence and recovery. We propose that there is a central homeostatic control circuit that governs this behaviour, and our experiments aim to discover where in the brain this is located, and how it controls physiology and behaviour. Discovering this circuit would open the door to a new generation of treatments of pain, fatigue and mood disorders, and offer a fundamental new approach to immunomodulation.

Our lab uses a combination of computational modelling, imaging, and behavioural experiments to understand how this system works. We have been building increasingly sophisticated models of defensive and recuperative behaviour, designed to capture the core computations that the brain makes in the face of threat and significant injury. We have discovered algorithms – that is, information processing systems – that can be shown to optimize efficient behaviour (in terms of reward harvesting) in dangerous environments (Mahajan et al, 2024). This allows, for example, animals to forage safely for food when exploring potentially dangerous new habitats or environments; and arises from having multi-module value memory systems in the brain, and employing a risk-sensitive decision-making. Overall, this allows us to begin to build a picture of what is under control, and this specificity should enable us to work backwards to identify the proposed central control circuit involved in changes in homeostasis (i.e. injury/illness allostasis).

Next, therefore, we have applied this to experiments probing how behaviour changes after an injury, using various behavioural tasks, and with concurrent neuroimaging. Using this computational approach, we can set out predictions for how the homeostatic priorities change, given optimal survival assumptions (Seymour, Crook and Chen, 2023). We have now applied this to experiments in humans with inflammatory arthritis (i.e. rheumatoid arthritis), and successfully shown that these theoretical models predict how behaviour is shaped in patients with inflammation and associated symptoms of fatigue and pain (see figure).

Using the computational signature alongside imaging data, we can then look across the brain to identify key areas involved in representation and control. However, as the brain is a complex information processing network, we use graph theory models to identify hubs of information flow. This approach has revealed a key role for the insula – an evolutionarily old brain regions closely associated with interoception (sensing the internal body), homeostasis and emotion; a region also closely connected to the hypothalamus and other key homeostatic structures (Mancini et al, 2024).

The next step in our research is to try and prospectively track these processes using longitudinal studies, whereby we can use temporal precedence to get closer to identifying causal circuits. We are also expanding this approach to develop partner rodent studies, where we can get a much finer handle on circuit architecture.

The lab has also been involved in capacity building activities. We have launched a fully-acredited course in neurotechnology for chronic pain (https://cpnn.ac.uk/neurotech-course/) which offers a series of 28 lectures and quizzes, in a free, online platform. We have been also running a series of practical workshops, again with UK-Japan collaboration build in. And lastly, we continue to actively pursue research that is diverse and inclusive, and in which the patient perspective plays a central role in the research process.

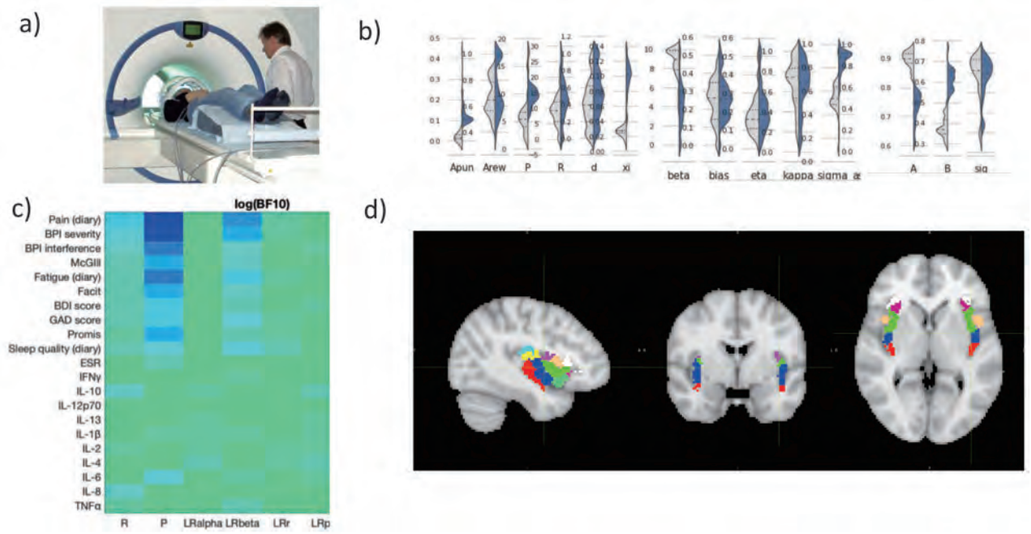


Figure.
Identifying potential injury-homeostatic circuits in the brain.
a) we use functional brain imaging to study brain activity whilst patients with inflammatory arthritis perform various motivation and decision-making tasks; b) computational analysis of behaviour yields a parameter profiles (computomic panels) that c) uniquely predict symptoms of inflammation (pain and fatigue); and d) these can be mapped to the brain using hub centrality metrics that derive from graph theoretic analysis of brain information networks.

Recent Publications

1. Mahajan P, Tong S, Lee SW and Seymour B. Balancing safety and efficiency in human decision making. bioRxiv, pp.2024-01 (2024).
2. Seymour B, Crook RJ and Chen ZS.. Post-injury pain and behaviour: a control theory perspective. Nat Rev Neurosci. 1-15 (2023).
3. Mancini F, Mahajan P, Guttesen AÁV, Onysk J, Scholtes I, Shenker N, Lee M. and Seymour B. Enhanced behavioural and neural sensitivity to punishments in chronic pain and fatigue. bioRxiv, pp.2024-04 (2024).
4. Onysk J, Gregory N, Whitefield M, Jain M, Turner G, Seymour B and Mancini F. Statistical learning shapes pain perception and prediction independently of external cues. eLife, 12 (2023).

Immunology and Cell Biology

Masaru Ishii, MD/PhD

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Associate Professor	Yasuhito Yahara
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	Kentaro Fujii
Postdoctoral Fellow	3
Research Assistant	2
Visiting Scientist	2
Support Staff	2

Crowds of people crossing streets, cars streaming along roadways, and trains that come and go carrying passengers: These are examples of ubiquitous, tightly organized human dynamics that support and vitalize societies. Similarly, in the human body, a variety of cells with different roles move and function according to their features and locations; collectively they comprise complex biological systems. A typical example is the immune system. Lymphocytes and macrophages migrate to every region of the body and gather in specific environments to exchange information and maintain normal immune responses. Thus, organisms are shaped by organizational dynamics. These dynamics are natural in life and do not occur following death. Understanding the dynamic cellular society by elucidating how cells move, or are moved, to maintain life is a fundamental theme in the life sciences. However, researchers have only recently succeeded in analyzing cellular movement. Recent advances in *in vivo* fluorescence imaging technologies have enabled researchers to look inside the bodies of live animals and analyze cellular and molecular dynamics. In our laboratory, we have developed a novel multiphoton excitation microscopy technique for studying the movement of immune cells in vivo using minimally invasive observational and analytical methods that maintained spatiotemporal information intact. Since we successfully observed the interior of live bone tissue, we have energetically pursued the analysis of cellular dynamics for various types of cells (Figure 1).

Especially as one of the most impacting achievements in the fiscal year of 2023, we demonstrated the spatiotemporal heterogeneity of inflammatory responses in the liver. The liver is the main gateway from the gut, and the unidirectional sinusoidal flow from portal to central veins constitutes heterogenous zones, including the periportal vein (PV) and pericentral vein zones; however, the functional differences in the immune system in each zone remain poorly understood. Here, intravital imaging revealed that inflammatory responses were suppressed in PV zones. The zone-specific single-cell transcriptomics detected an immunosuppressive macrophage subset enriched in PV zones that highly expresses IL-10 and Marco, a scavenger receptor sequestering pro-inflammatory PAMPs/DAMPs and consequently suppressing immune responses. Induction of the Marco+ immunosuppressive macrophages depended on gut microbiota, and especially, a specific bacterial family, *Odoribacteraceae*, was identified to induce this macrophage subset via its postbiotic, isoallo-lithocholic acid. Intestinal barrier leakage results in inflammation in PV zones, which was markedly augmented by Marco-deficient conditions. Chronic liver inflammatory diseases such as primary sclerosing cholangitis (PSC) and non-alcoholic steatohepatitis (NASH) showed decreased Marco+ macrophages. Functional ablation of Marco+ macrophages led to PSC-like inflammatory phenotypes related to colitis and exacerbated steatosis in NASH in animal experimental models. Collectively, commensal bacteria induce Marco+ immunosuppressive macrophages, consequently limiting excessive inflammation at the gateway. Failure of this self-limiting system promotes hepatic inflammatory disorders such as PSC and NASH (Figure 2).

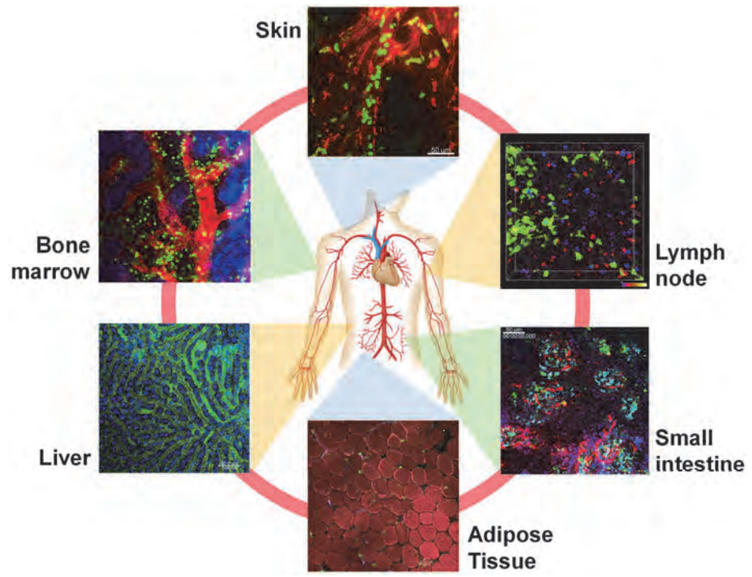

Figure 1.
A dynamic cellular system inside the living body.

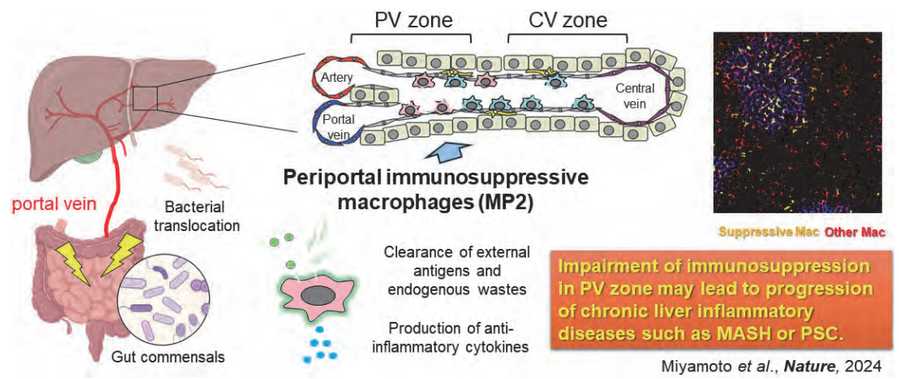

Figure 2.
Periportal macrophages protect against commensal-driven liver inflammation.

Recent Publications

1. Miyamoto Y, Kikuta J, Matsui T, Hasegawa T, Fujii K, Okuzaki D, Liu Y-C, Yoshioka T, Seno S, Motooka D, Uchida Y, Yamashita E, Kobayashi S, Eguchi H, Morii E, Tryggvason K, Shichita T, Kayama H, Atarashi K, Kunisawa J, Honda K, Takeda K, Ishii M. Periportal macrophages protect against commensal-driven liver inflammation. Nature. 629:901–909 (2024).

2. Shimizu K, Kikuta J, Ohta Y, Uchida Y, Miyamoto Y, Morimoto A, Yari S, Sato T, Kamakura T, Oshima K, Imai R, Liu Y-C, Okuzaki D, Hara T, Motooka D, Emoto N, Inohara H, Ishii M. Single-cell transcriptomics of human cholesteatoma identifies an activin A-producing osteoclastogenic fibroblast subset inducing bone destruction. Nat Commun. 14(1):4417 (2023).

3. Taniguchi S, Matsui T, Kimura K, Funaki S, Miyamoto Y, Uchida Y, Sudo T, Kikuta J, Hara T, Motooka D, Liu Y-C, Okuzaki D, Morii E, Emoto N, Shintani Y, Ishii M. In vivo induction of activin A-producing alveolar macrophages supports the progression of lung cell carcinoma. Nat Commun. 14(1):143 (2023).

Chemical Imaging Technique

Kazuya Kikuchi, PhD

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Assistant Professor	Tomoya Yamamoto
Postdoctoral Fellow	1
Research Assistant	1
Support Staff	2

"Elastic Polymer Coated Nanoparticles with Fast Clearance for ^{19}F MR Imaging"

Magnetic resonance imaging (MRI) is a critical noninvasive tool for molecular imaging, with ^{19}F MRI being particularly advantageous due to its high sensitivity and the 100% natural abundance of ^{19}F atoms. The low endogenous background signal of ^{19}F in the body enables high-contrast imaging, making it ideal for tracking ^{19}F-containing probes in deep tissues and monitoring enzyme activity in vivo. Our group has developed FLAME (FLuorine Accumulated silica nanoparticle for MRI Contrast Enhancement), consisting of perfluorocarbon (PFC)-encapsulated silica NPs. However, the efficacy of silica nanoparticles (NPs) as ^{19}F MRI probes has been hampered by their propensity to accumulate in the liver, leading to prolonged retention and potential side effects such as chronic inflammation and toxicity. To address this limitation, elastic nanomaterials, including nanogels and polymersomes, have emerged as attractive cargo for drug-delivery systems (DDSs). With advances in nanomaterial synthesis and the characterization of stiffness, the role of NP elasticity in biological systems has gained increased attention. Thus, we aim to develop PFC-encapsulated elastic NPs to improve delivery efficacy and reduce long-term accumulation.

The synthesis involved forming perfluoro-15-crown-5-ether (PFCE)-encapsulated micelles followed by surface-templated reversible addition-fragmentation chain transfer (RAFT) polymerization. This process used a trithiocarbonate-based chain transfer agent (CTA) for controlled polymerization. The NPs had an average size of 162 nm and a negative surface charge (−30.0 mV), similar to silica NPs. We confirmed the deformability of the obtained NPs with high-speed atomic force microscopy (HS-AFM). They could shrink and return to their original shape under pressure without leakage of the core compound, unlike stiff silica NPs that only slid on the substrate without significant morphological changes. The ^{19}F NMR spectrum showed a sharp peak corresponding to the encapsulated PFCE, with the transverse relaxation time (T_2) of the encapsulated PFCE in polymer NPs being slightly shorter than that of non-encapsulated PFCE, indicating well-dispersed NPs in aqueous solution and substantial sensitivity in ^{19}F NMR signal detection. The amounts of adsorbed serum proteins on the NP surfaces were quantitatively analyzed. Notably, the amounts of protein adsorbed on the polymer NPs were below the detection limit and significantly lower compared to the silica NPs. This indicates their potential for mitigating unwanted immune responses and improved behavior in biological systems. influence the biodistribution of nanomaterials.

We further visualized the biodistribution of elastic polymer NPs using ^{19}F MRI. The NPs were administered intravenously to a mouse and monitored for three weeks. Acute toxicity was not observed after injection. At 2 weeks after injection, the ^{19}F MRI signal intensity of the polymer NPs in the liver and spleen gradually decreased over time. Additionally, we compared the biodistribution of elastic polymer NPs with that of stiff silica NPs using multi-color ^{19}F MRI within a single mouse. The results demonstrated that the polymer-coated NPs exhibited a gradual decline in signal intensity post-administration, indicating effective clearance from the liver or degradation over time. This behavior contrasts with stiff silica NPs, which tend to accumulate and persist in the liver.

The development of these elastic polymer-coated NPs represents a significant advancement in the field of molecular imaging. The study highlights the importance of NP elasticity in enhancing clearance from the body, thereby reducing potential side effects associated with long-term accumulation. The ability to track NP dynamics in vivo using ^{19}F MRI offers a powerful tool for real-time and longitudinal monitoring, which is crucial for the clinical translation of nanoparticle-based imaging agents and therapeutics.

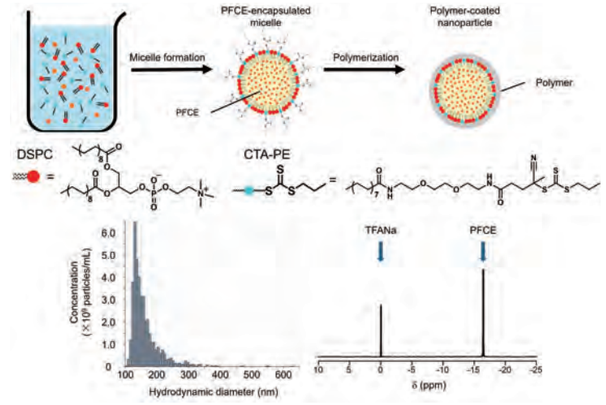

Figure 1.
(top) Schematic image of elastic polymer NPs containing PFCE prepared by polymerization on micelle. (bottom) Size distribution of polymer-coated NPs using a nanoparticle analyzer and ^{19}F NMR spectrum of polymer-coated NPs containing PFCE (TFANa was used as an internal standard).

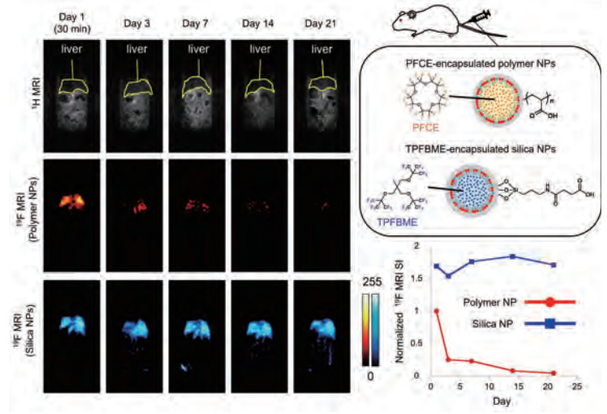

Figure 2.
^{1}H/^{19}F MRI images of a mouse injected with polymer and silica NPs. Time course of ^{19}F MRI signal intensity changes in liver. Normalized by ^{19}F MRI of polymer NPs on Day 1.

Recent Publications

1. Torii K, et al. No-wash Fluorogenic Labeling of Proteins for Reversible Photoswitching in Live Cells. Chem Sci. 15:1393-1401 (2024).
2. Konishi Y, et al. Elastic Polymer-coated Nanoparticles with Fast Clearance for ^{19}F MR Imaging. Angew Chem Int. Ed. 62: e202308565 (2023).
3. Minoshima M, et al. Development of a Versatile Protein Labeling Tool for Live-Cell Imaging Using Fluorescent β-Lactamase Inhibitor. Angew Chem Int. Ed. 62: e202301704 (2023).
4. Nishiura M, et al. Visualization of Multiple Localizations of GLUT4 by Fluorescent Probes of PYP-tag with Designed Unnatural Warhead. Chem Sci. 14:5925-5935 (2023).

Immune Response Dynamics

Kazuhiro Suzuki, MD/PhD

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Support Staff	3

Our research focus has been to discover novel mechanisms that control lymphocyte migration and elucidate their physiological and pathological significance. Our previous studies showed that inputs from adrenergic neurons to the β2-adrenergic receptor expressed on lymphocytes enhance the responsiveness of specific chemokine receptors and inhibit lymphocyte exit from lymph nodes (Nakai et al. J Exp Med. 2014). This mechanism was found to generate diurnal variations in lymphocyte numbers in lymph nodes and consequently the magnitude of adaptive immune responses in phase with the circadian oscillation of adrenergic neuron activity (Suzuki et al. J Exp Med. 2016). These studies provided insights into the molecular basis of the interaction between the nervous and immune systems.

In our efforts to elucidate the mechanism of the crosstalk between the β2-adrenergic receptor and chemokine receptors, we identified a protein complex consisting of copper metabolism MURR1 domain-containing (COMMD) 3 and COMMD8 (COMMD3/8 complex) as a positive regulator of chemokine receptor signaling. Our study demonstrated that the COMMD3/8 complex plays important roles in the control of B cell migration and the induction of humoral immune responses (Nakai et al. J Exp Med. 2019). However, the contribution of the COMMD3/8 complex to the pathogenesis of immunological disorders was unclear.

Based on the important role of the COMMD3/8 complex in humoral immune responses, we tested its involvement in collagen-induced arthritis, a B cell-dependent mouse model of rheumatoid arthritis. COMMD3/8 complex deficiency induced at the onset of arthritis inhibited disease progression. This was accompanied by a reduced humoral immune response to collagen. These findings indicated that the COMMD3/8 complex contribute to the pathogenesis of rheumatoid arthritis (Shirai et al. Sci Immunol. 2023).

Prompted by this finding, we performed a chemical screen to identify inhibitors of the COMMD3/8 complex that could be used for the treatment of autoimmune diseases. Since the function of the COMMD3/8 complex depends on the association between COMMD3 and COMMD8, we sought for compounds that disrupt the physical interaction between the two COMMD proteins. After screening of a chemical library that was relatively enriched in natural products, we identified celastrol as the most potent compound. Celastrol is a bioactive molecule extracted from a medicinal herb, *Tripterygium wilfordii*, and exhibits anti-inflammatory properties. However, its mechanism of action had been poorly understood. Celastrol disrupted the COMMD3/8 complex in living cells or in the purified form, indicating direct action of celastrol on the COMMD3/8 complex. Using site-directed mutagenesis, molecular dynamics simulations (Figure) and liquid chromatography-tandem mass spectrometry, we revealed that celastrol covalently binds to cysteine 170 (C170) on COMMD3 to dissociate the COMMD3/8 complex (Shirai et al. Sci Immunol. 2023).

We then asked whether celastrol reproduces the functional consequences caused by COMMD3/8 complex deficiency. Celastrol inhibited chemotactic migration of B cells in vitro and in vivo. Celastrol treatment suppressed antibody responses with reduced production of germinal center B cells and plasma cells. The progression of collagen-induced arthritis was blocked by celastrol treatment started at the disease onset. Thus, celastrol treatment phenocopied COMMD3/8 complex deficiency, suggesting that celastrol may target the COMMD3/8 complex in the context of humoral immune responses and autoimmunity.

Since alanine substitution of C170 (C170A) on COMMD3 rendered the COMMD3/8 complex resistant to celastrol while preserving the function of the protein complex, we generated a mouse strain expressing COMMD3^{C170A} from the endogenous *Commd3* locus and examined whether the effects of celastrol are abolished in these mice. B cells isolated from COMMD3^{C170A} mice showed complete resistance to celastrol in chemotactic migration. Humoral immune responses and collagen-induced arthritis in the mutant mice were not suppressed by celastrol treatment. These findings indicated that the COMMD3/8 complex is a major target of celastrol (Shirai et al. Sci Immunol. 2023).

Our study suggests that the COMMD3/8 complex is involved in the progression of antibody-mediated autoimmunity. However, the point of action of the COMMD3/8 complex in the pathogenesis of autoimmune diseases has not been clearly defined. Additionally, since most of our knowledge about the COMMD3/8 complex has been obtained in mice, the relevance of our findings to autoimmunity in humans remains to be established. Future studies will overcome these limitations and realize the development of a novel therapy targeting the COMMD3/8 complex in immune disorders.

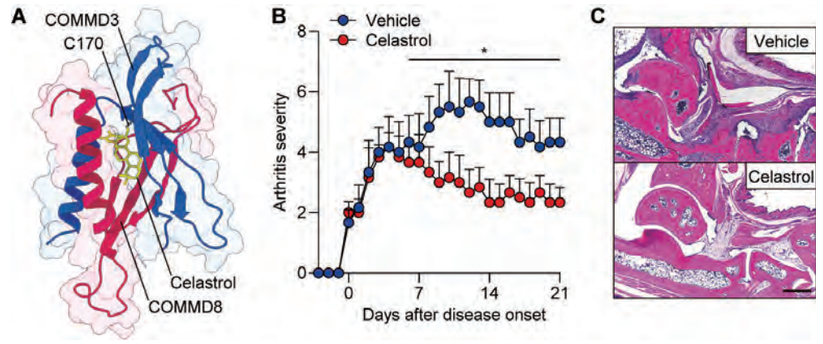

Figure.
Celastrol inhibits the COMMD3/8 complex and alleviates autoimmunity. (**A**) An *in silico* model of the interaction between celastrol and the COMMD3/8 complex. (**B** and **C**) Effects of celastrol on the severity (B) and histopathology (C) of collagen-induced arthritis. Scale bar, 500 μm.

Recent Publications

1. Shirai T, Nakai A, Ando E, Fujimoto J, Leach S, Arimori T, Higo D, van Eerden FJ, Tulyeu J, Liu Y-C, Okuzaki D, Murayama MA, Miyata H, Nunomura K, Lin B, Tani A, Kumanogoh A, Ikawa M, Wing JB, Standley DM, Takagi J, Suzuki K. Celastrol suppresses humoral immune responses and autoimmunity by targeting the COMMD3/8 complex. Sci Immunol. 8: eadc9324 (2023).

2. Nakai A, Fujimoto J, Miyata H, Stumm R, Narazaki M, Schulz S, Baba Y, Kumanogoh A and Suzuki K. The COMMD3/8 complex is a determinant of GRK6 specificity for chemoattractant receptors. J Exp Med. 216:1630-1647 (2019).

3. Suzuki K, Hayano Y, Nakai A, Furuta F and Noda M. Adrenergic control of the adaptive immune response by diurnal lymphocyte recirculation through lymph nodes. J Exp Med. 213:2567-2574 (2016).

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Biophotonics

Nicholas Isaac Smith, PhD

Associate Professor	Nicholas Isaac Smith
Assistant Professor	Alison Hobro
Support Staff	1

The Biophotonics laboratory develops tools for label-free analysis of single cells. Single-cell analysis is a popular target for a variety of research fields, usually pursued by labeling surface markers, by introducing fluorescent dyes into the cell, or by invasive, yet comprehensive, techniques such as single cell RNA sequencing. In contrast, our tools are based on label-free optical methods, which aim to produce some of the same discriminatory capability as the more invasive methods. Additionally, label-free methods are based on endogenous contrasts of the cell, and can also find novel features that can be used to discriminate between cell phenotypes or cell states.

In the last year we were fortunate to complete several projects. We have proposed that unlabeled Raman measurement of single cells can have sufficient sensitivity to provide discrimination which has previously needed labels or sequencing to achieve. Now, we established more clear limits on exact levels of sensitivity when used for phenotyping cells and established safe laser power regimes where we demonstrate that no adverse effects on cells are occurring. While optical techniques usually fall into categories of either imaging, or non-imaging single measurement (as in flow cytometry), we showed that cell phenotyping can be done in imaging or non-imaging modes, or even partway between the two extremes, where some amount of spatial information is captured, increasing phenotype discrimination accuracy compared to single point measurement, while still allowing for higher throughput than full imaging which is typically too slow for biologically relevant phenotyping. By comparing 4 different possible modes of measurement, and assessing each in terms of the discrimination power, as well as any induced change in the sample (denoted as differential spectra in the figure at different power and exposure regimes), we showed how to maximize the measured features of each cell while ensuring that no laser-induced changes are occurring.

We also used Raman imaging to elucidate both morphological and biochemical changes occurring in the liver in mouse fed with a high-fat diet, leading to the onset of NASH (a type of fatty liver disease). While it is known that a high fat diet leads to aggregation of lipids in the liver, we used Raman imaging to assess how they were distributed, and whether they were correlated with those in the diet itself. We found that oleic acid dominated the lipid buildup in the liver, as a direct result of the diet, while other dietary components, such as sucrose and glucose were not deposited. This type of unlabeled imaging using no a priori information can be used for a 3-way quantitative comparison of components across a) diet, b) diseased tissues and c) healthy tissues, giving weights for each component that denote their actual strength. The possibility to identify emergent differences also exists, and in this study we were able to observe lipid peroxidation occurring in the NASH liver samples, as a distinct component not present to the same degree in control samples.

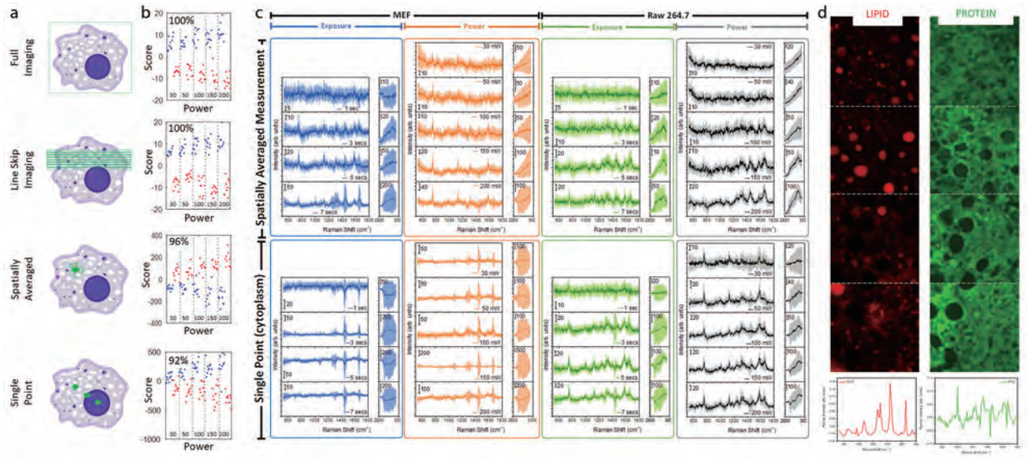
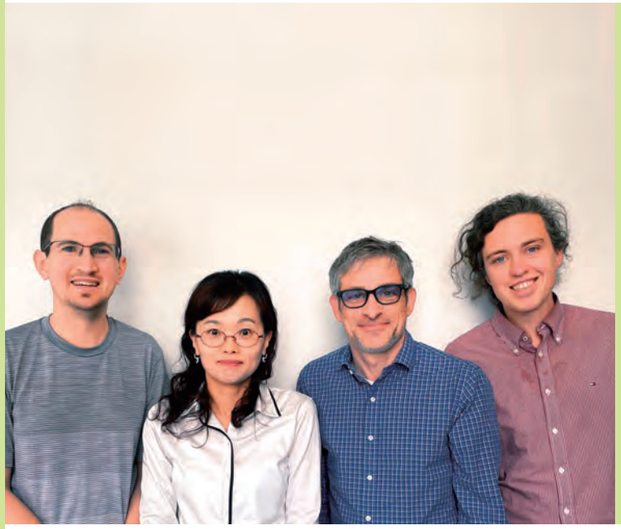


Figure.
For single-cell Raman phenotyping, we created several techniques, including no spatial information for the fastest throughput, using full spatial information for the greatest amount of data, and several hybrid techniques that blend the ability to read intracellular components across regions in the cell while still allowing high throughput. The left four panels (a) show the different modalities, compared by their ability to classify MEF vs Raw264 cells (b). At higher throughputs, we can forgo imaging completely, and use only a single point illumination, but the ability to characterize each still is still improved by spatially averaging the information across each individual cell. Spectra shown in (c) are differential spectra showing any changes induced across sequential measurements with the ideal result there being no significant change induced by the measurement itself. The spatially averaged measurement system we developed is significantly better than single point measurement for single-cell phenotyping. In (d) we see the full Raman imaging of NASH-affected liver, where the building of lipids, in this case primarily oleic acid occurs in holes in the distribution of proteins and other components. The identification of the components requires no a priori information or labeling.

Recent Publications

1. Hobro AJ, et al. Imaging vs non-imaging Raman spectroscopy for high-throughput single-cell phenotyping. Analytical Chemistry. (2024).
2. Lelliott PM, et al. Cellular Adhesion Is a Controlling Factor in Neutrophil Extracellular Trap Formation Induced by Anti-Neutrophil Cytoplasmic Antibodies. ImmunoHorizons. 6 (2):170-183 (2022).
3. Pavillon N, and Smith NI. Deriving accurate molecular indicators of protein synthesis through Raman-based sparse classification. Analyst. 146, pp. 3633-3641 (2021).
4. Sugiyama T, et al. Label-free Raman mapping of saturated and unsaturated fatty acid uptake, storage, and return toward baseline levels in macrophages. Analyst. 146 (4):1268-1280 (2020).
5. Pavillon N, Hobro AJ, Akira S and Smith NI. Noninvasive detection of macrophage activation with single-cell resolution through machine learning. Proc Natl Acad Sci USA. 115(12):E2676-E2685 (2018).

Systems Immunology



Daron Standley, PhD

Professor	Daron Standley
Associate Professor	Kazutaka Katoh
	Songling Li
	Soyoung Park
Postdoctoral Fellow	1
Research Assistant	2
Visiting Scientist	4

Classification of health/disease from Adaptive Immune Receptors

We have formulated the problem of disease diagnosis from Adaptive Immune Receptors (AIRs) as a classification problem that can be solved by ML. The classifier, MyImmune, required the development of a more useful representation of AIRs than the conventional "clonotype" definition, that could group AIRs from different individuals (Fig. 1A). The resulting clusters were found to be >95% specific in terms of targeted antigens (Fig. 1B). We then validated MyImmune on three classes of disease: infectious (COVID-19, HIV), autoimmune (Autoimmune Hepitaitis, Type-1 diabetes) and cancer (non-small cell lung cancer, colorectal cancer). MyImmune demonstrated significant improvement over seven alternative classifiers using the same inputs: mean ROC AUC 0.893 vs 0.777, respectively. Moreover, we observed linear improvement on MyImmune's performance with the size of the training data (Fig. 1C). A surprising finding for cancer is that healthy individuals harbor a reservoir of potential tumor-infiltrating lymphocytes that can be identified by MyImmune feature importance.

Molecular function from simulation of protein dynamics

Drawbridge model for T cell receptor activation. Despite the critical importance of the T cells in immune responses, it is still unclear how T cell receptor (TCR) binding to specific peptide-MHC (pMHC) complexes results T cell activation. Based on extensive multiscale molecular dynamics simulations we formulated the drawbridge model (Figure 2A) in which the TCR receptor releases a brake on CD3 proteins upon pMHC binding. This model is consistent with a wide range of experimental data, including mutagenesis of the hinge and FG loop residues, as well as recent cryo-EM analysis.

Mechanism of activity enhancing antibodies in SARS-CoV-2. SARS-CoV2 infection enhancing antibodies, discovered in the Arase Laboratory, exert their effect in an Fc-independent manner. Together, we hypothesized that this mechanism involves crosslinking two neighboring spike proteins. Long-range molecular dynamics simulations on the Fugaku computer support a model in which such crosslinking decouples the N-terminal domain from the receptor-binding domain (RBD), allowing the RBD to transition from the "down" to the "up" orientation, where it can engage with the host entry receptor ACE2.

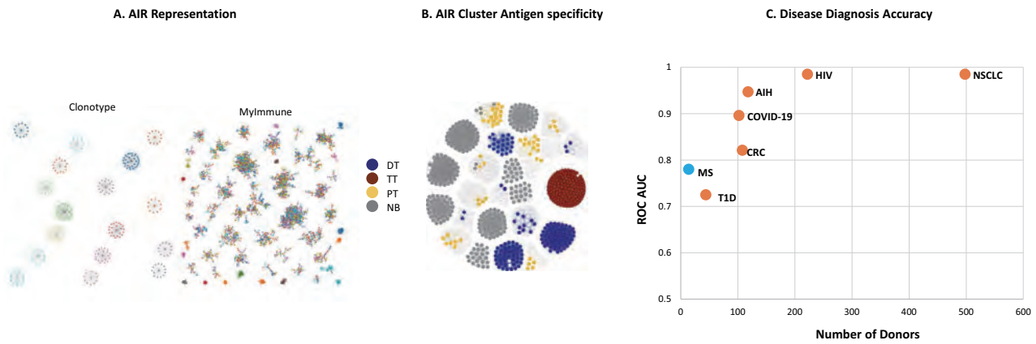


Figure 1.
MyImmune Disease Classification. **A,** B cell receptor (BCR) heavy chain networks were constructed for 10 COVID-19 patients; in these networks, nodes are colored by donor, and edges represent clonotype or MyImmune similarity. Clonotype networks are typically small and do not connect different donors. MyImmune networks are much larger and generally connect many donors. **B,** BCR networks derived from single-cell immune profiling of diphtheria-tetanus-pertussis (DTP)-vaccinated donors were demonstrated by ELISA to exhibit 96% antigen purity, surpassing the apparent 82% purity achieved by assigning antigens to the same B cells using fluorescently labeled DTP antigen probes. **C,** The performance of MyImmune on new indivuduals (i.e. not seen by the ML training) was benchmarked for six diseases in three categories using the Area Under the Receiver Operating Characteristic Curves (ROC AUC). The MyImmune performance increased linearly with the number of donors up to a threshold value of ~200, after which the classifier was nearly perfect.

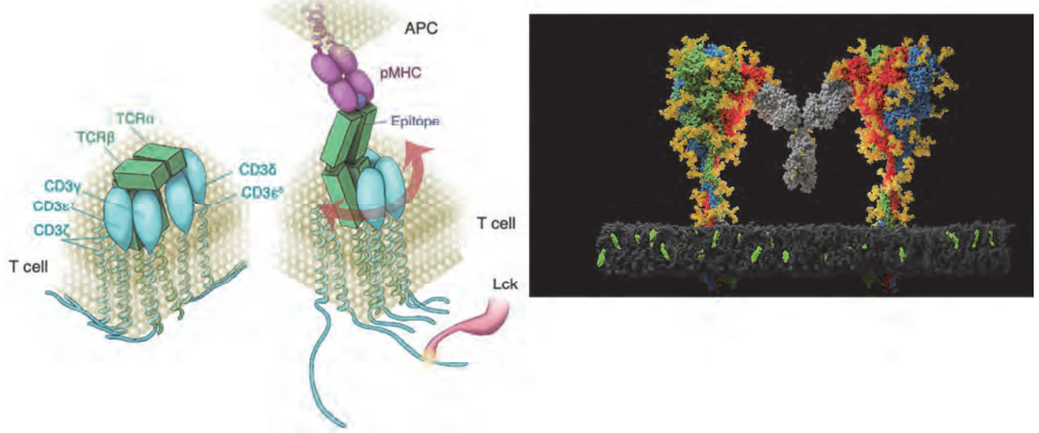


Figure 2.
A, The drawbridge model for TCR triggering. The TCR elongates upon TCR-pMHC binding, resulting in an increased mobility of the CD3 proteins, which carry the ITAM signaling domains. **B,** Infection-enhancing antibodies crosslink neighboring spike proteins via their NTD domains, resulting in decoupling of the NTD and RBD domains, thereby catalyzing the RBD down to up transition, which is an important part of the infection process.

Recent Publications

1. Saputri DS, et al. Deciphering the antigen specificities of antibodies by clustering their complementarity determining region sequences. mSystems 8: e0072223 (2023).
2. Millius A, et al. Circadian ribosome profiling reveals a role for the Period2 upstream open reading frame in sleep. Proc Natl Acad Sci U S A 120: e2214636120 (2023).
3. Mitsui Y, et al. Expression of the readthrough transcript CiDRE in alveolar macrophages boosts SARS-CoV-2 susceptibility and promotes COVID-19 severity. Immunity 56:1939-1954 e1912 (2023).
4. Kitagawa Y, Akizuki S, Ito Y, et al. Construction of a T cell receptor signaling range for spontaneous development of autoimmune disease. J Exp Med. 220 (2023).
5. Eerden FJ v, et al. TCR-pMHC complex formation triggers CD3 dynamics. eLife (2023).

Statistical Immunology

Yukinori Okada, MD/PhD

Professor	Yukinori Okada
Assistant Professor	Yuya Shirai
Research Assistant	5
Support Staff	2

Goal of our laboratory

Genetic backgrounds of individuals have substantial impacts on risk of a wide range of immune-related diseases. Statistical immunology is a research field that evaluates causality of human genetic variations on immune-related diseases, using statistical and bioinformatics approaches. The goal of our laboratory is to develop such methods and apply to the latest large-scale disease genome and multi-layer omics data.

Single cell sequencing with human genome data of severe COVID-19

Coronavirus disease 2019 (COVID-19) represents a serious global public health issue. Toward next pandemic, immunological mechanisms of the dysfunctional immune response in severe COVID-19 infection are elusive. We analyzed single-cell transcriptomes and T and B cell receptors (BCR) of ~900,000 peripheral blood mononuclear cells from 150 Japanese subjects with COVID-19 as well as healthy controls along with host genome-wide genotype data (Figure 1). We found a series of immunological features: (i) COVID-19 patients showed a low fraction of nonclassical monocytes. (ii) Cell-cell communication analysis inferred decreased cellular interactions involving nonclassical monocytes in severe COVID-19. (iii) Clonal expansions of BCR were evident in the COVID-19 patients rather than TCR. (iv) Disease risk genes identified by COVID-19 genome-wide association study showed cell type-specific expressions in monocytes and dendritic cells. (v) Further, a COVID-19-associated risk variant at *IFNAR2* had COVID-19-specific and monocyte-specific expression quantitative trait loci effects (Edahiro R et al. *Nat Genet* 2023).

Human genomes in non-humans from human body

Sharrow human DNA included in faecal samples can result in a small number of human reads in gut shotgun metagenomic sequencing data. It has been unclear whether and how much personal information can be quantitatively reconstructed from such contaminated reads. Based on metagenome resources of Japanese, we constructed the genomic approaches to reconstruct personal information from the faecal metagenomes. Genetic sex could be accurately predicted based on the sequencing depth of sex chromosomes. Individuals could be re-identified from the matched genotype data based on human reads recovered from the faecal metagenomic data. The method enabled us to predict the genetic ancestries of the samples. We then performed ultra-deep shotgun metagenomic sequencing of the faecal samples, which demonstrated that the genotypes of both common and rare variants could be reconstructed from faecal samples. This included clinically relevant variants. Our study showed that human reads within gut metagenomic data is personal identifiable information (Tomofuji Y et al. *Nat Microbiol* 2023).

Deconvoluting heterogeneity across autoimmune rheumatic diseases

Deconvolution of immunological and clinical heterogeneity across autoimmune rheumatic diseases (AIRDs) is of needs. We conducted large-scale and cohort-wide immuno-phenotyping of 46 peripheral immune cells from >1,000 Japanese patients of 11 AIRDs. Multimodal clustering of immune-phenotypes deciphered underlying disease-cell type network, providing novel immune cell type specificity shared or distinct across AIRDs. Individual patient-level clustering deconvoluted the AIRD patients into several clusters with different immunological features. Of these, RA- or SLE-like clusters were exclusively dominant, showing immunological polarization between RA and SLE across AIRDs. By adopting RA as a flagship disease, in depth clinical analysis revealed that such patient clusters differentially defined clinical heterogeneity in disease activity and treatment responses, such as treatment resistance in the RA patients with SLE-like immuno-phenotypes. Inborn human genetics represented by polygenic risk score based on the RA case-control genome-wide association study (GWAS) and within-case stratified GWAS were associated with patient characteristics, disease activity, and immune-phenotypes such as dendritic cells for RA-interstitial lung disease. Our study demonstrated a value of cohort-wide and cross-disease immuno-phenotyping to elucidate clinically heterogenous patient subtypes existing within the single disease in an immune cell type-specific manner. (Tanaka H et al. Ann Rheum Dis 2024).

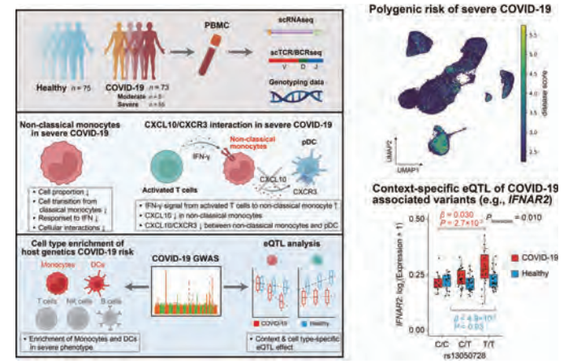


Figure 1.
Large-scale Single cell sequencing with human genome data of severe COVID-19 in Japanese.

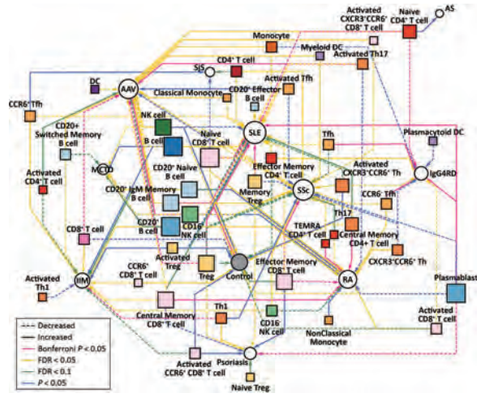


Figure 2.
Deconvoluting network across peripheral immune cells and autoimmune rheumatic diseases.

Recent Publications

1. Yamamoto K, Sonehara K, Namba S, Konuma T, Masuko H, Miyawaki S, BioBank Japan Project, Kamatani Y, Hizawa N, Ozono K, Yengo L, Okada Y. Genetic footprints of assortative mating in the Japanese population. Nat Hum Behav. 7:65-73 (2023).

2. Edahiro R, Shirai Y, Takeshima Y, Sakakibara S, Yamaguchi Y, Murakami T, Morita T, Kato Y, Liu YC, Motooka D, Naito Y, Takuwa A, Sugihara F, Tanaka K, Wing JB, Sonehara K, Tomofuji Y; Japan COVID-19 Task Force, Namkoong H, Tanaka H, Lee H, Fukunaga K, Hirata H, Takeda Y, Okuzaki D, Kumanogoh A, Okada Y. Single-cell analyses and host genetics highlight the role of innate immune cells in COVID-19 severity. Nat Genet. 55:753-767 (2023).

3. Tomofuji Y, et al. Reconstruction of the personal information from human genome reads in gut metagenome sequencing data. Nat Microbiol. 8:1079-1094 (2023).

4. Tomofuji Y, et al. Analysis of gut microbiome, host genetics, and plasma metabolites reveals gut microbiome-host interactions in the Japanese population. Cell Rep. 42:113324 (2023).

5. Tanaka H, Okada Y, Nakayamada S, Miyazaki Y, Sonehara K, Namba S, Honda S, Shirai Y, Yamamoto K, Kubo S, Ikari K, Harigai M, Sonomoto K, Tanaka Y. Extracting immunological and clinical heterogeneity across autoimmune rheumatic diseases by cohort-wide immunophenotyping. Ann Rheum Dis. 83:242-252 (2023).

Quantitative Immunology

Associate Professor Diego Diez

Our group applies computational and single cell genomics techniques to understand the immune system. We develop computational methods to analyze single cell data. We integrate experimental data, including transcriptome, chromatin accessibility, protein expression, immune repertoire, and spatial transcriptomics, with publicly available information into network models of immune regulation. We apply this framework to study gene regulatory networks controlling immune cell development and function.

Development of computational methods

An important problem in single cell genomics is how to combine different datasets while correcting for batch effects. A key focus is on preserving the original cell population structure while not introducing bias. We have developed Canek, a method that leverages a fuzzy logic framework that enables efficient batch correction without bias. Another problem is the identification of marker genes. In collaboration with Alexis Vandenbon at Kyoto University, we have developed *singleCellHaystack*, a method to identify differentially expressed genes from multi-dimensional representations of single cell genomics data.

Mathematical modeling

The large number of cells obtained in single cell genomics experiments opens the door to approaches that study the immune system using mathematical modeling and machine learning. Transcriptional regulatory networks are critical determinants of cell identity and function. We use machine learning to model immune transcriptional regulatory networks. Using the expression level of the regulators as a proxy for their activities we apply these methods to study how transcriptional networks change during immune cell differentiation and disease.

Applications to immunology

Using single cell transcriptomics, protein expression, immune repertoire, and chromatin accessibility we study the differentiation of NKT cells in the thymus and spleen of SKG and WT mice. SKG mice have a mutation in ZAP70 that weakens TCR signaling, resulting in a bias towards development of NKT1 compared to NKT2 in the wild type (BALB/c). This approach enables us to understand how changes in regulatory networks during development effect NKT specification. In a clinical setting, we apply single cell genomics to get insight into IgA nephritis onset and therapies. We use transcriptome, protein expression and immune repertoire of immune cells in PBMCs and tonsils from IgAN patients before and after tonsillectomy and steroid immunosuppression. We collaborate with other groups at IFReC to study diverse aspects of immune responses in a basic and clinical setting.

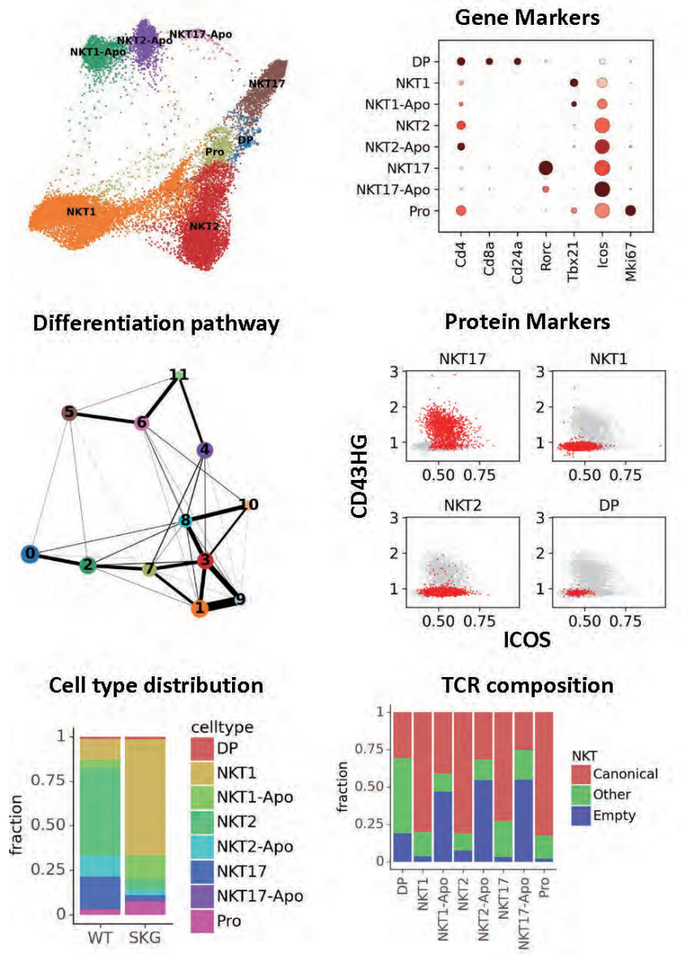


Figure.
Single cell genomics identifies RNA and TCR features of differentiating NKT cells.

Recent Publications

1. Vandenbon A & Diez D. A universal tool for predicting differentially active features in single-cell and spatial genomics data. Sci Rep. 13:11830 (2023).
2. Loza M, Teraguchi S, Standley DM & Diez D. Unbiased integration of single cell transcriptome replicates. NAR Genom Bioinform. 4, lqac022 (2022).
3. Diez D, Morte B & Bernal J. Single-Cell Transcriptome Profiling of Thyroid Hormone Effectors in the Human Fetal Neocortex: Expression of SLCO1C1, DIO2, and THRB in Specific Cell Types. Thyroid. 31:1577-1588 (2021).
4. Vandenbon A and Diez D. A clustering-independent method for finding differentially expressed genes in single-cell transcriptome data. Nat Commun. 11(1):4318 (2020).
5. Teraguchi S, Saputri DS, Llamas-Covarrubias MA, Davila A, Diez D, Nazlica SA, Rozewicki J, Ismanto HS, Wilamowski J, Xie J, Xu Z, Loza-Lopez MJ, van Eerden FJ, Li S, Standley DM. Methods for sequence and structural analysis of B and T cell receptor repertoires. Comput Struct Biotechnol J. 18:2000-2011 (2020).

Events & Outreach Activities

International Symposium on Microbiology and Immunology

The 13th International Symposium of IFReC

This symposium was co-organized with the Center for Infectious Disease Education and Research (CiDER) at Osaka University, the project for "Self-referential immune perception" (Grant-in-Aid for Transformative Research Areas, JSPS). Centering on basic research achievements relating to microbiology and immunology, this symposium consists of 12 lectures by world-leading scientists. This symposium was a significant opportunity for the participants to share ideas and expertise for further development of microbiology, immunology, and life science.

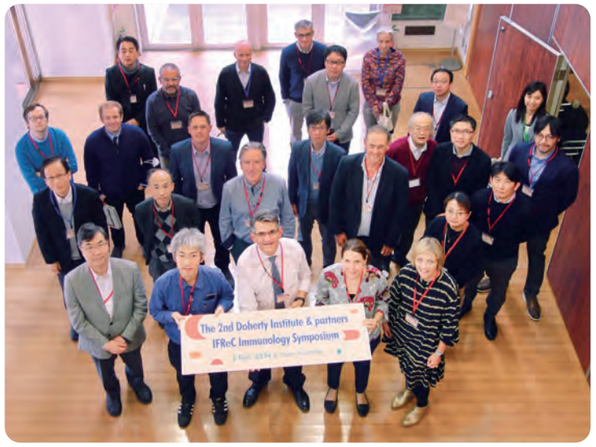
- **Date:** February 9, 2024
- **Venue :** Yuichi Yamamura Life Hall, Senri Life Science Center, Suita-city, Osaka

Speaker (order of presentation)	Title
Kazuhiro Suzuki (IFReC, Osaka University)	'Targeting B cell migration in the pathogenesis of autoimmunity'
Hisako Kayama (IFReC, Osaka University)	'The role of microbial metabolites in development of ulcerative colitis'
Ana Ivonne Vazquez Armendariz (LIMES-Institute, University of Bonn)	'Complex murine and human organoid systems to model pulmonary diseases'
Sho Yamasaki (IFReC, Osaka University)	'Immune response to host metabolites'
Joseph Craft (Yale School of Medicine)	'Tissue Adaptation and Tissue Damage in Autoimmunity'
Wataru Ise (CiDER, Osaka University)	'Regulation of plasma cell survival and migration to the survival niches for establishing durable antibody response'
Laura McCoy (University College London)	'Preservation of memory B cell homeostasis in an individual producing broadly neutralizing antibodies against HIV-1'
Raul Andino (University of California San Francisco)	'Studies on enterovirus particle structure and functions'
Leyuan Ma (University of Pennsylvania)	'Boosting Chimeric Antigen Receptor T cell therapy via a synthetic vaccine'
Lynette Beattie (University of Melbourne)	'A dual role for gamma delta T cell-derived IL-4 in CD8 T cell priming'
Eric Huseby (University of Massachusetts)	'MHC-II polymorphisms regulate non-cognate negative selection to CD4 T cell orchestrators of type-1 diabetes'
Adrian Hayday (The Francis Crick Institute, London)	'Regulating tissue immunosurveillance by innate and adaptive uses of the T cell receptor'

The 2nd Doherty Institute & partners – IFReC Immunology Symposium

Date: February 8, 2024

Venue : Taniguchi Memorial Hall, Osaka University



Speaker (order of presentation)	Title
Sammy Bedoui (UoM/Doherty Institute)	'T cell memory, metabolism and the microbiome'
Nobuhiko Kamada (IFReC)	'Decoding the Functional Impact of Gut Microbiota in Inflammatory Bowel'
Andrew Brooks (UoM/Doherty Institute)	'Polymorphism in Killer cell immunoglobulin-like Receptor genes diversifies Natural Killer Cell responses'
Yasutaka Okabe (IFReC)	'Stromal-immune cell interactions in non-classical lymphoid tissues'
Sidonia Eckle (UoM/Doherty Institute)	'Antigen recognition and associated immune functions of MAIT cells'
Daron M. Standley (IFReC)	'Medical diagnosis using adaptive immune receptor repertoires'
Axel Kallies (UoM/Doherty Institute)	'Cytotoxic CD8 T cells in chronic infection and cancer'
Atsushi Tanaka (IFReC)	'Regulation of TCR signaling molecules in Treg cells'
Scott Mueller (UoM/Doherty Institute)	'Dissecting the tissue contexts of immunity'
Masahiro Yamamoto (IFReC)	'The novel VeDTR mouse system reveals roles of immune cells in anti-tumor immunity'
Jose Villadangos (UoM/Doherty Institute)	'Antigen-presenting cells and molecules, the initiators of adaptive immunity'

Finnish-Japanese Immunology Symposium Emerging concepts in immunology - From basic mechanisms to treating immune mediated diseases

Date: August 29-30, 2023

Venue : University of Turku, Finland

Speakers (order of presentation)
1. Akihiko Yoshimura (Keio University)
2. Riitta Lahesmaa (University of Turku)
3. Satu Mustjoki (University of Helsinki)
4. Shimon Sakaguchi (Osaka University)
5. Akira Takeda (University of Turku)
6. Tomohiro Kurosaki (IFReC)
7. Hisashi Arase (IFReC)
8. Cecilia Naucler (University of Turku and Karolinska Institutet)
9. Shizuo Akira (IFReC)
10. Hiroshi Ohno (RIKEN)
11. Hisako Kayama (IFReC)
12. Kazuyo Moro (IFReC)
13. Olli Silvennoinen (University of Helsinki)
14. Hayato Takahashi (Keio University)
15. Laura Airas (University of Turku)

Speakers (order of presentation)
16. Kazuhiro Suzuki (IFReC)
17. Sirpa Jalkanen (University of Turku)
18. Mika Rämet (Tampere University)

Joint workshop with ImmunoSensation at the University of Bonn to promote joint research

Date: June 14-15, 2023

Venue : University of Bonn, Germany

A joint workshop was held to promote collaboration between IFReC at Osaka University and ImmunoSensation at the University of Bonn. Ten PIs from IFReC and 16 PIs from ImmunoSensation participated in this workshop, the aim of which was to promote matching for further joint research in addition to existing ones. The researchers shared information about their research in advance, and gave short talks at the workshop introducing their research, followed by individual interviews with potential collaborators. As a result, more than ten new joint research projects were established. The workshop commenced with a presentation by Masahiko Hayashi, Director of the JSPS Bonn Office where he introduced the competitive funding provided by the JSPS, which is available to support the joint research.

The 2nd ImmunoSensation² - IFReC International School on Advanced Immunology

The 2nd International School on Advanced Immunology was held in Germany. Fifteen leading immunologists were invited as lecturers, and 49 exceptional participants with remarkable research achievements were selected from a large number of worldwide applicants. The high level of research by the participants and the active exchanges to promote interaction among them were well received by both lecturers and participants.

Date: September 17-21, 2023

Venue: Seehotel Maria Laach, Germany

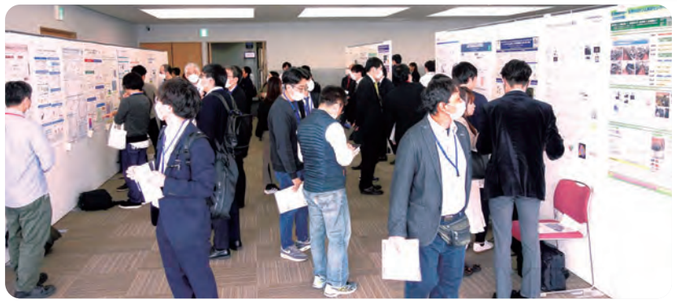
Lecturers
Zeinab Abdullah (University of Bonn, Germany)
Julie Magarian Blander (Weill Cornell Medicine, USA)
Marco Colonna (Washington University School of Medicine, USA)
James Chen (UT Southwestern Medical Center, USA)
Kate Fitzgerald (UMass Chan Medical School, USA)
Matthias Geyer (University of Bonn, Germany)
Masaru Ishii (Osaka University, Japan)
Raphaela T. Goldbach-Mansky (National Institutes of Health-NIAID/DIR, USA)
Naoki Hosen (Osaka University, Japan)
Kazuyo Moro (Osaka University, Japan)
Ann Marshak-Rothstein (UMass Chan Medical School, USA)
Joachim Schultze (University of Bonn, Germany)
Hans Stauss (UCL Institute of Immunity & Transplantation, UK)
Brigitta Stockinger (Francis Crick Institute, UK)
Kiyoshi Takeda (Osaka University, Japan)

NGS Expo 2023

The "NGS Expo", which started in 2022 is an academic event for users and potential users of next generation sequencer, and organized by IFReC and RIMD, Osaka University. About 800 people participated in this symposium including online. The organizers incorporated "online salon", a communication tool between participants, and nearly 50,000 page views were obtained.

Date: November 15-16, 2023

Venue : Osaka International Convention Center (Grand Cube Osaka)



Colloquia and Seminars

IFReC Colloquia : Important events allowing IFReC researchers to gather together, and have been held every two months.

Date	Speakers
May 25, 2023	**James Badger Wing** (Human Single Cell Immunology) **Mohamad Alkadi & Daisuke Okuzaki** (Human Immunology (Single Cell Genomics)) **Nicholas Isaac Smith** (Biophotonics)
July 20, 2023	**Diego Diez** (Quantitative Immunology Research Unit) **Burcu Temizoz, Takayuki Shibahara, and Ken Ishii** (Vaccine Science)
September 28, 2023	**Rei Watanabe & Manabu Fujimoto** (Cutaneous Immunology) **Shunya Ikeda & Naoki Hosen** (Cellular Immunotherapy)
November 8, 2023	**Fumitaka Muramatsu & Nobuyuki Takakura** (Signal Transduction) **Oluwaseun Fatoba & Toshihide Yamashita** (Molecular Neuroscience)
January 25, 2024	**Takuya Koike & Tomohiro Kurosaki** (Lymphocyte Differentiation) **Taichi Nakatani & Takashi Nagasawa** (Stem Cell Biology and Developmental Immunology)
March 21, 2024	**David Calianese & Shigekazu Nagata** (Biochemistry & Immunology) **Shimpei Kawamoto & Eiji Hara** (Aging Biology)

IFReC Seminar & IFReC ImmunoSeminar*

A wide variety of scientists has been invited as speakers.

*Inviting world-prestigious immunologists mainly online.

Date	Speakers
April 12, 2023	**Bernard Malissen*** (Group leader at Centre d'imuunologie de Marseille-Lumity/Founding-director of Center for Immunophenomics, Marseille, France)
May 16, 2023	**Keisuke Nagao** (Senior Investigator, Cutaneous Leukocyte Biology Section, NIAMS, NIH, USA)
May 24, 2023	**Arthur Weiss*** (Ephraim P. Engleman Distinguished Professor/Professor of the Department of Medicine, UCSF, USA)
July 21, 2023	**Yosuke Kumamoto** (Assistant Professor, Department of Pathology, Immunology and Laboratory Medicine, Rutgers, the State University of New Jersey, USA)
August 8, 2023	**Kai-Michael Toellner** (Professor, Adaptive Immunology, Institute of Immunology and Immunotherapy, University of Birmingham, UK)
September 19, 2023	**Olivier Lantz*** (Director, the Clinical Immunology Laboratory at Institut Curie's Paris hospital, France)
September 29, 2023	**Hiroki Kato** (Professor, Institute of Cardiovascular Immunology, Medical Faculty, University of Bonn, Germany)
November 2, 2023	**Andreas Schlitzer** (Professor, Quantitative Systems Biology, LIMES-Institute, University of Bonn, Germany)
January 22, 2024	**Ruth A. Franklin** (Assistant Professor, Stem Cell and Regenerative Biology, Harvard University, USA)
February 19, 2024	**H. Suenaga, R. Ito, T. Takahashi, and M. Suzuki** (PIs, Central Institute for Experimental Animals (CIEA), Japan)

Outreach Activities

In 2023, we have organized various outreach events by online and face-to-face. An interaction with the general public is a good stimulus for researchers. Especially, an approaching to high school students and high school teachers is important for the future development of science and technology.

The 12th WPI Science Symposium

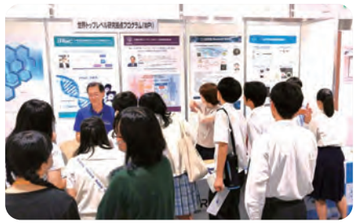
- Date : November 23, 2023
- Venue : Akira Suzuki Memorial Hall, Hokkaido University, Sapporo
- Speakers : Hisashi Yamamoto (ICReDD), et al.

Science Café at the Nakanoshima Festival “The heart; present & the future”

- Date : December 3, 2023
- Venue : Lecture Hall A, Osaka University School of Medicine
- Speakers : Moyu Hasegawa and Kenji Miki (WPI-PRIMe, Osaka University)

Introduction of IFReC and WPI program for Super Science High School Students

- July 15, 2023@Meijo University, Nagoya
- December 27, 2023@Okazaki Conference Hall, Aichi



Life Science Seminar for High School Students

- Date : August 7, 2023
- Venue : Taniguchi Memorial Hall, RIMD and IFReC
- Speakers : Yoko Fukushima (Osaka University School of Medicine), et al.

Online Seminars for High School Teachers

- August 1, 2023: “A Molecule that controls winter depression and inflammation”
  Speakers : Taiichiro Shirai (WPI-IFReC) and Takashi Yoshimura (WPI-ITbM, Nagoya University)
- March 27, 2024: “The forefront of genetic analysis”
  Speakers : Daisuke Okuzaki (IFReC) and Daisuke Motooka (RIMD Osaka University)
- March 28, 2024: “Mathematical approach to life phenomena”
  Speakers : Takahiro Nemoto (WPI-PRIMe) and Kantaro Fujiwara (WPI-IRCN, University of Tokyo)

SpringX Super School - Online Lecture series for protecting our lives from infectious diseases – by CiDER and IFReC

Speakers :

- Tokiko Watanabe: “A wide variety of virus research” (April 28, 2023)
- Kazuhiro Suzuki: “Regulation of Autoimmunity” (July. 28, 2023)
- Yasutaka Okabe: “Macrophages protect our health” (Oct. 27, 2023)
- Kiyoharu Fukushima: “Interstitial pneumonia/NTM diseases” (Jan. 26, 2024)

Japanese Language Class

Message from Ms. Tajima, an instructor of the class 2024.

“Hi. I’m Kaori Tajima. It’s been very fun to talk with you in the class. I can’t wait to see new members, too. The class is focused on speaking. You are expected to talk about yourself, using the grammar and the vocabulary we learn in the class. I’m really looking forward to seeing you!”

Information

Major Awards

Highly Cited Researchers (HCR) 2023 by Clarivate™

Nobuhiko Kamada
Shimon Sakaguchi


Debrecen Award for Molecular Medicine 2023

Shimon Sakaguchi

Karl Meyer Lectureship Award 2023 by Society for Glycobiology

Taroh Kinoshita

"Rising Fellow" by Toyota Physical and Chemical Research Institute

Soyoung Park

Mochida Memorial Award 2023

Tomohiro Kurosaki

The Ishidate-Ueno Award 2023 by Chugai Foundation

Yukinori Okada

The 14th Ikushi Prize by Japan Society for the Promotion of Science

Emi Ito

Osaka University Prize 2023

Kiyoharu Fukushima
Hiroko Kitamoto


Advanced Postdoc Program at IFReC

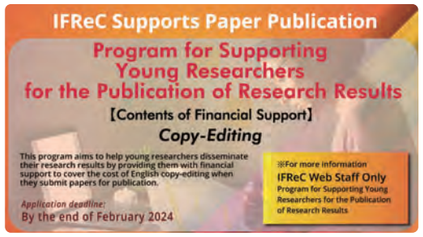
IFReC has been recruiting post-doctoral researchers for its Advanced Postdoc Program. This program offers three-year employment and funding (3 million JPY per year) for original research to promising young researchers. Selected applicants have access to continually upgraded state-of-the-art facilities at IFReC for their research, including equipment for single-cell analysis.

Original Support Programs for Young Researchers

To strengthen our international research network and our basis for international collaborative research, IFReC has established two kinds of financial support programs for researchers. 1) "IFReC Kishimoto Foundation Fellowship," which has been used to invite international researchers to Osaka. 2) "Program for International Circulation of Young Talented Researchers" for those who wish to participate in overseas research activities. Since 2009, about 150 researchers have received these grants.

Support for Paper Submission

This program aims to support the dissemination of research results by young researchers of IFReC.



Grant for Next Generation Principal Investigators

This program aims to foster the next generation of principal investigators at IFReC. In particular, challenging research that has the potential to create a new field of study in immunology is selected. The grant helped to generate excellent research achievements in 2023.

Common Facilities (IFReC, RIMD, Animal Resource Center)

IFReC and its parent institution, the Research Institute for Microbial Diseases (RIMD) are located on the same site, constituting a large research complex. The complex contains the Core Instrumentation Facility, the Animal Resource Center and the Network Administration Office, all of which are jointly operated by IFReC and RIMD. The Core Instrumentation Facility is equipped with various highly advanced instruments and skilled technicians provide in-house services to IFReC and RIMD researchers. The Animal Resource Center consists of three buildings for specific pathogen-free (SPF) animals and the live immuno-imaging facility. With a largecapacity animal-breeding facility in IFReC, researchers are able to choose animal rooms suitable for their experiment purpose. Using these common facilities, IFReC researchers are able to effectively and smoothly carry out their experiments to promote their world-leading research at IFReC.

1. IFReC Research Building
2. Integrated Life Science Building
3. Main Building, Research Institute for Microbial Diseases, RIMD
4. South Building, Research Institute for Microbial Diseases, RIMD
5. Cutting-Edge Research Building for Infectious Diseases
6. Animal Resource Center for Infectious Diseases

Animal Resource Center for Infectious Diseases

- Specific pathogen-free (SPF) animal facility
- Sperm/ embryo freezing and preservation
- In vitro fertilization and embryo transplantation
- Intracytoplasmic sperm injection
- Transgenic and knock-out animals
- Genome editing in experimental animals

Live immuno-imaging facility

- SPF animal experiment facility with 11.7T MRI, in vivo imager & two-photon microscope.

Network Administration Office

- Provision and maintenance of network infrastructure: LAN system and servers (web, mail, mailing lists, etc.)

Core Instrumentation Facility

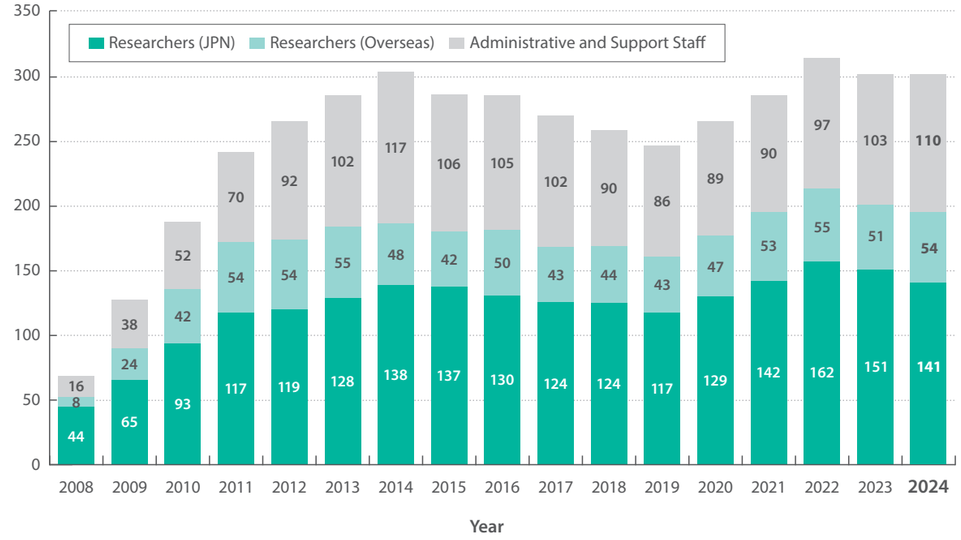
- Basic and advanced instruments
- In-house service
- DNA sequencing, cell sorting, electron microscopy, mass spectrometry and next-generation sequencing analysis
- Radio isotope facility

Composition & Finance

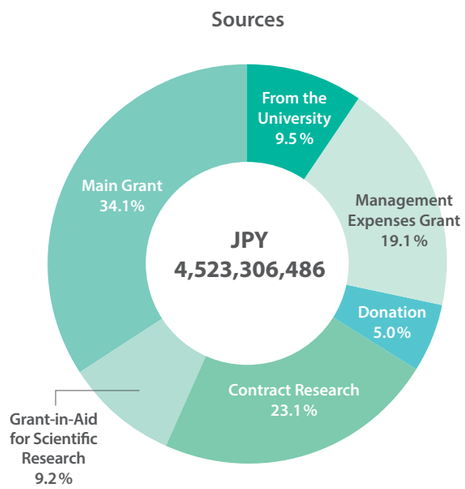
Composition

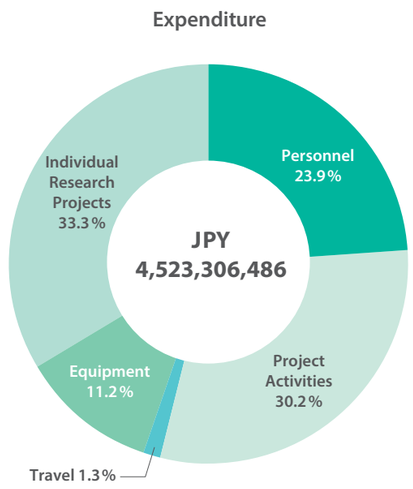
Composition since the establishment of IFReC

as of Feb. 1, 2024



Finance





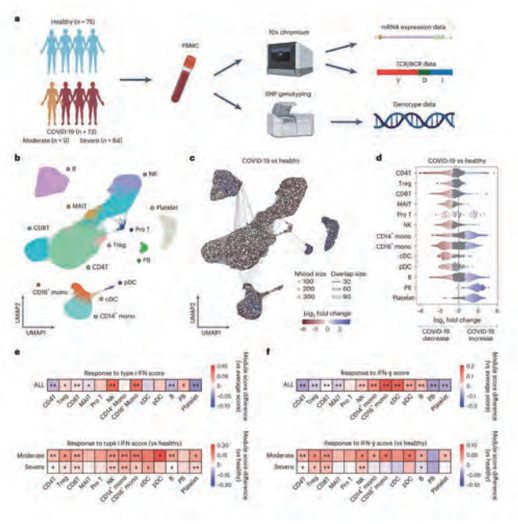
Selected Articles

Single-cell analyses and host genetics highlight the role of innate immune cells in COVID-19 severity.

Edahiro R, Shirai Y, Takeshima Y, Sakakibara S, et al.

Nat Gen. 55:753-767 (2023).

Mechanisms underpinning the dysfunctional immune response in severe acute respiratory syndrome coronavirus 2 infection are elusive. Atsushi Kumanogoh (Graduate School of Medicine, Osaka University/IFReC), Yukinori Okada (Graduate School of Medicine, Osaka University/IFReC/RIKEN/Graduate School of Medicine, University of Tokyo) and the research group found that CD14+CD16++ monocytes, a rare subset among monocytes, are involved in COVID-19 severity. Further, COVID-19 severity-associated genes such as IFNAR2, have specific function mainly in monocytes and dendritic cells.

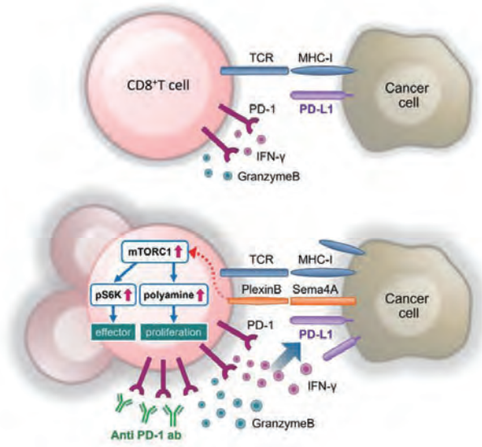


Tumor-derived semaphorin 4A improves PD-1–blocking antibody efficacy by enhancing CD8+ T-cell cytotoxicity and proliferation.

Naito Y, Koyama S, Masuhiro K, Hirai T, et al.

Sci Adv. 9 (20): eade0718 (2023).

Although immune checkpoint inhibitors have brought major advances in cancer treatment, their effectiveness is still far from satisfactory. We need to develop novel biomarkers and therapies that strengthen their effectiveness. Atsushi Kumanogoh (Graduate School of Medicine, Osaka University/IFReC), and the research group shows that histologically Semaphorin 4A (Sema4A)-positive non-small cell lung cancer (NSCLC) responded significantly better to anti-programmed cell death 1 (PD-1) antibody than Sema4A-negative NSCLC.



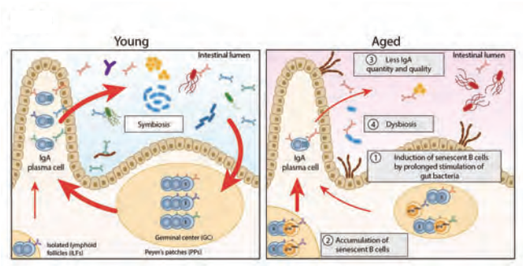
Schematic of the role of Sema4A in the tumor microenvironment.
Sema4A expressed by tumor cells ameliorated anti-tumor function and proliferation of CD8+ T cells by promoting mTORC1-S6K signaling and polyamine synthesis.

Bacterial induction of B cell senescence promotes age-related changes in the gut microbiota.

Kawamoto S, Uemura K, Hori N, Takayasu L, et al.

Nat Cell Biol. 25:865-876 (2023).

We all experience aging, a phenomenon in which our biological functions deteriorate. A research group led by Eiji Hara (Aging Biology, IFReC) has revealed that long-term stimulation by gut microbiota causes the induction of cellular senescence in immunoglobulin A (IgA)-producing B cells, resulting in changes in the production and diversity of IgA, which in turn causes dysbiosis of gut microbiota. This research clarifies the mechanism of changes in the gut microbiota with aging. Furthermore, it could lead to the development of methods to improve age-related dysbiosis of the gut microbiota, which is thought to be one of the causes of various age-related diseases.

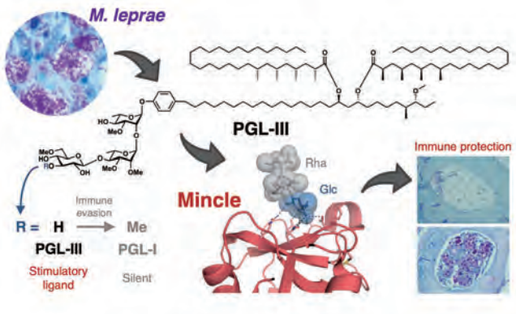


PGL-III, a rare intermediate of Mycobacterium leprae phenolic glycolipid biosynthesis, is a potent Mincle ligand.

Ishizuka S, van Dijk JHM, Kawakita T, Miyamoto Y, et al.

ACS Cent Sci. 9 (7):1388-1399 (2023).

Although leprosy (Hansen's disease) is one of the oldest known diseases, the pathogenicity of Mycobacterium leprae (M. leprae) remains enigmatic. Indeed, the cell wall components responsible for the immune response against M. leprae are as yet largely unidentified. The research group of Sho Yamasaki (Molecular Immunology, IFReC/RIMD/CiDER, Osaka University) identified phenolic glycolipid-III (PGL-III) as a M. leprae-specific ligand for the immune receptor Mincle.

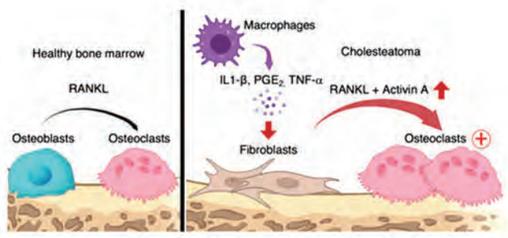


Single-cell transcriptomics of human cholesteatoma identifies an activin A-producing osteoclastogenic fibroblast subset inducing bone destruction.

Shimizu K, Kikuta J, Ohta Y, Uchida Y, et al.

Nature Commun. 14:4417 (2023).

Cholesteatomas are made up of cysts or bumps in the ear that consist of skin, collagen fibers, skin cells, fibroblasts, keratin, and dead tissue. However, the exact mechanism for the creation of cholesteatomas remains unknown. The research group of Masaru Ishii (Graduate School of Medicine/Graduate School of Frontier Biosciences/IFReC) revealed the cause of cholesteatomas, which may help in developing new therapies for patients who are suffering from this disease.



Schematic of osteoclastogenesis induced by cholesteatoma fibroblasts expressing activin A.

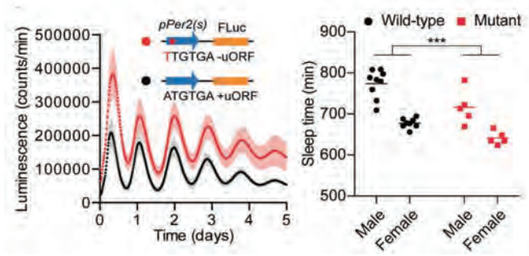
IL-1β, PGE2, and TNF-α secreted from infiltrating CD45+ cells, particularly macrophages, induced activin A-expressing pathogenic fibroblasts; the activin A acted in conjunction with RANKL to promote ectopic osteoclastogenesis. Construction of a T-cell receptor signaling range for spontaneous development of autoimmune disease.

Circadian ribosome profiling reveals a role for the Period2 upstream open reading frame in sleep.

Millius A, Yamada R, Fujishima H, Maeda K, et al.

Proc Natl Acad Sci USA. 120(40):e2214636120(2023).

Circadian rhythms, the internal biological clocks that regulate our daily activities, are essential for maintaining health and well-being. The research group of Arthur Millius (RIKEN/present: IFReC), Kazuhiko Maeda (Host Defense, IFReC), Daron Standley (System Immunology, IFReC), and Hiroki Ueda (RIKEN) showed how translation and post-transcriptional processes influence the body's internal clock and its impact on sleep patterns.



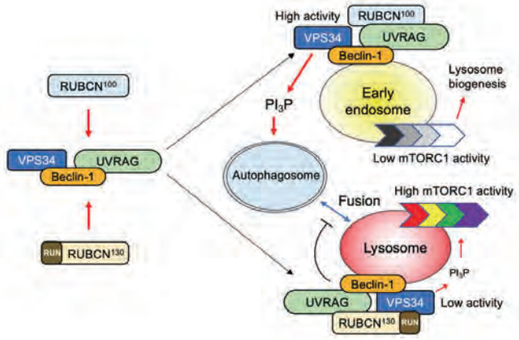
Disruption of the Per2 uORF disrupts the amplitude of circadian rhythms (left) and reduces sleep in mice (right).

Opposing roles of RUBCN isoforms in autophagy and memory B cell generation.

Tsai C, Sakakibara Y, Kuan Y, Omori H, et al.

Sci Signal. 16:803 (2023).

Memory B cells depend on autophagy for their survival, but the protein Rubicon is thought to hinder this process. Dr. Chao-Yuan Tsai and the researchers of Osaka University have discovered a shorter isoform of Rubicon called RUBCN100, which enhances autophagy in B cells. Mice that lacked the longer isoform, RUBCN130, produced more memory B cells in a way that relied on autophagy. These findings provide further insight into the role of Rubicon in autophagy.



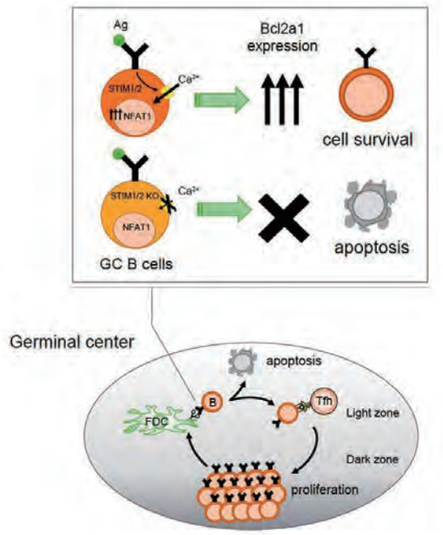
A model for the functional interaction between RUBCN100 and RUBCN130 in regulating autophagy.

STIM-mediated calcium influx regulates maintenance and selection of GC B cells.

Yada Y, Matsumoto M, Inoue T, Baba A, et al.

J Exp Med. 221 (1): e20222178 (2024).

Germinal centers (GCs) are specialized microenvironments where antigen-specific B cells undergo antibody affinity maturation and clonal expansion. However, the requirements of BCR signaling in GC B cells remain poorly understood. Yoshihiro Baba (Kyushu University), Tomohiro Kurosaki (Lymphocyte Differentiation, IFReC) and the research group showed that stromal interacting molecule (STIM)-deficient B cells have reduced B cell competitiveness compared to wild-type B cells during GC responses.

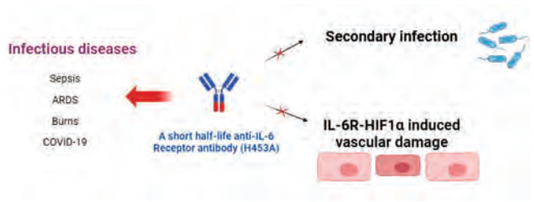


Gp130-HIF1α axis-induced vascular damage is prevented by the short-term inhibition of IL-6 receptor signaling.

Kang S, Onishi S, Ling Z, Inoue H, et al.

Proc Natl Acad Sci USA. 121 (2) : e2315898120 (2024).

Interleukin (IL)-6, an essential indicator of cytokine release syndromes (CRS), regulates vascular homeostasis and inflammation. Inhibition of IL-6 receptor (IL-6R) signaling is beneficial for various CRS; however, it is limited by adverse effects related to poor understanding of mechanisms involved. Tadamitsu Kishimoto and Sujin Kang, and their research group discovered that hypoxia-inducible factor (HIF1) α signaling is activated by IL-6R trans-signaling in endothelial cells, which promotes vascular inflammatory responses and endothelial permeability by glycolysis.



Giving medication a short half-life anti-IL-6 to patients of Sepsis, ARD, Burns, etc. is expected to suppress vascular damage or secondary infection.

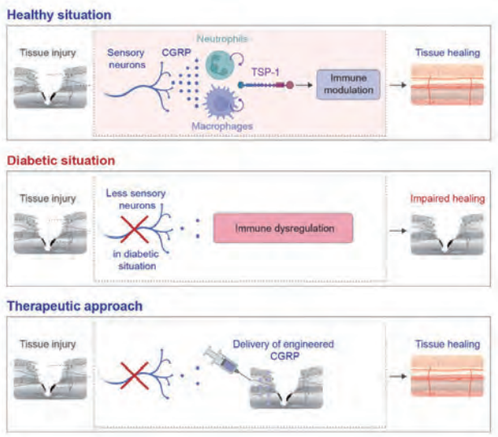
CGRP sensory neurons promote tissue healing via immune cells.

Lu YZ, Nayer B, Singh SK, et al.

Nature. (2024).

Nociceptive sensory neurons have a crucial role as immunoregulators and exert both protective and harmful effects depending on the context. However, how neuro–immune interactions affect tissue repair and regeneration following acute injury is unclear.

Collaborating with Shizuo Akira (Host Defense, IFReC), a research team led by Mikaël Martino (Monash University) demonstrated that the removal of a specific subtype of sensory neurons containing the Nav1.8 ion channel significantly impairs skin wound repair and muscle regeneration following injury.



Graphical summary of the study by Mikaël Martino.

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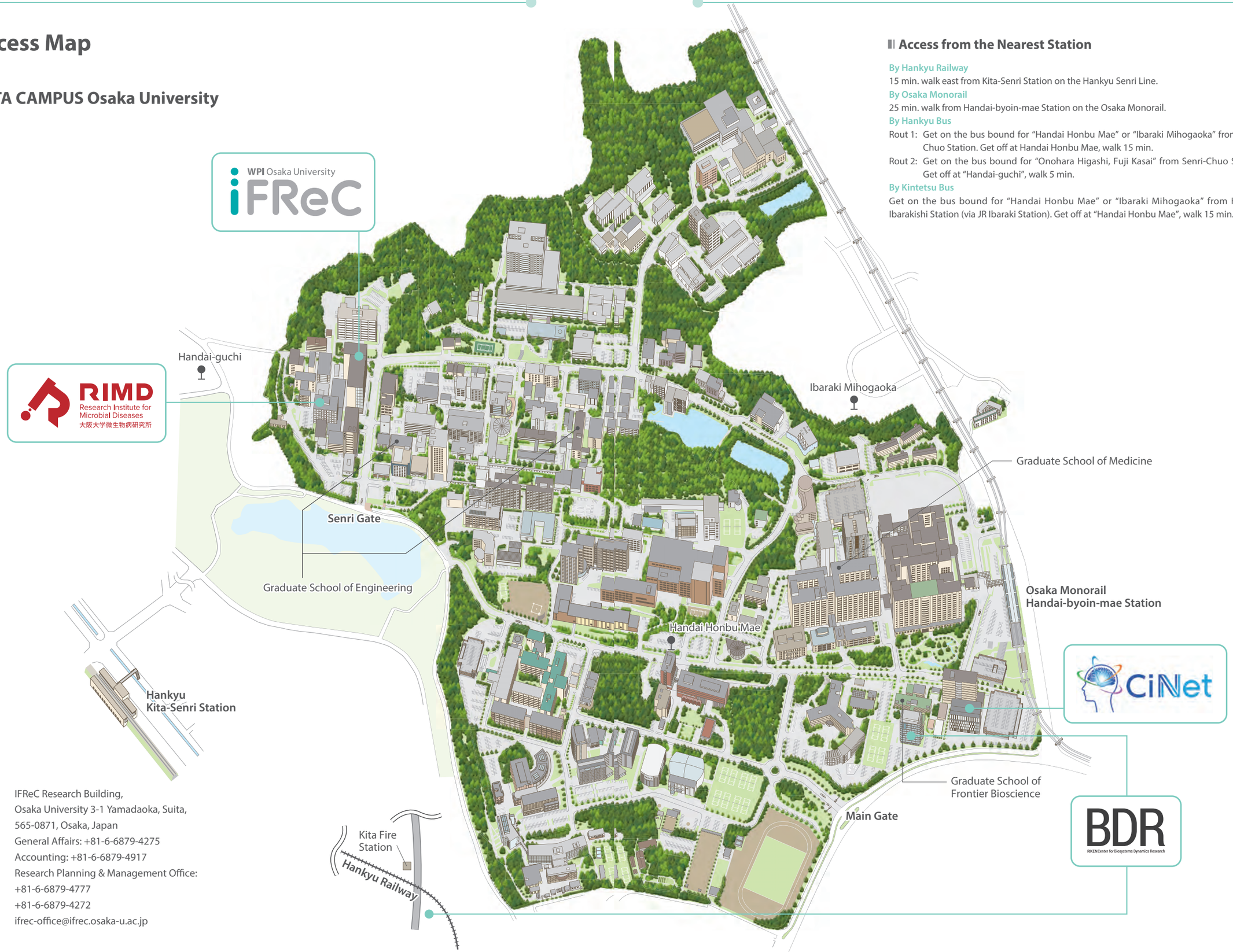

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