Osaka University

Immunology Frontier Research Center

WPI Immunology Frontier Research Center 2022-2023

Osaka University

Annual Report of IFReC 2022-2023

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wpi
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 2022. Since its inception in 2007, IFReC has established itself as a high-profile international research center for immunology with the broad support of many people. Furthermore, since IFReC joined the WPI Academy in 2017, we have continued efforts to create history as a new research center funded through a unique academic-industry partnership agreement, which is paving the way for new possibilities in collaborative research.

After the start of the COVID-19 pandemic in late 2019, the major waves of COVID-19 in the past several years led to a significant number of cases in Japan. However, as the COVID-19 pandemic began to subside in late 2022, various regulations have been eased or lifted completely. As a result, the activities in many regions have returned to pre-COVID-19 levels and in-person classes and events have resumed at the university.

Against this backdrop, the first "International School on Advanced Immunology," co-organized as a research retreat by IFReC and ImmunoSensation2 (University of Bonn), was held on Awaji Island in November 2022. It was the first research retreat held in three years since the last "Winter School on Immunology" that IFReC jointly held in 2019 with the Singapore Immunology Network as an annual event starting in 2011. This "revived international school" provided an opportunity for young immunologists and senior researchers from around the world to meet, discuss, and exchange ideas.

Internationalization is an important mission of IFReC, and we made progress in our relationships with overseas research centers in FY2022. We held joint symposia with the University College of London and the University of Melbourne in the UK and Australia, respectively. Also in Osaka, "the International Symposium on Microbiology and Immunology - The 12th International Symposium of IFReC" was held, and we enjoyed discussions at a high level.

Although the COVID-19 pandemic appears to be subsiding, there is still the possibility of another unknown infectious disease causing another pandemic. IFReC plays a central role in the research of immunology and infectious disease that transcends the boundaries between research departments and between institutes. Our researchers collaborate with the researchers at the Center for Infectious Diseases Education and Research (CiDER), which was established in response to the outbreak of COVID-19, and the Center for Advanced Modalities and DDS (CAMaD), which is a new vaccine research center established at Osaka University.

IFReC welcomed two new female researchers in FY2022 as principal investigators. One is Dr. Sujin Kang, who studies the regulation of metabolism and inflammation from an immunological perspective. The other is Dr. Yumi Matsuoka, who is a researcher in infectious diseases and allergies as well as a clinician in dermatology. She aims to elucidate the mechanisms of interaction between host immunity and microorganisms in the skin of allergy patients.

We continue our efforts to conduct basic research in immunology and to seek ways to contribute to society, and through research and education, we will continue to contribute to the advancement of science and grow to lead the world in immunology research.

Kiyoshi Takeda

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

Organization

Organization Chart

As of March 2023

Osaka University
International Advanced Research Institute (IARI)

MEXT
WPI Initiative Program Committee
JSPS

WPI Osaka University
iFReC

International Scientific Advisory Board

Research Groups
- Immunology Groups
- Imaging Groups
- Informatics Groups
- Unit for Combined Research Fields

Director

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

Committees & Advisory Board for IFReC

The World Premier International Research Center Initiative (WPI)

Program Director

As of November 2022

Akira UKAWA	WPI Program Director and Academy Director

Program Committee Members

Michinari HAMAGUCHI (Chairperson)	President, Japan Science and Technology Agency (JST) Chairperson, The Japanese National Commission for UNESCO
Hiroshi AMANO	Professor, Nagoya University, Tokai National Higher Education and Research System Nobel laureate in Physics (2014)
Mariko HASEGAWA	President, The Graduate University for Advanced Studies
Kazuhiko ISHIMURA	President, National Institute of Advanced Industrial Science and Technology
Maki KAWAI	Director General, Institute for Molecular Science (IMS) National Institutes of Natural Sciences (NINS)
Kiyoshi KUROKAWA	Professor Emeritus, National Graduate Institute for Policy Studies
Hiroshi MATSUMOTO	President, RIKEN
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Rita COLWELL	Distinguished University Professor, University of Maryland, USA
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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 2023

Lewis LANIER	University of California, San Francisco, USA
Anne O'GARRA	The Francis Crick Institute, UK
Jeffrey RAVETCH	Rockefeller University Immunology, 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 2023

Host Defense

Shizuo Akira, MD/PhD

Professor	Shizuo Akira
Associate Professor	Kazuhiko Maeda
Assistant Professor	Hiroki Tanaka
	Kiyoharu Fukushima
Postdoctoral Fellow	2
Research Assistant	6
Visiting Scientist	5
Support Staff	5

The innate immune system, which has been conserved throughout evolution, is activated by pattern recognition receptors (PRRs) that identify specific structures on microorganisms. Toll-like receptors (TLRs) are a class of PRRs that can detect a diverse range of microorganisms, including bacteria, fungi, protozoa, and viruses. Each TLR recognizes different components of microorganisms, leading to distinct patterns of gene expression and activation of the innate immune system. Our research aims to gain insight into host defense mechanisms against various pathogens, including acquired immunity, in order to develop effective therapeutic strategies against immune-related diseases. Specifically, we are focusing on the RNA-binding protein (RBP) Regnase-1, which is involved in the development of autoimmune diseases, and Rbm7 (RNA-binding motif protein 7), which is another RBP involved in pulmonary fibrosis.

The Role of Endoribonuclease Regnase-1 in Immune and Non-Immune Cells

Regnase-1 is RBP with endonuclease activity (Maeda & Akira, *Int. Immunol*., 2018; Akira & Maeda, *Annu. Rev. Immunol*., 2021). After Regnase-1 is deleted, mice develop autoimmune diseases with enlarged spleens and lymph nodes. Our recent studies have shown that when Regnase-1 is deleted in the NK cells of mice (*Regnase-1*$^{\Delta NK}$ mice), these mice produce more perforin and interferon-γ, and as a result, show higher resistance to tumor progression and metastasis. Furthermore, Regnase-1 regulates its own expression by binding to its own *3'-untranslated region* (*3'UTR*). To investigate the importance of this negative feedback mechanism, we generated *3'UTR* mutant mice that abolished Regnase-1-mediated self-regulation. Currently, we are analyzing these mutant mice in various disease models.

Phosphorylation of Regnase-1

Phosphorylation of Regnase-1 by IKK at a DSGxxS motif results in βTrCP-dependent ubiquitination and degradation (Iwasaki et al., *Nat. Immunol*., 2011), but the role of other signal transduction pathways to regulate Regnase-1 function remains largely unknown. Phosphorylation of Regnase-1 after IL-17 stimulation also occurs in *Regnase-1* mutants that lack the IKK phosphorylation site (*Regnase-1*$^{AA/AA}$ and *Regnase-1*$^{\Delta CTD/\Delta CTD}$), which suggests that other phosphorylation sites may post-translationally modify Regnase-1 activities. We have shown that IL-17 induces Regnase-1 phosphorylation in an Act1-TBK1-IKKi-dependent manner, particularly in non-hematopoietic organs (Tanaka et al, *J. Exp. Med*., 2019). Phosphorylated Regnase-1 is released from the endoplasmic reticulum into the cytoplasm, thereby losing its mRNA degradation function and allowing IL-17 target genes to be expressed (Figure. 1). Thus, Regnase-1 plays an important role in the pathogenesis of IL-17-mediated inflammatory diseases via the Act1-TBK1-IKKi axis, and blocking the phosphorylation site of Regnase-1 may be a promising treatment for T helper 17-related diseases.

Diversity of Monocyte Subsets and their Role in Lung Fibrosis in Humans and Mice

Fibrosis is a life-threatening disease with unknown causes, and effective therapies are few. The activation of monocytes and macrophages is associated with the development of fibrosis, but the pathogenesis is still poorly understood. We have identified a new macrophage subset called SatM (segregated-nucleus-containing atypical monocytes), which shares granulocyte characteristics and is regulated by C/EBPβ). These cells play a critical role in fibrosis. We have also identified Rbm7, a component of the NEXT complex, whose increased expression in the fibrotic phase leads to the suppression of fibrosis when lost in non-hematopoietic cells. Aberrant *Rbm7* expression results in apoptosis via nuclear degradation of non-coding RNA (ncRNA) *Neat1*, which is involved in the development of fibrosis by producing chemokines that attract SatM and lead to lung fibrosis. Thus, inhibition of RBM7 may be an effective therapy for fibrosis in patients. We found that fibrosis-promoting macrophages derived from different monocyte subsets closely interact with other immune and non-immune cells, releasing essential factors that maintain the fibrosis niche (Fukushima et al., *Immunity* 2020). Our findings provide a comprehensive understanding of the mechanisms involved in the development and maintenance of fibrosis (Figure. 2).

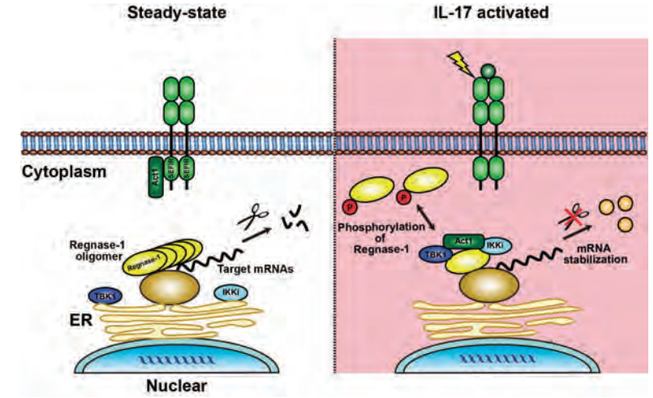


Figure 1.
IL-17 induces Regnase-1 phosphorylation in an Act-1/TBK1/IKKi–dependent manner. Phosphorylation of Regnase-1 is released from the endoplasmic reticulum into the cytosols, resulting in loss of the mRNA degradation function.

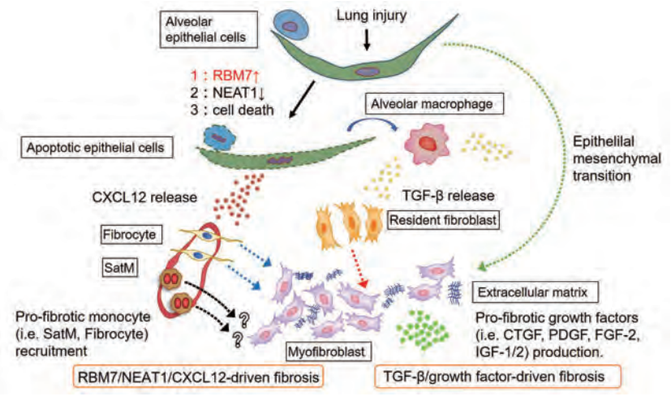


Figure 2.
RBM-7 regulates fibrosis formation through ncRNA decay and cell death. RBM-7 destabilizes ncRNA *NEAT1* and induces cell death in lung epithelial cells, resulting in recruitment of SatM and fibrocytes into the lung to promote lung fibrosis.

Recent Publications

1. Morisaka H, Takaishi M, Akira S, Sano S. Keratinocyte Regnase-1, a downregulator of skin inflammation, contributes to protection against tumor promotion by limiting cyclooxgenase-2 expression . J Invest Dermatol. (2022).
2. Akira S and Maeda K. Control of RNA stability in immunity. Annu. Rev. Immunol. 39:481-509 (2021).
3. Fukushima K, Satoh T, Sugihara F, Sato Y, 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).
4. Tanaka H, Arima Y, Kamimura D, Tanaka Y, Takahashi N, Uehata T, Maeda K, Satoh T, Murakami M, Akira S. Phosphorylation-dependent Regnase-1 release from endoplasmic reticulum is critical in IL-17 response. J Exp Med. 216:1431-1449 (2019).
5. Nagahama Y, Shimoda M, Mao G, Singh SK, Kozakai Y, Sun X, Motooka D, Nakamura S, Tanaka H, Satoh T, Maeda K, Akira S. Regnase-1 controls colon epithelial regeneration via regulation of mTOR and purine metabolism. Proc Natl Acad Sci USA. 115:11036-11041 (2018).

Immunoglycobiology

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

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

Our major interest has been biosynthesis and deficiencies of glycosylphosphatidylinositols (GPIs), which are a class of glycolipids acting as membrane-anchors of more than 150 various cell surface proteins and also being present as free glycolipids. In FY2022, we made the following progress.

Mechanism of GPI biosynthesis up-regulation

It has been unclear how cells upregulate GPI biosynthesis when needed. In cells defective in GPI transamidase that transfers GPI to proteins, precursors of GPI-APs are usually degraded by ER-associated degradation (ERAD). We previously reported that when both ERAD and GPI transamidase are defective in HEK293 cells, undegraded GPI precursors remain and GPI biosynthesis increases 8- to 10-fold (Wang Y et al, Nat Commun, 2020). We investigated mechanisms of this GPI biosynthesis up-regulation and found that accumulated precursors of specific GPI-APs, such as CD55, CD48 and PLET1, upregulate GPI biosynthesis. We showed that their C-terminal GPI attachment signal peptides are functional elements. We then found that ARV1, an ER-membrane protein implicated in lipid homeostasis and GPI biosynthesis, is required for this GPI upregulation (Figure 1). There must be a mechanism that senses the presence of specific GPI-AP precursors that remain, which signifies GPI shortage and triggers upregulation of GPI biosynthesis (Liu Y-S et al, J. Cell Biol., 2023).

Discovery of non-conventional mode of GPI-attachment

Until recently, it was believed that proteins are always attached to GPI-anchors by making an amide bond with ethanolamine-phosphate (EtN-P) linked to the third mannose (Man3), so-called "bridging EtN-P." The core glycan of GPI precursor has three mannoses, which in mammals, are all modified by EtN-P. Whereas EtN-P on Man3 acts as the bridge to the protein and EtN-P on Man1 remains as a sidechain, EtN-P on Man2 is removed after GPI-protein attachment. However, EtN-P on Man2 may not be always transient, as mutations of *PIGG*, the enzyme that transfers EtN-P to Man2, cause inherited GPI deficiency, characterized by neuronal dysfunctions. We showed in 2022 that EtN-P on Man2 is the preferential bridge in some GPI-APs, such as ect-5′-nucleotidase (NT5E) and netrin G2 (NTNG2) (Figure 2). We also found that all CD59, a GPI-anchored complement regulator, in *PIGB*-knockout cells, in which GPI lacks Man3, and a small fraction of CD59 in wild type cells are attached via EtN-P on Man2 to GPI-anchors. Our findings modified the previous view of GPI anchoring and provided a mechanistic basis of inherited GPI deficiency caused by *PIGG* mutations (Ishida M et al, EMBO Rep., 2022).

Gene therapy of inherited GPI deficiency model mice

To establish effective therapies for inherited GPI deficiency, we generated model mice by knocking in pathogenic mutations into the *Pigo* gene. The model mice had symptoms similar to some of those seen in human individuals with inherited *Pigo* deficiency. Adeno-associated virus PHP.eB (AAV-PHP.eB)-based gene therapy that aimed to systemically restore normal *Pigo* mRNA in the model mice was effective in ameliorating some symptoms (Kuwayama R et al, Nat. Commun., 2022).

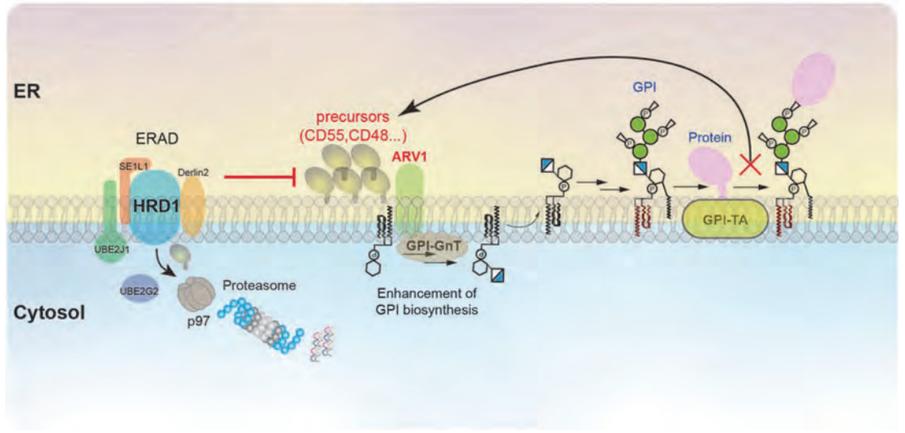


Figure 1.
Model of GPI biosynthesis upregulation mechanism in cells defective in GPI transamidase and ERAD (Adopted from Liu Y-S et al, J. Cell Biol., 2023).

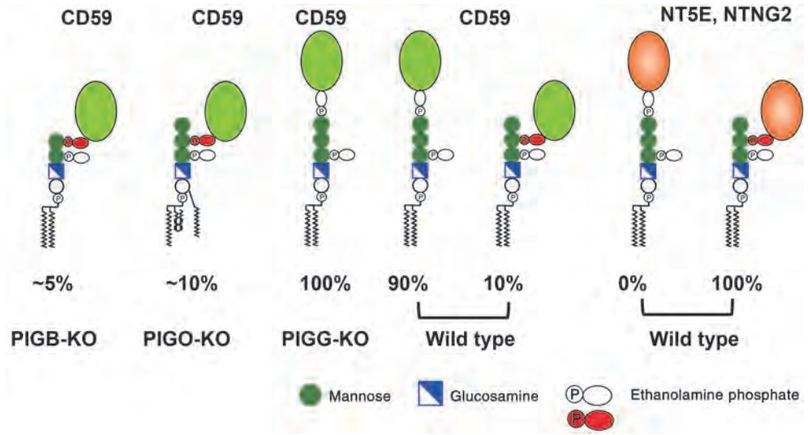


Figure 2.
Ethanolamine-phosphate on the second mannose is a preferential bridge for some GPI-anchored proteins, such as NT5E and NTNG2 (Adopted from Ishida M et al, EMBO Rep., 2022).

Recent Publications

1. Liu LS, 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).

2. 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).

3. 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).

4. 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).

5. Hirata T, Kobayashi A, Furuse T, et al. Loss of the N-acetylgalactosamine side chain of the GPI-anchor impairs bone formation and brain functions and accelerates the prion disease pathology. J Biol Chem. 298(3):101720 (2022).

Immunopathology

Atsushi Kumanogoh, MD/PhD

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

Our team adopts a two-pronged approach to research: basic immunology and clinical immunology. Our proposed study aims to investigate 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 to the diagnosis/therapy for human immunological disorders, such as autoimmunity, allergy, immune deficiency, cancer/metastasis, and neurodegenerative diseases. We recently focus on the crosstalk among neuronal, immune and metabolic systems since some of the semaphorins' expression are regulated by a metabolic sensor, mTOR, in which we investigated the biological and pathological significance of Lamtor1/p18, an amino acid sensor localized at the lysosome.

Lamtor1 is an essential component of the Ragulator complex. Although the Ragulator complex has a clearly established role as a regulator of cellular metabolic states by coordinating mTORC1 and AMPK activities, recent research has focused on its other functions. The Ragulator complex is a platform for maintaining cellular homeostasis, with roles in integrin signaling via Lamtor2–MEK, acidification of lysosomes via V-type ATPase, lysosome biogenesis by enhanced TFEB nuclear translocation, endomembrane damage repair or organelle homeostasis, and the regulation of migration through interactions with the myosin phosphatase Rho-interacting protein. In addition to these regulatory effects on cellular functions, we have uncovered a variety of roles of the Ragulagtor complex as an inflammatory signaling hub. However, the precise role of the Ragulator complex in NLRP3 inflammasome activation requires elucidation. Here, we show the vital role of the Ragulator complex in NLRP3 inflammasome activation. Deficiency of Lamtor1 abrogated NLRP3 inflammasome activation in murine macrophages and human monocytic cells. Myeloid-specific Lamtor1-deficient mice showed marked attenuation of NLRP3-associated inflammatory disease severity, including LPS-induced sepsis, alum-induced peritonitis, and monosodium urate (MSU)-induced arthritis.

Mechanistically, Lamtor1 interacted with both NLRP3 and histone deacetylase 6 (HDAC6). HDAC6 enhances the interaction between Lamtor1 and NLRP3, resulting in NLRP3 inflammasome activation. DL-all-rac-a-tocopherol, a synthetic form of vitamin E, inhibited the Lamtor1– HDAC6 interaction, resulting in diminished NLRP3 inflammasome activation. Further, DL-all-rac-a-tocopherol alleviated acute gouty arthritis and MSU-induced peritonitis. These results provide novel insights into the role of lysosomes in the activation of NLRP3 inflammasomes by the Ragulator complex.

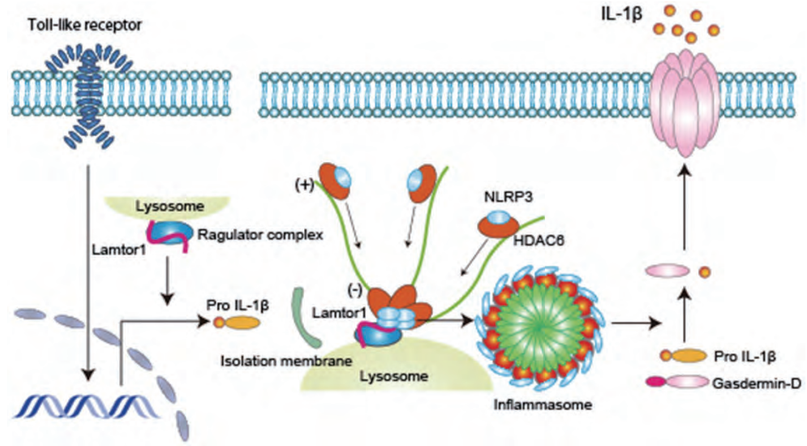


Figure.
How inflammasome activation is orchestrated by specific organelles remains unclear. Here, the lysosomal Ragulator complex is shown to enhance NLRP3 inflammasome activation via histone deacetylase 6 (HDAC6). Deficiency of Lamtor1, an essential component of the Ragulator complex, abrogated NLRP3 inflammasome activation *in vitro* and in mice. The Ragulator complex interacted with HDAC6, which facilitated the interaction between the Ragulator complex and NLRP3, and that both interactions were required for the activation of the NLRP3 inflammasome.

Recent Publications

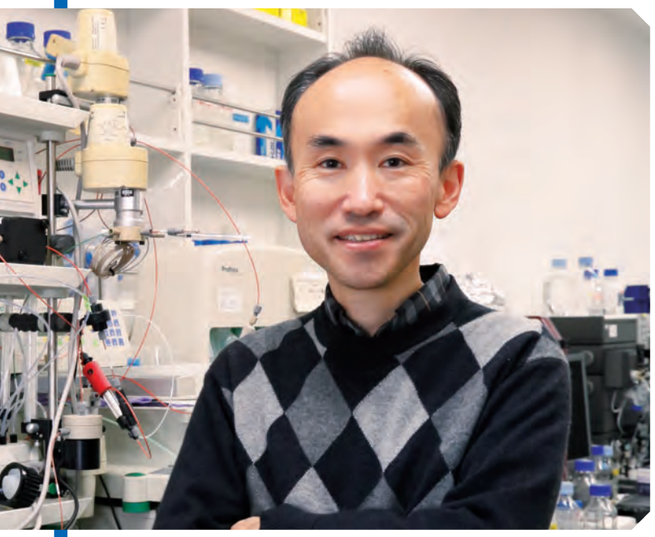
1. Tsujimoto K, Jo T, Nagira D, Konaka H, et al. The lysosomal Ragulator complex activates NLRP3 inflammasome in vivo via HDAC6. EMBO J. 42(1):e111389 (2023).

2. Nakatani T, Tsujimoto K, Park J, Jo T, Kimura T, Hayama Y, Konaka H, Morita T, Kato Y, Nishide M, Koyama S, Nada S, Okada M, Takamatsu H, Kumanogoh A. The lysosomal Ragulator complex plays an essential role in leukocyte trafficking by activating myosin II. Nat Commun. 12(1):3333 (2021).

3. Tsuda T, Nishide M, Maeda Y, Hayama Y, Koyama S., et al. Pathological and therapeutic implications of eosinophil-derived semaphorin 4D in eosinophilic chronic rhinosinusitis . J Allergy Clin Immunol. 145(3):843-854 (2020).

4. Kang S, Nakanishi Y, Kioi Y, Okuzaki D, et al. Semaphorin 6D reverse signaling controls macrophage lipid metabolism and anti-inflammatory polarization. Nature Immunol. 19:561-570 (2018).

Immunochemistry



Hisashi Arase, MD/PhD

Professor	Hisashi Arase
Assistant Professor	Wataru Nakai
	Hui Jin
Postdoctoral Fellow	1
Research Assistant	2
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, it has remained unclear how MHC class II molecules are involved in autoimmune disease susceptibility. We found that misfolded cellular autoantigens are rescued from protein degradation by MHC class II molecules (*Int. Immunol*. 2013). Furthermore, we found that misfolded proteins complexed with MHC class II molecules are 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). Furthermore, autoantibody binding to misfolded proteins transported to the cell surface by MHC class II molecules was strongly correlated with susceptibility to autoimmune diseases. Further analyses revealed that self-antigens complexed with MHC II molecules abrogate self-tolerance to induce autoimmune response (*Science Advances* 2022). In addition, we found that not only misfolded protein but also DNA is presented on MHC class II molecules with SLE-risk allele but not with SLE-protective allele (*Arthritis Rheumatol*. 2022). These findings demonstrated that misfolded proteins, which normally would 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 evolved with infectious diseases, indicating that studies on host-pathogen interaction are important to understand the immune system. We found that PILRα, one of a pair of receptors, plays an important role in the regulation of immune response (*Nat. Immunol*. 2012; *Int. Immunol*. 2015; *Eur. J. Immunol*. 2016) as well as HSV-1 infection (*Cell* 2008; *J. Virol*. 2009). Similarly, Siglec-4 and Siglec-7, which are a pair of receptors, are involved in VZV infection (*PNAS* 2010; *BBRC* 2022). LILR is another type of paired receptor family. We found that activating LILRA2 is involved in the detection of immunoglobulin abnormalities in microbial infection (*Nature Microbiology* 2016). Furthermore, we found that RIFINs, products of multigene family of *Plasmodium falciparum*, are involved in immune evasion through binding to inhibitory LILRB1 and LILRB2 (*Nature* 2017; *Nature* 2020; *BBRC* 2021). These findings demonstrated that paired receptors play an important role in immune regulation as well as viral infection.

SARS-CoV-2 causes severe pneumonia in some infected individuals. Therefore, it is important to elucidate the factors that cause severe COVID-19. Antibodies against the receptor-binding domain (RBD) of spike protein play an important role in the defense against SARS-CoV-2 infection. However, we found that some antibodies against the N-terminal domain (NTD) of spike protein cause conformational changes in spike protein and enhance infectivity. In particular, anti-NTD antibodies that enhance infectivity were found more in severe patients, suggesting that the antibodies may be involved in the development of severe disease (*Cell* 2021, Figure 2). These findings indicated that the infectivity-enhancing antibodies must be considered in antibody response against SARS-CoV-2.

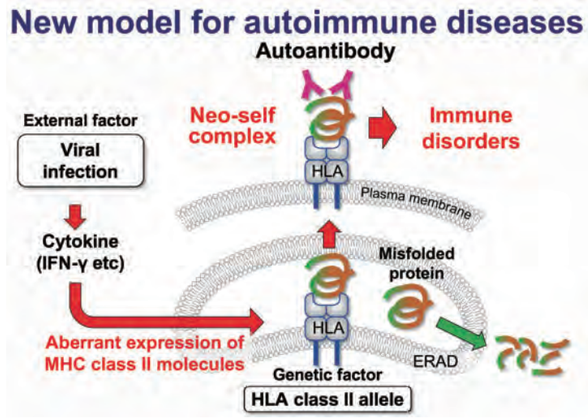


Figure 1.
Misfolded proteins transported to the cell surface by MHC class II molecules are targets for autoantibodies.
Misfolded cellular proteins are generally degraded in the cells and are not transported to outside the cells. Therefore, misfolded proteins transported to the cell surface by MHC class 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).

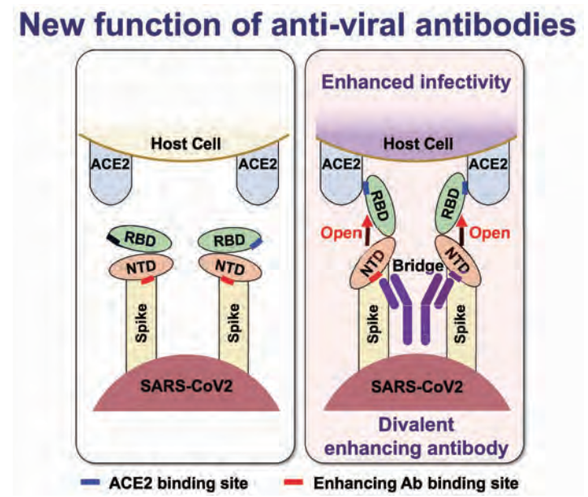


Figure 2.
SARS-CoV-2 infectivity enhancing antibodies.
Some antibodies against NTD of spike protein induces conformational change in spike protein to induce the open form of RBD and enhance the infectivity of SARS-CoV-2 (*Cell* 2021).

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 Advances. 8:eabj9867. (2022).
2. Tsuji H, Ohmura K, Jin H, et al. Anti-Double-Stranded DNA Antibodies Recognize DNA Presented on HLA Class II Molecules of Systemic Lupus Erythematosus Risk Alleles. Arthritis Rheumatol. 74:105-111 (2022).
3. 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).
4. Saito F, Hirayasu K, Satoh T, Wang CW, et al. Immune evasion of Plasmodium falciparum by RIFIN via inhibitory receptors. Nature. 552:101-105 (2017).
5. 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).
6. 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 (Co-PI)

Professor	Tadamitsu Kishimoto
Associate Professor	Sujin Kang
Postdoctoral Fellow	1
Support Staff	5

Endothelial gp130-HIF1α signaling on the onset of cytokine release syndrom

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). Thus, tocilizumab was recently approved by the United States Food and Drug Administration for the treatment of SARS-CoV-2 infection-induced pneumonia. In our lab, to clarify the downstream pathway of IL-6 trans-signaling in endothelial activation, we performed an RNA-seq analysis of primary human vascular endothelial cells (HUVECs). We found that expression of HIF1α was substantially increased by IL-6 trans-signaling in HUVECs. Additionally, inhibition of HIF1α activity in HUVECs decreased the expression of IL-6, IL-8, MCP-1, and plasminogen activation inhibitor-1 (PAI-1) in response to IL-6 trans-signaling. Moreover, knockdown of gp130 suppressed expression of glycolysis-related enzymes including HK2 and PFKFB3, which are target genes of HIF1α transcriptional activity, indicating that IL-6 signaling induces vascular inflammation via glycolysis activation in endothelium. Next, we generated the endothelial-specific gp130 knockout mice ($gp130^{ECKO}$) to delineate the role of gp130-HIF1α signaling on endothelium during cytokine storm *in vivo*. At the onset of sepsis, $gp130^{ECKO}$ mice showed resistance to survival rate, and decreased inflammatory cytokines production and endothelial permeability. Consistently, lung endothelial cells from $gp130^{ECKO}$ mice displayed decreased levels of HIF1α expression. Thus, our findings demonstrated that endothelial gp130-HIF1α signaling promotes vascular inflammation and sepsis progression. However, the long-lasting and discriminatory suppression of IL-6 signaling in the acute critical care setting raises concerns about the increased risk of secondary infections. To overcome the limitations of IL-6 signaling blockade in acute inflammatory diseases, we generated an anti-IL-6R antibody (silent anti-IL-6R antibody) with a short half-life to reduce Fc-mediated side effects. We found that the treatment of CRS mouse models including thermal injury and sepsis models with the silent anti-IL-6R antibody significantly improved the survival rate compared with controls. Additionally, we found that silent anti-IL-6R antibody treatment prevented endothelial damage by inhibiting glycocalyx degradation, increasing endothelial permeability, and PAI-1 production. Consequently, treatment with silent anti-IL-6R antibodies showed significant efficacy in several acute inflammatory diseases by controlling endothelial injury (Fig. 1).

Threonine phosphorylation of STAT1 controls the host inflammatory response

The STAT1 working paradigm has focused on interferon (IFN) mediated JAK-Tyr701 phosphorylation. However, IFN-JAK signaling does not explain the full spectrum of STAT1 functions against inflammatory stimuli. Our recent work identified the novel Thr749 (Thr748 in mouse) phosphorylation of STAT1, which triggered distinct gene-regulatory functions of STAT1 independent of the canonical Tyr701 phosphorylation. **1) Disruption of Thr748 phosphorylation of STAT1 protects the host against lipopolysaccharide (LPS)-induced septic shock**: We generated genetically engineered mice expressing a non-phosphomimetic threonine748-to-alanine (T748A) mutant STAT1. We found that STAT1T748A and heterozygous mice exhibited higher survival compared with control mice against LPS-induced septic shock, suggesting pThr STAT1 promoted the host inflammatory response in sepsis. Additionally, STAT1T748A mice were resistant to LPS-induced lethality, albeit to a lesser extent than their STAT1-deficient littermates. Notably, expression of STAT1 and other STAT proteins was unperturbed in STAT1T748A mice, suggesting that threonine748 phosphorylation of STAT1 regulates inflammatory response against LPS-induced septic shock. We found that monocytes derived macrophages (MDMs) from STAT1T748A mice decreased expression of inflammatory cytokines including IL-6, IL-12. In contrast, MDMs from STAT1T748A mice increased expression of antiviral genes, RSAD2, IFIT2, and increased IL-10 expression. Our work indicates that T748A mutation of STAT1 may induce anti-inflammatory characteristics of macrophages, which alleviates the inflammatory response against sepsis. **2) Disruption of Thr748 phosphorylation of STAT1 promotes colitis**: A deficiency in STAT1 leads to severe DSS-induced colitis. However, colon samples from patients with ulcerative colitis exhibited increased expression of JAK-mediated pTyr STAT1. Tofacitinib, a JAK inhibitor, has been approved for the treatment of patients with ulcerative colitis. A potential explanation of these paradoxical findings is that the protective role of STAT1 in ulcerative colitis is largely independent of the canonical JAK-STAT1 pathway. We evaluated the intestinal inflammation of wild-type (WT) and STAT1T748A mice following DSS administration. Notably, STAT1T748A mice exhibited severe colitis compared to WT mice by weight loss, colon lengths, and histopathological analysis. We assessed the intestinal inflammation of STAT1KO and their STAT1T748A littermates by DSS administration. STAT1T748A mice phenotype completely mimicked the detrimental phenotype of STAT1KO littermates. We found that epithelial cells, not immune cells, are responsible for the phenotype observed in T748A mice. Our data indicate that threonine748 phosphorylation on STAT1 is a key driver for STAT1 protective function against intestinal inflammation. Consequently, we found that pThr STAT1 contributed to STAT1 functions to a different extent in vivo in various disease models, and our findings suggest that cell-dependent threonine kinase pathways contribute to the specificity of STAT1 functions, especially those not driven by canonical JAK pathway (Fig. 2).

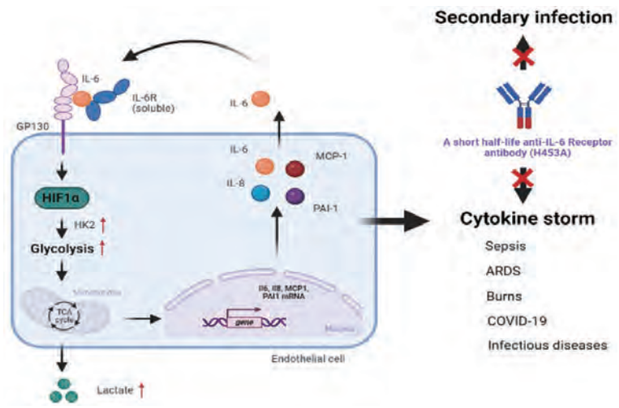


Figure 1.
Targeting of anti-IL-6R antibody for infectious diseases by inhibition of endothelial injuries.

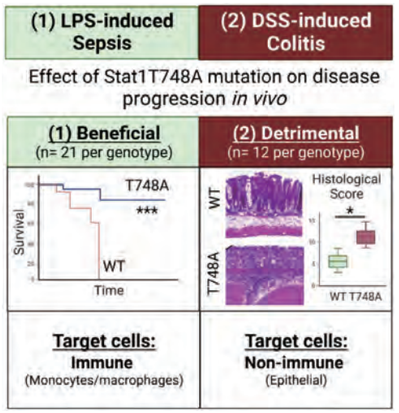


Figure 2.
Role of novel phosphorylation site of STAT1 *in vivo*.

Recent Publications

1. 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). (*equally contributed).
2. Narazaki M, Kishimoto T. Current status and prospects of IL-6-targeting therapy. Expert Rev Clin Pharmacol. 15(5):575-592 (2022).
3. Hashimoto S, Kishimoto T. Roles of RNA-binding proteins in immune diseases and cancer. Semin Cancer Biol. 86(Pt 3):310-324 (2022).
4. Nyati KK, Kishimoto T. Recent Advances in the Role of Arid5a in Immune Diseases and Cancer. Front Immunol. 19;12:827611 (2022).

Mucosal Immunology

Kiyoshi Takeda, MD/PhD

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Inflammatory bowel disease (IBD), which consists of Crohn's disease (CD) and ulcerative colitis (UC), involves chronic inflammation of digestive tract. Despite a plethora of studies emphasizing the involvement of T cells in the pathogenesis of IBD, no disease-specific T-cell subset that plays an essential role in the progression of IBD has been identified. In this study, we performed deep profiling of T cells in the intestinal mucosa of IBD and identified a particular subset of $CD4^+$ tissue-resident memory T cell (Trm) subset that is prevalent in CD showing unique inflammatory properties. Our results highlight the importance of $CD4^+$ Trm in the pathogenesis of CD, which can be applicable to the development of T cell-directed strategies.

CD and UC are characterized by distinct T-cell subsets

To explore the specific molecular properties of each subtype of IBD, we applied mass cytometry panels using antibodies against 28 cell surface markers, which allow us to capture various types of T-cell subsets. We profiled the intestinal lamina propria mononuclear cells isolated from inflamed gut mucosa of patients with CD, UC, and unaffected mucosal regions of colon cancer patients as a control. Overall, landscape of adaptive immunity in CD and UC were clearly distinguished from each other by the marked changes in the specific clusters. $CD4^+$ Trm were significantly increased in CD, whereas follicular helper T cells were significantly increased in UC. Heatmaps of the mean expression levels of T-cell markers revealed that a particular fraction of $CD4^+$ Trm abundant in the gut of CD (CD-enriched population: CDpop) exhibited high expression of CD103, CD161 and CCR5 compared with other T-cell fractions. Furthermore, $CD4^+$ Trm expressing CCR5 or CD161 produced significantly higher IFN-γ levels upon phorbol 12-myristate 13-acetate (PMA)/ionomycin stimulation than their negative counterparts that did not express these markers.

$CD4^+$ Trm are reprogrammed in CD gut to initiate effector and innate functions

To identify the transcriptional landscape of IBD gut, single-cell targeted RNA-sequencing (scRNA-seq) analysis of $CD3^+$ T cells was performed. Multi-resolution Reconciled Tree, which reflect the extent of transcriptional distinctions among cell groups, applied to scRNA-seq datasets revealed that the transcriptional signature of $CD4^+$ CD8- Trm is more closely related to that of $CD8^+$ T cells rather than other $CD4^+$ T cell subsets and enriched in innate immune-like cytotoxic pathways. Notably, a particular subset of CDpop is highly specific to CD patients. This CD-specific $CD4^+$ Trm highly expressed genes associated with cytotoxicity, such as *NKG6*, *GZM*, and *GNLY*, and also significantly upregulated Th1-related genes, such as *IFNG* and *TBX21*. In vitro evaluation of CDpop revealed that it secretes IFN-γ by cytokine stimulation without exogenous TCR ligation, implicating the innate aspect of this subset. Cytokines that stimulated CDpop includes IL-12, IL-18, IL-7, and IL-15, which are known to be elevated in the intestinal mucosa of IBD patients. Thus, the effector function of CDpop can be maximally enhanced by the unique cytokine milieu in IBD gut. Furthermore, CDpop was the predominant producer of type-1 inflammatory cytokines upon stimulation by both cytokine cocktail and PMA/ionomycin stimuli among all $CD4^+$ T cell subsets.

Cytotoxic effect of CD-predominant T cells on epithelial layer is maximized by their localization properties

Immunohistochemical evaluation of CD4 and CD103 double-positive cells including CDpop in the lamina propria revealed that they localized adjacent to the epithelia. Coculture of human-derived spheroids with CDpop stimulated with a cytokine cocktail resulted in the epithelial damage accompanied by the elevation of LDH levels in the culture supernatant that is comparable to the findings observed when IFN-γ was added to the spheroids. In contrast, coculture with CDpop without cytokine cocktail did not disrupt the spheroid structure or induce LDH release. This response was inhibited by the addition of a neutralizing antibody against IFN-γ. Finally, the positive correlation between the abundance of CDpop in the gut biopsy samples and a clinical activity score suggests that the accumulation of this T-cell subset is a pathological hallmark of CD.

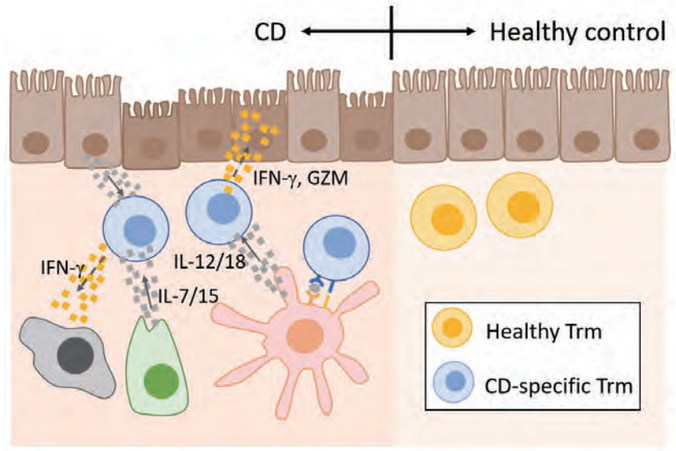

Figure.
In the lamina propria of Crohn's disease (CD) patients, $CD4^+$ $CD103^+$ $CCR5^+$ $CD161^+$ tissue resident memory T cells (Trm), which localize adjacent to the gut epithelial cells are expanded. These Crohn's disease-specific Trm are stimulated by cytokines which are abundant in the gut of CD patients, and secrete IFN-γ and GZM. This in turn activate inflammatory immune cells and damage adjacent epithelial cells, exacerbating the disease.

Recent Publications

1. Nii T, Maeda Y, Motooka D, et al. Genomic repertoires linked with pathogenic potency of arthritogenic Prevotella copri isolated from the gut of rheumatoid arthritis patients. Annals Rheum. Dis. In press.
2. Yokoi T, Murakami M, Kihara T, et al. Identification of a unique subset of tissue resident memory $CD4^+$ T cells in Crohn's disease. Proc. Natl. Acad. Sci. USA 120, e2204269120 (2023).
3. Otake-Kasamoto Y, Kayama H, Kishikawa T, et al. 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, et al. The ATP-hydrolyzing ectoenzyme E-NTPD8 attenuates colitis through modulation of P2X4 receptor-dependent metabolism in myeloid cells. Proc. Natl. Acad. Sci. USA118, e2100594118, (2021).
5. Morita N, Umemoto E, Fujita S, Hayashi A, et al. GPR31-dependent dendrite protrusion of intestinal CX_3CR1^+ cells by bacterial metabolites. Nature 566,110-114 (2019).

Experimental Immunology

Shimon Sakaguchi, MD/PhD

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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 manipulating the mechanism of immunologic self-tolerance; and (iii) the cause and pathogenetic mechanism of systemic autoimmune diseases, such as rheumatoid arthritis, by utilizing an animal model established in our laboratory.

Tregs, which specifically express the transcription factor Foxp3, are actively engaged in the maintenance of immunological self-tolerance and homeostasis. They are abundant in tumor tissues, hampering effective anti-tumor immune responses; and their depletion is indeed able to evoke/enhance tumor immunity. However, one of the difficulties to specifically eliminate tumor Tregs by targeting a molecule expressed by Tregs is that a majority of such candidate molecules are commonly shared by tumor-infiltrating Tregs and activated conventional T cells. For example, CTLA-4 and PD-1 are both expressed by Tregs and $CD8^+$ CTLs in tumor tissues. We have previously shown that high-ADCC/ADCP anti-CTLA-4 mAb treatment effectively evoked anti-tumor immune responses in tumor-bearing mice only when mAb administered first to deplete Tregs and then tumor antigen vaccination several days later to spare activated $CD8^+$ CTLs from cell depletion by the mAb treatment (Ha et al., PNAS 2019). In contrast, anti-PD-1 blocking mAb not only activate $PD\text{-}1^+$ $CD8^+$ CTLs, but also occasionally drive $PD\text{-}1^+$ Tregs to proliferate and enhance their suppressive activity, hindering tumor immunity and even causing rapid cancer progression called hyper-progressive disease (HPD) (Kamada et al., PNAS 2019).

These findings have prompted us to search for a molecule that is more specifically expressed by Tregs but not by activated $CD8^+$ CTLs in tumor tissues, enabling specific depletion of tumor Tregs but not activated $CD8^+$ CTLs. In 2022, assuming that tumor Tregs would clonally expand when they were activated by tumor-associated antigens to suppress anti-tumor immune responses, we performed single-cell analysis on tumor Tregs to characterize them by T-cell receptor (TCR) clonotype and gene expression profiles. We found that multi-clonal Tregs present in tumor tissues predominantly expressed the chemokine receptor CCR8. In mice and humans, $CCR8^+$ Tregs constituted 30-80% of tumor Tregs in various cancers and less than 10% of Tregs in other tissues, whereas most tumor-infiltrating conventional T cells (Tconvs) were $CCR8^-$. $CCR8^+$ tumor Tregs were highly differentiated, functionally stable, and potently suppressive. One-time administration of cell-depleting anti-CCR8 mAb indeed selectively eliminated multi-clonal tumor Tregs, leading to cure of established tumors in mice. The treatment resulted in the expansion of $CD8^+$ effector Tconvs, including tumor-antigen-specific ones, that were more activated and less exhausted than those induced by anti-PD-1 immune checkpoint blockade. Anti-CCR8 mAb treatment also evoked strong secondary immune responses against the same tumor cell line inoculated several months after tumor eradication, indicating that elimination of tumor-reactive multi-clonal Tregs was sufficient to induce memory-type tumor-specific effector Tconvs. Despite induction of such potent tumor immunity, anti-CCR8 mAb treatment elicited minimal autoimmunity in mice, contrasting with systemic Treg depletion, which eradicated tumors but induced severe autoimmune disease. Thus, specific removal of clonally expanding Tregs in tumor tissues for a limited period by cell-depleting anti-CCR8 mAb treatment can generate potent tumor immunity with long-lasting memory and without deleterious autoimmunity. Cancer immunotherapy with cell-depleting anti-CCR8 mAb is envisaged in the clinic.

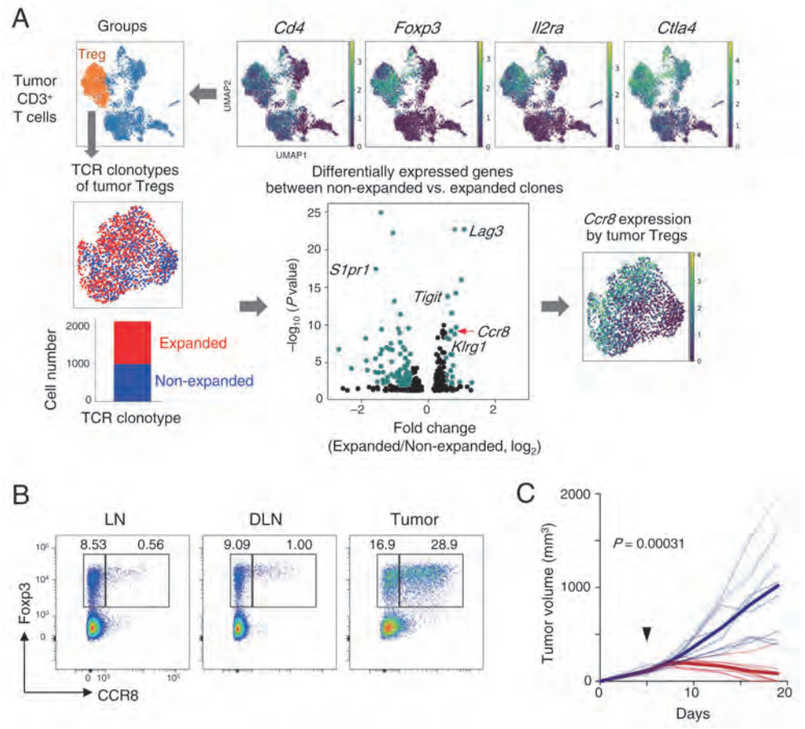
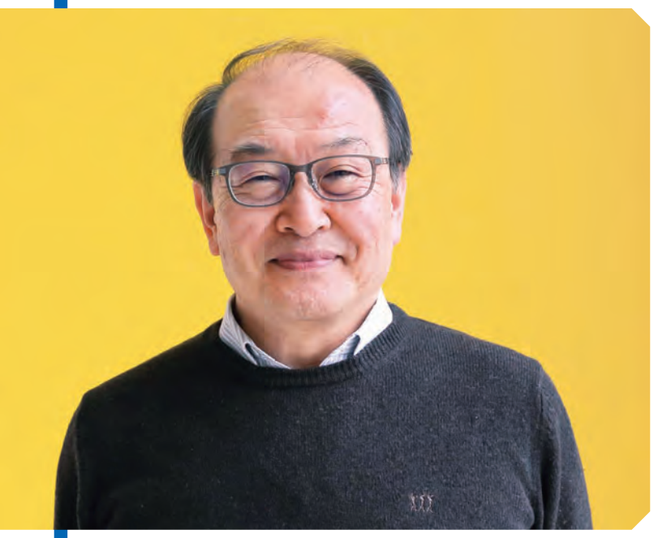


Figure.
(A) Search for the genes specifically expressed by clonally expanding tumor Tregs. Tumor-infiltrating $CD3^+$ T cells in CT26-bearing mice on day18 after tumor cell inoculation were analyzed for gene expression by single-cell RNA-seq. The Treg population defined by the expression of Treg function-associated genes was divided into the clonally expanded population (i.e., more than two Tregs commonly expressing a particular TCR clonotype) (red dots) and the clonally non-expanded one (i.e., every Treg expressing a non-replicated unique TCR) (blue dots) by TCR clonotyping. Volcano plot shows gene expression profiles of the two cell populations. (B) CCR8 protein expression by $CD3^+CD4^+$ T cells in LNs, draining LNs (DLNs) and tumors. (C) Tumor growth in tumor-bearing mice treated with anti-CCR8 or control mAb. Antibodies were administered on day 5 to mice with CT26 colon tumor.

Recent Publications

1. Tanaka A, Maeda S, Nomura T, et al. Construction of a T-cell receptor signaling range for spontaneous development of autoimmune disease. J. Exp. Med. 220(2):e20220386 (2023). doi: 10.1084/jem.20220386.
2. Kidani Y, Nogami W, Yasumizu Y, et al. CCR8-targeted specific depletion of clonally expanded Treg cells in tumor tissues evokes potent tumor immunity with long-lasting memory. Proc Natl Acad Sci USA. 119(7):e2114282119 (2022). doi: 10.1073/pnas.2114282119.
3. Tekguc M, James Badger Wing JB, Osaki M, Long J, Sakaguchi S. Treg-expressed CTLA-4 depletes CD80/CD86 by trogocytosis, releasing free PD-L1 on antigen-presenting cells. Proc Natl Acad Sci USA. 118(30):e2023739118 (2021). doi: 10.1073/pnas.2023739118.
4. Kawakami R, Kitagawa Y, et al. Coordinated activation of distinct Foxp3 enhancer elements for Treg development, maintenance, and immunological self-tolerance. Immunity. 54(5):947-961.e8 (2021). doi: 10.1016/j.immuni.2021.04.005.
5. Ohkura N, Yasumizu Y, Kitagawa Y, Tanaka A, Nakamura Y, Motooka D, Nakamura S, Okada Y, Sakaguchi S. Regulatory T cell-specific epigenomic region variants are a key determinant of susceptibility to common autoimmune diseases. Immunity. 52(6):1119-1132.e4 (2020). doi: 10.1016/j.immuni.2020.04.006.

Cell Signaling



Takashi Saito, PhD

Professor Takashi Saito

The objective of our team is to determine the molecular mechanisms of T cell activation, differentiation and function. Ultimately, we wish to elucidate the onset of and to modulate T cell function/activation to prevent immune diseases such as autoimmunity and allergic inflammation. For this purpose, we analyzed the regulation of T cell activation/function from a signaling perspective.

1. Regulation of T cell function by innate signaling.

We found that Receptor-interacting serine/threonine-protein kinase 1 (RIPK1) plays a critical role in T cell function and particularly on cellular senescence and following systemic inflammation during the analyses on the modulation of T cell function by innate-like signals. RIPK-1 has been known to function both on cell death – apoptosis and necroptosis - and cell survival. We generated and analyzed T cell-specific RIPK1-deficient (tKO) mice to analyze its function in T cells on metabolism and aging. RIPK1-tKO mice induces various inflammatory diseases with ageing (inflammaging) such as opacity, sarcopenia, cardiovascular inflammation, anemia, hypoglycemia, lymphopenia, and early death. T cells in KO mice show senescence phenotype from early life through constitutive activation of Caspase-8, RIPK3 and mTORC1. Inhibition of mTORC1, or caspase-8/RIPK3 restored T cell senescence and age-related diseases. Therefore, caspase-8/RIPK3 induced basal activation of mTORC1 and senescence program, which leads to inflammaging (Figure). Since it has been suggested that senescent cells influence neighbor cells to induce senescence, when senescent T cells were transferred into normal mice, transferred senescence T cells returned to normal, whereas normal T cells become senescent when transferred into RIPK1-KO mice.

2. Negative regulation of T cell activation

Our finding that TCR-microclusters (MC) initiate T cell activation led us to analyze the dynamics of signaling molecules at the immune synapse. Similar to our previous studies on negative regulation of T cell activation through CTLA4 and PD-1, we have analyzed the dynamic regulation of another inhibitory co-stimulator, LAG3. LAG3 was also colocalized with the TCR-MC upon TCR stimulation to mediate inhibition of T cell activation. Since the association between TCR-MC and LAG3 cluster is critical for the inhibitory function, anti-LAG3 Ab induced separation of LAG3 cluster from TCR-MC and consequently enhancement of T cell activation. Our analyses provide a dynamic view of signal regulation to define inhibitory mechanism. Furthermore, we have also analyzed negative regulation of T cell activation by autoimmune-related PTPN22. Its deficiency resulted in enhanced activation and an increase in effector/memory T cells. Analysis of the associated proteins revealed that PTPN22 was recruited to the TCR-MC to comprise an “inhibitory complex” with other inhibitory molecules to inhibit activation. A PTPN22 mutant causing susceptibility to autoimmune diseases was defective in recruitment to the TCR-MC. These studies help define the autoimmune susceptibility caused by the mutation.

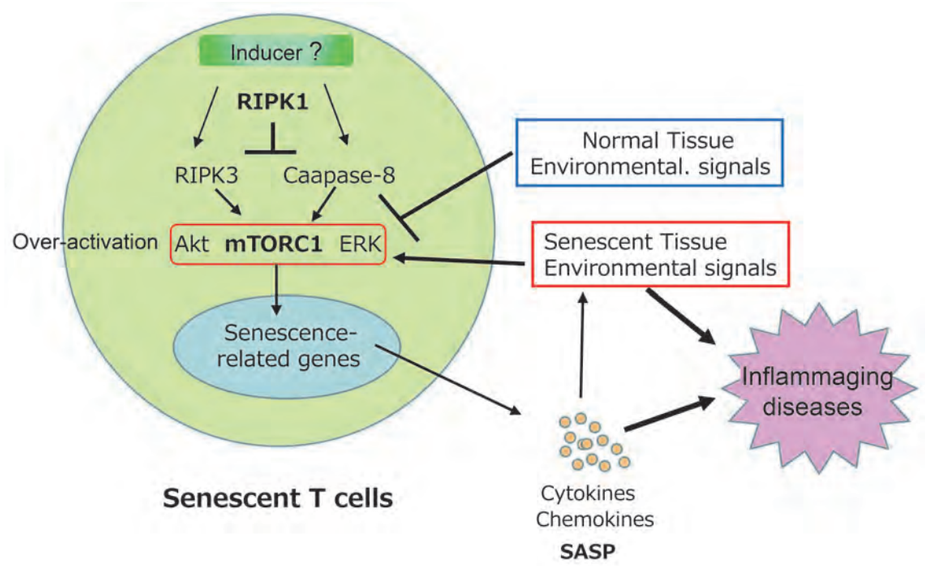


Figure.
Regulatory mechanism of T cell senescence and consequent inflammation and age-related diseases by RIPK1
RIPK1 inhibits excess activation of mTORC1 by inhibiting RIPK3 and Caspase-8. Overactivation of mTORC1 induces expression of senescence-related genes, promotes T cell senescence which produce various cytokines, chemokines, and senescence-associated secretory phenotype (SASP), which promote tissue senescence and induces age-related diseases. Senescent tissue environmental signals enhance mTORC1 activation, exacerbates ageing and age-related diseases. On the other hand, normal tissue environmental signals inhibit mTORC1 activation and T cell senescence and inflammaging.

Recent Publications

1. Imanishi T, Unno M, Yoneda N, Motomura Y, Mochizuki M, Sasaki T., Moro K., Pasparakis M. and Saito T.: RIPK1 blocks T cell senescent regulated by RIPK3 and caspase-8. Sci Advances. 25(9): eadd6097 (2023).

2. Takemori T, Sugimoto-Ishige A, Nishitsuji H, Futamura Y, Harada M, Kimura-Someya T, Matsumoto T, Honma T, Tanaka M, Yaguchi M, Isono K, Koseki H, Osada H, Miki D, Saito T, Tanaka T, Fukami T, Goto T, Shirouzu M, Shimotohno K, Chayama K. Establishment of a monoclonal antibody against human NTCP that blocks HBV infection. J Virology 96:e0168621(2022).

3. Kumagai A, Nara T., Uematsu M, Kakinuma Y, Saito T,Masuda. Development and characterization of a unique anti-IgE monoclonal antibody cross-reactive between human and canine IgE. Immun. Inflamm. Dis. 9:1740-1748 (2021).

4. Imanishi T, Unno M, Kobayashi W, Yoneda N, Akita S and Saito T. mTORC1 signaling controls TLR2-Mediated T-cell activation by inducing TIRAP expression. Cell Reports. 32:107911-107911 (2020).

5. Imanishi T and Saito T. T Cell co-stimulation and functional modulation by innate signals. Trends Immunol. 41:200-212 (2020).

Lymphocyte Differentiation

Tomohiro Kurosaki, MD/PhD

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Since the outbreak of COVID-19 in late 2019, several SARS-CoV-2 variants of concern have continuously emerged in the past years. Among them, the Omicron BA.1 (B.1.1.529) variant, harboring ~15 mutations in the spike receptor-binding domain (RBD), showed a profound effect on evading the neutralizing antibody responses in those received 2-dose of mRNA vaccination (Pfizer-BioNTech BNT162b2 or Moderna mRNA-1273). In fact, epidemiologic data suggested that weak or undetectable neutralizing antibodies against Omicron variant were induced in serum IgG after a 2-dose of mRNA vaccination. In contrast, individuals boosted with a third dose of mRNA vaccine encoding the original Wuhan spike protein induced potent neutralizing serum activity against Omicron, and were highly protected from infection.

In addition to the antibody induction, mRNA vaccination elicits the generation of SARS-CoV-2-specific memory B cells which represent a second layer of immune protection through quick differentiation into antibody-secreting plasma cells upon re-encountering antigens. Indeed, a recall response from memory B cells was highlighted as a key factor for the protection from severe pathology in the lungs of nonhuman primates. Furthermore, memory B cells can persist for a long period and evolve due to the progressive acquisition of somatic hypermutations (SHM) through germinal center (GC) reaction. In fact, SARS-CoV-2 mRNA vaccination induced a robust and persistent GC response in humans. Hence, memory B cells are able to possess a diverse antibody repertoire, allowing for an adaptive response against the pathogen upon re-infection, particularly in the case of variant pathogen infections.

We have addressed the underlying mechanism of the differential neutralizing antibody responses against the Omicron variant between the second and the third vaccine dose. For this purpose, we analyzed memory Our results suggest that acutely produced Omicron-non-cross-reactive antibodies help skew the memory B cells towards more Omicron-cross-reactivity during a 2-dose immune response, thereby at least partly, contributing to the generation of Omicron-neutralizing antibodies upon a third vaccine dose.

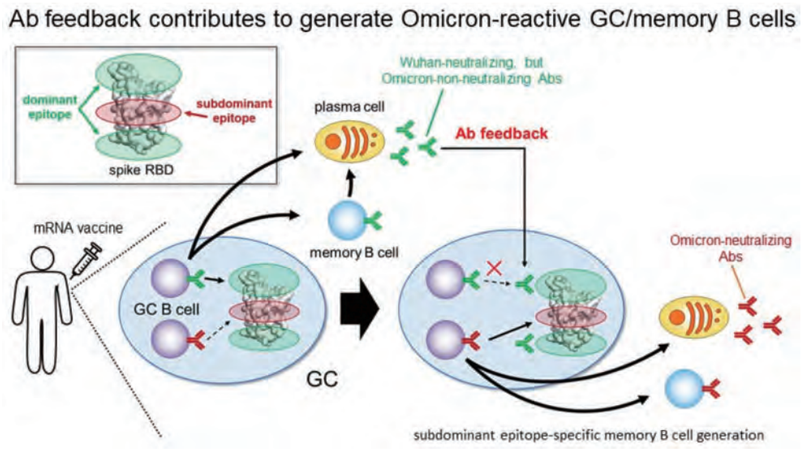

Figure.
Model for antibody feedback regulation of B cell responses upon mRNA vaccination. Before or just after the 2nd vaccination, B cells specific for immunodominant epitopes are preferentially selected for antibody production. Antibodies induced by the 2nd vaccine dose diversifies the immunogenicity of memory B cells during the sustained GC responses, likely by epitope masking, so that immuno-subdominant epitope-specific memory B cells are generated more frequently and can produce Omicron-neutralizing antibodies upon the 3rd vaccine dose.

Recent Publications

1. Inoue T, Kurosaki T. Memory B cells. Nat. Rev. Immunol. in press.
2. Koike T, Fujii K, Kometani K, Butler NS, Funakoshi K, Yari S, Kikuta J, Ishii M, Kurosaki T, Ise W. Progressive differentiation toward the long-lived plasma cell compartment in the bone marrow. J Exp Med. 220(2):e20221717 (2023).
3. Inoue T, Shinnakasu R, Kawai C, Yamamoto H, Sakakibara S, Ono C, Itoh Y, Terooatea T, Yamashita K, Okamoto T, Hashii N, Ishii-Watabe A, Butler NS, Matsuura Y, Matsumoto H, Otsuka S, Hiraoka K, Teshima T, Murakami M, Kurosaki T. Antibody feedback contributes to facilitating the development of Omicron-reactive memory B cells in SARS-CoV-2 mRNA vaccinees. J Exp Med. 220(2):e20221786 (2023).
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Malaria Immunology

Cevayir Coban, MD

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Research Assistant	3
Visiting Scientist	2

Our lab focuses on the elucidation of host-pathogen interactions. We mainly work on malaria disease but cover various infectious organisms such as Leishmania parasites, and respiratory viruses, to be able to understand their way of causing pathology and eventually create successful vaccines against them. Our recent topics include how to bolster B cell memory responses against pathogens.

Elucidation of malaria-mediated pathologies:

Malaria is caused by *Plasmodium* parasites which often lead to severe complications such as cerebral malaria and death. According to a recent WHO report (*WHO Malaria Report, 2022*), Covid19 pandemic brought many challenges to malaria intervention services including diagnostics and treatment. In 2021, the global malaria cases reached 247 million with 619,000 deaths (50% more child death than the previous year). Why and how malaria infection causes severe outcomes have been intensely researched in our laboratory. We have investigated the spatial distribution of the *Plasmodium berghei* ANKA (*Pb*ANKA) parasites in the whole-brain microvessels by utilizing the new tissue-clearing method CUBIC (Clear, Unobstructed, Brain/Body Imaging Cocktails and Computational analysis) with light-sheet fluorescent microscopy (LSFM). We reported that parasites significantly accumulated in the olfactory bulb (OB) of mice (*Matsuo-Dapaah et al., Int Immunology, 2021*). With ongoing studies, we have further discovered several important genes involved in olfactory pathology with the same experimental cerebral malaria model (*manuscript in preparation*).

Novel adjuvant discovery and development:

Adjuvants are known as must-have vaccine components for the potentiation of vaccine responses. As a member of IMSUT International Vaccine Design Center (https://vdesc.ims.u-tokyo.ac.jp/en/), we have been involved in the screening of herbal medicine extracts as safe and ready-to-use adjuvants for current human vaccines (*Hioki et al., Frontiers Immun., 2022*). We have been systematically screening innate and adaptive immune signaling molecules taking part in the mode of action (MOA) of adjuvants and vaccines. One of the recent findings involves understanding how the combination of TLR9 and STING agonists synergistically induce innate and adaptive responses to become an advantageous type 1 adjuvant while suppressing type 2 immunity which leads to the generation of robust anti-tumor responses (*Temizoz et al., Int. Immunol., 2022*).

Our recent projects focus on the investigation of B cell development and pathways involved in the germinal center (GC) formation for the generation of potent antibody responses. We found that TANK-binding kinase-1 (TBK1), the famous innate immune signaling kinase for controlling anti-viral immune responses and nucleic-acid mediated type-I interferon responses, is very important for the generation of GC which confers sterile immunity to reinfections. In the absence of TBK1 in B cells, B cells failed to form GC despite normal T-follicular helper (Tfh) cell differentiation. Notably, memory B cells generated from TBK1-deficient B cells fail to confer sterile immunity upon malaria re-infection, suggesting that TBK1 determines B cell fate to promote long-lasting humoral immunity (*Lee et al., J Exp. Medicine, 2022*). Figure 1 depicts the current mechanistic understanding of TBK1-related signaling events involved in B differentiation. Importantly, these findings were not only specific to malaria infection but rather general, and even during vaccination. We currently follow these studies to understand the role of TBK1 in B cells in a tissue-specific manner.

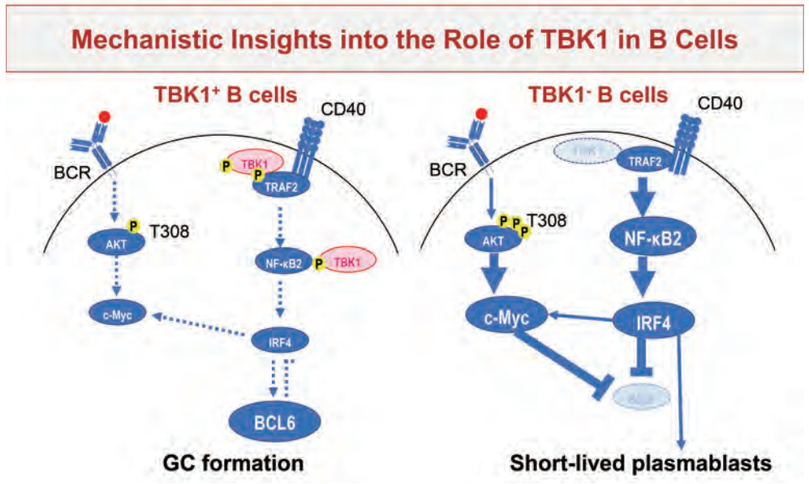

Figure.
Mechanistic insights into the role of TBK1 in B cells (*Lee et al., J Exp. Medicine, 2022*).
TBK1 drives the fate decision of B cell differentiation into either germinal center (GC) B cell or short-live plasmablast through the regulation of CD40 and BCR signaling. TBK1 phosphorylation increases as naïve B cell differentiates into GC B cell. TBK1 is involved in CD40-induced TRAF2 phosphorylation in B cell and negatively regulates downstream non-canonical NFκB signaling to limit IRF4 expression. TBK1 also negatively regulates the downstream of BCR activation-induced AKT phosphorylation at T308 site to suppress c-Myc expression. In the absence of TBK1, high c-Myc and IRF4 expressions driven by activated BCR and CD40 signalings drive plasmablast differentiation, while on the other hand suppress BCL6, which is the master regulator of GC B cell differentiation.

Recent Publications

1. Hioki K, Hayashi T, Natsume-Kitatani Y, Kobiyama K, Temizoz B, Negishi H, Kawakami H, Fuchino H, Kuroda E, Coban C, Kawahara N, Ishii KJ. Machine learning-assisted screening of herbal medicine extracts as vaccine adjuvants. Front Immunol. 13:847616 (2022). doi: 10.3389/fimmu.2022.847616.

2. Temizoz B, Hioki K, Kobari S, Jounai N, Kusakabe T, Lee MSJ, Coban C, Kuroda E, Ishii KJ. Anti-tumor immunity by the transcriptional synergy between TLR9 and STING activation. Int Immunol. 34(7):353-364 (2022). doi: 10.1093/intimm/dxac012.

3. Lee MSJ, Inoue T, Ise W, Matsuo-Dapaah J, Wing JB, Temizoz B, Kobiyama K, Hayashi T, Patil A, Sakaguchi S, Simon AK, Bezbradica JS, Nagatoishi S, Tsumoto K, Inoue JI, Akira S, Kurosaki T, Ishii KJ, Coban C. B cell intrinsic TBK1 is essential for germinal center formation during infection and vaccination in mice. J Exp Med. 219(2):e20211336 (2022). doi: 10.1084/jem.20211336.

4. Matsuo-Dapaah J, Lee MSJ, Ishii KJ, Tainaka K, Coban C. Using a new three-dimensional CUBIC tissue-clearing method to examine the brain during experimental cerebral malaria. Int Immunol. dxab060 (2021). doi: 10.1093/intimm/dxab060.

5. Coban C. The host targeting effect of chloroquine in malaria. Curr Opin Immunol. 66:98-107 (2020). doi: 10.1016/j.coi.2020.07.005.

Vaccine Science

Ken J. Ishii, MD/PhD

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Assistant Professor	Burcu Temizoz
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Primary goal of our laboratory is to understand the immunological mechanisms of the intra- and inter-cellular signaling pathways that mediate the immunogenicity of successful vaccines, as well as biological responses to adjuvants. Such knowledge will enable us to develop novel concepts, modalities and next generation immuno-preventive and/or therapeutic agents against infectious diseases, cancer and allergy as well as other non-communicable diseases.

1. Making innate sense of mRNA vaccine adjuvanticity.

Successful vaccines contain two essential immunological components: a protective antigen and an adjuvant. Adjuvants are essential for optimal antigen-specific immune responses, the so-called 'immunogenicity', but are often a cause of reactogenicity (even toxicity) that results in local and systemic inflammation. Therefore, to ensure vaccine efficacy and safety, it is critical to understand the molecular and cellular mechanism(s) by which adjuvants provoke the immune system. By introducing papers, we describe that there seems to be more room to improve the immunogenicity and reduce the reactogenicity of LNP-mRNA vaccine formulations by further study of immunization methods (including delivery systems and devices) and their built-in adjuvanticity.

2. Anti-tumor immunity by transcriptional synergy between TLR9 and STING activation

Agonists for TLR9 and stimulator of IFN genes (STING) offer therapeutic applications as both anti-tumor agents and vaccine adjuvants, though their clinical applications are limited; the clinically available TLR9 agonist is a weak IFN inducer and STING agonists induce undesired type 2 immunity. Yet, combining TLR9 and STING agonists overcame these limitations by synergistically inducing innate and adaptive IFNγ to become an advantageous type 1 adjuvant, suppressing type 2 immunity, in addition to exerting robust anti-tumor activities when used as a monotherapeutic agent for cancer immunotherapy. Here, we sought to decipher the immunological mechanisms behind the synergism mediated by TLR9 and STING agonists and found that their potent anti-tumor immunity in a Pan02 peritoneal dissemination model of pancreatic cancer was achieved only when agonists for TLR9 and STING were administered locally, and was via mechanisms involving CD4 and CD8 T cells as well as the co-operative action of IL-12 and type I IFNs. Rechallenge studies of long-term cancer survivors suggested that the elicitation of Pan02-specific memory responses provides protection against the secondary tumor challenge. Mechanistically, we found that TLR9 and STING agonists synergistically induce IL-12 and type I IFN production in murine APCs. The synergistic effect of the TLR9 and STING agonists on IL-12p40 was at protein, mRNA and promoter activation levels, and transcriptional regulation was mediated by a 200 bp region situated 983 bp upstream of the IL-12p40 transcription initiation site. 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.

3. Machine Learning-Assisted Screening of Herbal Medicine Extracts as Vaccine Adjuvants

Adjuvants are important vaccine components, composed of a variety of chemical and biological materials that enhance the vaccine antigen-specific immune responses by stimulating the innate immune cells in both direct and indirect manners to produce a variety cytokines, chemokines, and growth factors. It has been developed by empirical methods for decades and considered difficult to choose a single screening method for an ideal vaccine adjuvant, due to their diverse biochemical characteristics, complex mechanisms of, and species specificity for their adjuvanticity. We therefore established a robust adjuvant screening strategy by combining multiparametric analysis of adjuvanticity in vivo and immunological profiles in vitro (such as cytokines, chemokines, and growth factor secretion) of various library compounds derived from hot-water extracts of herbal medicines, together with their diverse distribution of nano-sized physical particle properties with a machine learning algorithm. By combining multiparametric analysis with a machine learning algorithm such as rCCA, sparse-PLS, and DIABLO, we identified that human G-CSF and mouse RANTES, produced upon adjuvant stimulation in vitro, are the most robust biological parameters that can predict the adjuvanticity of various library compounds. Notably, we revealed a certain nano-sized particle population that functioned as an independent negative parameter to adjuvanticity. Finally, we proved that the two-step strategy pairing the negative and positive parameters significantly improved the efficacy of screening and a screening strategy applying principal component analysis using the identified parameters. These novel parameters we identified for adjuvant screening by machine learning with multiple biological and physical parameters may provide new insights into the future development of effective and safe adjuvants for human use.

4. A-910823, a squalene-based emulsion adjuvant, induces T follicular helper cells and humoral immune responses via α-tocopherol component

A-910823 is a squalene-based emulsion adjuvant used for S-268019-b, a novel vaccine against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that is currently in clinical development. Published evidence has demonstrated that A-910823 can enhance the induction of neutralizing antibodies against SARS-CoV-2 in humans and animal models. However, the characteristics and mechanisms of the immune responses induced by A-910823 are not yet known. Here, we found that the novel adjuvant A-910823 is capable of robust Tfh cell and humoral immune response induction, even when used as a booster dose, and we also emphasized that the adjuvant activity of A-910823 is driven by α-tocopherol.

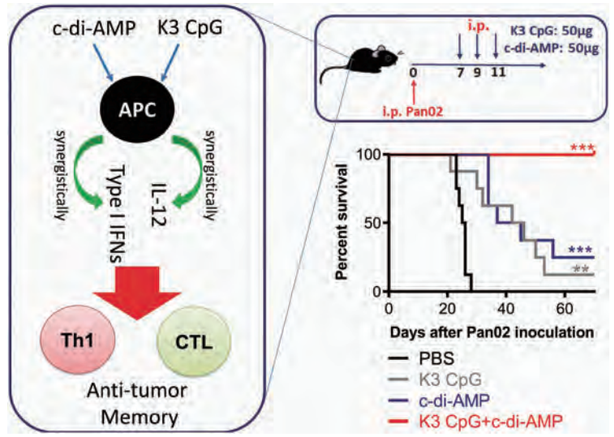

Figure.
How TLR9 and STING agonists synergize to protect against cancer
Agonists of TLR9 and STING can induce anti-tumor responses and, in combination, they appear to be much more effective than individually. To delineate the mechanisms, we use the Pan02 peritoneal dissemination model of pancreatic cancer. Local (i.p.) K3 CpG (TLR9 agonist) or c-di-AMP (STING agonist) generate anti-tumor responses; the combination is even more effective, prolonging host survival compared with single treatments or systemic (i.v.) inoculation of the combination. This antigen-free therapy allows 100% of mice to survive and even protects them from rechallenge with this aggressive tumor. Levels of IL-12 and type I interferons (IFNs) are increased by the combination, which is required for induction of potent anti-tumor $CD4^+$ and $CD8^+$ T cell responses along with robust NK cell activity. IL-12 is crucial and synergistically coordinates with type I IFNs to regulate the anti-tumor effect of the combination. The synergism operates within individual antigen-presenting cells and is regulated at the level of promotor activation, transcription and translation of IL-12.

Recent Publications

1. Yoshioka Y, Kobiyama K, Hayashi T, et al. A-910823, a squalene-based emulsion adjuvant, induces T follicular helper cells and humoral immune responses via α-tocopherol component. Front Immunol. 14:1116238 (2023). doi: 10.3389/fimmu.2023.1116238.
2. Temizoz B, Hioki K, Kobari S, et al. Anti-tumor immunity by transcriptional synergy between TLR9 and STING activation. Int Immunol. 34(7):353-364 (2022). doi: 10.1093/intimm/dxac012.
3. Hioki K, Hayashi T, Natsume-Kitatani Y, et al. Machine Learning-Assisted Screening of Herbal Medicine Extracts as Vaccine Adjuvants. Front Immunol. 13:847616 (2022). doi: 10.3389/fimmu.2022.847616.
4. Koiyama K, Ishii KJ. Making innate sense of mRNA vaccine adjuvanticity. Nat Immunol. Apr;23(4):474-476 (2022). doi: 10.1038/s41590-022-01168-4.

Immunoparasitology

Masahiro Yamamoto, PhD

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Toxoplasma gondii is an obligatory protozoan parasite that can infect cells with nuclei from virtually all warm-blooded animals. Although it is estimated that one-third of the world's population is infected with *T. gondii*, most infections are asymptomatic. Infection with *T. gondii*, however, can lead to life-threatening toxoplasmosis in immunocompromised humans and animals. Moreover, *T. gondii* is ranked among the top five human pathogens that cause economic loss and life impairment via foodborne illness in the United States. Thus, *T. gondii* is an important human and animal pathogen.

T. gondii forms parasitophorous vacuoles (PVs) in infected cells, which are membranous structures originating from host cell plasma membranes and are generated during invasion by the parasite. *T. gondii* can proliferate only inside the PV membrane (PVM), which is an interface between the host and the parasite. During infection, *T. gondii* secretes various molecules from secretory organelles such as rhoptry and dense granules into the host cell cytoplasm and nucleus, onto the PVM, and within the PV space. An important function of rhoptry proteins (ROPs) and dense granule proteins (GRAs) is to downregulate the host immunity that is dependent on interferon gamma (IFN-γ). IFN-γ robustly stimulates expression of hundreds of genes encoding a variety of proteins related to anti-*T. gondii* cell-autonomous immunity. IFN-γ-stimulated nitric oxide production and tryptophan degradation are important for suppression of *T. gondii* growth. IFN-inducible GTPases such as p47 immunity-related GTPases (IRGs) and p65 guanylate-binding proteins (GBPs) are required for disruption of PVM of avirulent type II *T. gondii* to kill the pathogen in a cell-autonomous fashion. IRGs and GBPs are coordinately accumulated on the *T. gondii* PVM. Among them, Irgb6 has been shown to act as a pioneer that detects *T. gondii* PVM, subsequently leading to recruitment of other IRGs such as Irga6 and Irgb10, GBPs, and effectors, including p62/Sqstm1 and ubiquitin. Irgb6-deficient mice are susceptible to type II *T. gondii* to the same extent as IFN-γ-deficient mice. Thus, Irgb6 plays a fundamental role in IFN-γ-dependent anti-*T. gondii* cell-autonomous host defense.

Targeting Irgb6 is one of the most effective virulence mechanisms of virulent type I *T. gondii*. Rhoptry kinases such as ROP17 and ROP18, and a pseudokinase ROP5 are secreted into the host cell cytoplasm and localized at the PVM to directly phosphorylate and inactivate IFN-inducible GTPases to inhibit their accumulation on the *T. gondii* PVM. Loss of ROP18 from a virulent type I *T. gondii* strain results in decreased virulence in mice. Thus, targeting host Irgb6 via ROP18 is an important counter-defense system for virulent *T. gondii* in IFN-γ-activated cells. However, little is known about the mechanism of how ROP18 is prepared at the transcriptional, translational, and post-translational levels inside type I *T. gondii* itself.

A genome-wide loss-of-function screen in a type I *T. gondii* strain identified >350 genes that determine fitness in IFN-γ-activated murine macrophages. Some of the highly ranked genes, such as GRA45, TGGT1_263560 and TGGT1_269950 (encoding putative GRAs), were characterized to determine the fitness-related mechanisms. However, most of the remaining genes remain uncharacterized. Here, to explore mechanisms by which *T. gondii*-derived molecules target host Irgb6-dependent cell-autonomous immunity, we tested the involvement of several type I *T. gondii* proteins encoded by uncharacterized genes in suppression of Irgb6-depedent host defense. We found that a putative transcription factor called IWS1 is important for ROP18-mediated virulence of type I *T. gondii*. Loss of IWS1 from type I parasites led to markedly increased accumulation of Irgb6 as well as other IFN-inducible GTPases at the PVM, and severely decreased the virulence of the parasite in mice. Global gene profile analysis revealed that IWS1 deficiency resulted in downregulation of various genes, including ROP18. Moreover, ROP18 overexpression in IWS1-deficient *T. gondii* reduced recruitment of IFN-inducible GTPases to the PVM and resulted in restoration of *in vivo* virulence in wild-type mice. Taken together, this study demonstrates that the *T. gondii* transcription factor IWS1 is important for ROP18 mRNA expression in the virulent parasite to subvert host IFN-inducible GTPase-mediated cell-autonomous immunity; this determines the fitness of the parasite in IFN-γ-activated cells and mice.

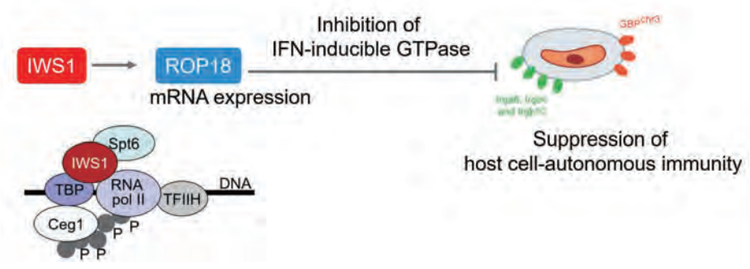


Figure.
Transcriptional regulation of a key Toxoplasma virulence gene by IWS1.

Recent Publications

1. Hashizaki E, Sasai M, Okuzaki D, Nishi T, Kobayashi T, Iwanaga S, Yamamoto M. Toxoplasma IWS1 Determines Fitness in Interferon-γ-Activated Host Cells and Mice by Indirectly Regulating ROP18 mRNA Expression. mBio. 14(1):e0325622 (2023). doi: 10.1128/mbio.03256-22.

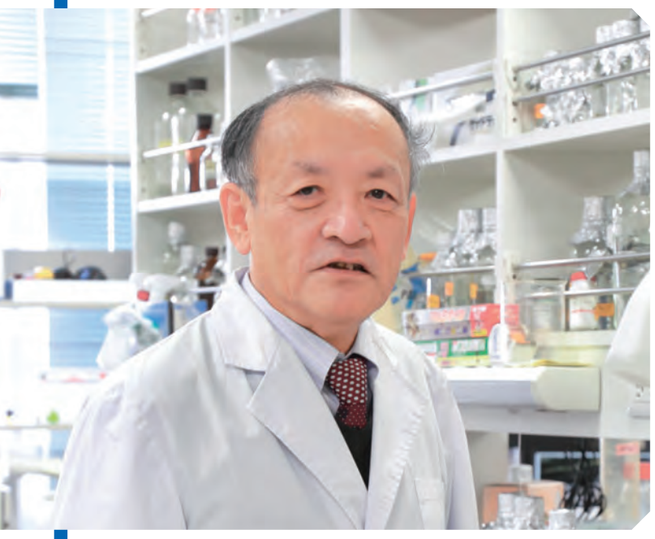
2. Sasai M, Ma JS, Okamoto M, Nishino K, Nagaoka H, Takashima E, Pradipta A, Lee Y, Kosako H, Suh PG, Yamamoto M. Uncovering a novel role of PLCβ4 in selectively mediating TCR signaling in CD8+ but not CD4+ T cells. J Exp Med. 218:e20201763 (2021). doi: 10.1084/jem.20201763. PMID: 33970189.

3. Pradipta A, Sasai M, Motani K, Ma JS, Lee Y, Kosako H, Yamamoto M. Cell-autonomous Toxoplasma killing program requires Irgm2 but not its microbe vacuolar localization. Life Sci Alliance. 4:e202000960 (2021). doi: 10.26508/lsa.202000960.

4. Sakaguchi N, Sasai M, Bando H, Lee Y, Pradipta A, Ma JS, Yamamoto M. Role of Gate-16 and Gabarap in Prevention of Caspase-11-Dependent Excess Inflammation and Lethal Endotoxic Shock. Front Immunol. 11:561948 (2020). doi: 10.3389/fimmu.2020.561948.

5. Bando H, Pradipta A, Iwanaga S, Okamoto T, Okuzaki D, Tanaka S, Vega-Rodríguez J, Lee Y, Ma JS, Sakaguchi N, Soga A, Fukumoto S, Sasai M, Matsuura Y, Yuda M, Jacobs-Lorena M, Yamamoto M. CXCR4 regulates Plasmodium development in mouse and human hepatocytes. J Exp Med. 216:1733-1748 (2019). doi: 10.1084/jem.20182227.

Biochemistry & Immunology



Shigekazu Nagata, PhD

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Assistant Professor	Takaharu Sakuragi
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Phospholipids are asymmetrically distributed between the inner and outer leaflets of plasma membranes with exclusive phosphatidylserine (PtdSer) localization in the inner leaflet. This asymmetrical distribution of phospholipids is maintained by ATP-dependent flippases, which translocate PtdSer from outer to inner leaflets. When cells undergo apoptosis, the asymmetrical distribution of phospholipids is disrupted by a scramblase(s) that non-specifically scrambles phospholipids between the two leaflets, leading to PtdSer-exposure.

We previously identified two P4-type ATPases (ATP11A and 11C) and their subunit CDC50A as flippases at plasma membranes (Segawa et al. Science 2014) and TMEM16F as Ca^{2+}-dependent scramblases (Suzuki et al. Nature 2010). The *TMEM16F* gene is mutated in human Scott syndrome, a congenital bleeding disorder, indicating that TMEM16F is responsible for exposing PtdSer in the platelets to activate blood clotting factors (Suzuki et al. Nature 2010). We also found that XKR8 forms a binary complex with Basigin or Neuroplastin, an Ig-super family protein and works as a caspase-dependent scramblase in apoptotic cells in various cells (Suzuki et al. Science 2013; PNAS 2016). The PtdSer, thus on the dead cell's surface, serves as an "eat me" signal. The null mutation of Xkr8, thus, causes inefficient engulfment of apoptotic cells leading to the activation of autoimmunity and male infertility (Kohno et al. PNAS 2019; Yamashita et al. MCB 2019), as found in the lack of the PtdSer-recognition system (Hanayama et al. Science 2004).

A high concentration of ATP (~ 4 mM) is present in the cells, while its extracellular concentration is low (less than 30 nM). However, it increases by several hundred μM in the inflamed tissues or tumour 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 necrotic or pyroptosis and releasing inflammatory cytokines. During CRISPR/Cas9 screening for the molecules involved in ATP-induced PtdSer-exposure in mouse T cells, we found that XK, a paralogue of XKR9, 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.

Cell fusion occurs in various biological systems, such as myoblasts to myotubes, and trophoblasts into syncytiotrophoblasts. Before the fusion, PtdSer is exposed on the cell surface, and blocking PtdSer inhibits cell fusion, indicating that the PtdSer exposed on the cell surface is essential for cell fusion. The ATP11A and ATP11C flippases in the plasma membrane are present ubiquitously in most types of cells except for the placenta, which expresses only ATP11A. Accordingly, the *Atp11a*-null mice are embryonic lethal, while the epiblast-specific deletion of the *Atp11a* gene did not affect the development, confirming that ATP11A has a non-redundant essential role in the placenta. Syncytiotrophoblasts were not well developed in the *Atp11a*-null placenta probably due to the constitutive PtdSer-exposure in trophoblasts (Figure 2). These results indicated that the PtdSer exposed at the pre-fusion state must be withdrawn during the fusion process.

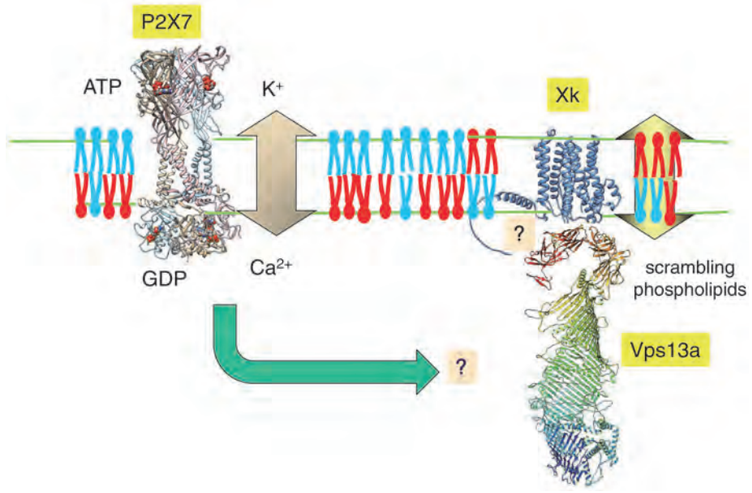

Figure 1.
Association of XK with VPS13A at the plasma membrane to scramble phospholipids.
VPS13A, a giant cytoplasmic protein (human VPS13A: 3174 aa), is associated with XK in the plasma membrane. P2X7 is a receptor for extracellular ATP and serves as a cation transporter. The binding of ATP to P2X7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

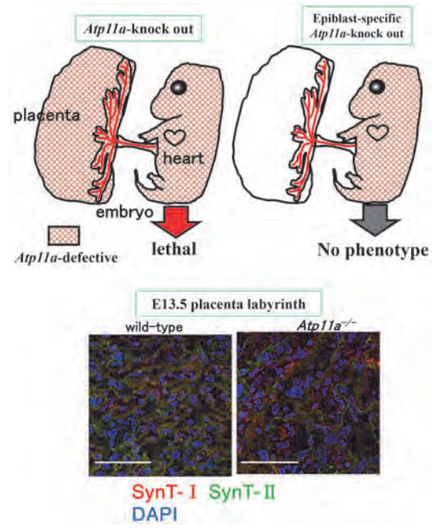

Figure 2.
Embryonic lethality of *Atp11a*$^{-/-}$ mice due to poor placenta development. Top panels: *Atp11a*$^{-/-}$ mouse embryos died at E14.5. However, epiblast-specific *Atp11a* deletion did not affect mouse development, suggesting the inability of *Atp11a*$^{-/-}$ placentas to support the embryos. Bottom panel: Two syncytiotrophoblasts (SynT-I and SynT-II) tightly adhere and separate the maternal and fetal blood compartments in the wild-type placenta labyrinth layer. In the *Atp11a*$^{-/-}$ placenta, the two syncytiotrophoblasts were not well developed, and the labyrinth was sparse.

Recent Publications

1. Sakuragi T. and Nagata S. Regulation of phospholipid distribution in the lipid bilayer by flippases and scramblases. Nat Rev Mol Cell Biol. (2023). https://doi.org/10.1038/s41580-023-00604-z.

2. Ochiai Y, Suzuki C, Segawa K, Uchiyama Y, and Nagata S. Inefficient development of syncytiotrophoblasts in the Atp11a-deficient mouse placenta. Proc Nat Acad Sci USA. 119, e2200582119 (2022).

3. Ryoden Y, Segawa K, and Nagata S. Requirement of Xk and Vps13a for the P2X7-mediated phospholipid scrambling and cell lysis in mouse T cells. Proc Nat Acad Sci USA 119: e2119286119 (2022).

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Molecular Neuroscience

Toshihide Yamashita MD/PhD

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	Oluwaseun Fatoba
	Hiroshi Tsujioka
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	Yosuke Ohtake
	Toru Nakanishi
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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.

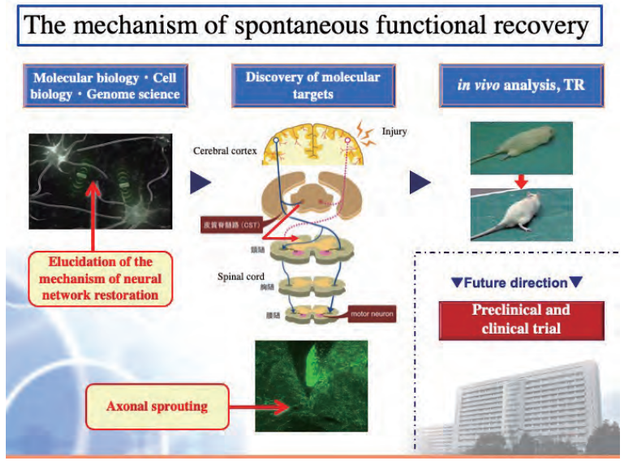

Figure 1.
The mechanism of spontaneous functional recovery.

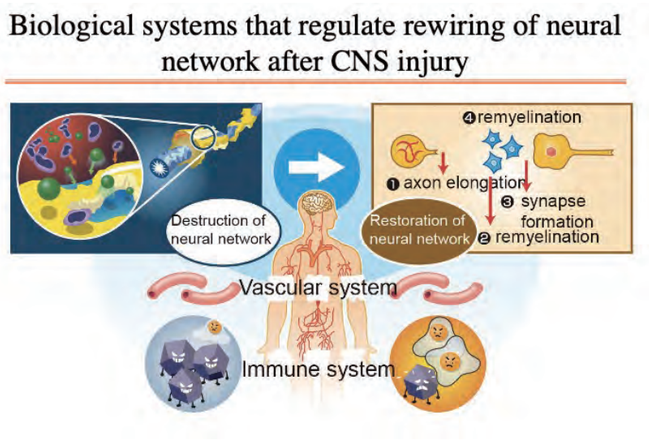

Figure 2.
Biological systems that regulate rewiring of neural network after CNS injury.

Recent Publications

1. Iwamoto S, Itokazu T, Sasaki A, et al. RGMa signal in macrophages induces neutrophil-related astrocytopathy in NMO. An Neurol. 91:532-547 (2022).
2. Ito M, Muramatsu R, Kato Y, Sharma B, Uyeda A, Tanabe S, Fujimura H, Kidoya H, Takakura N, Kawahara Y, Takao M, Mochizuki H, Fukamizu A & Yamashita T. Age-dependent decline in myelination capacity is mediated by apelin-APJ signaling. Nat Aging 1:284-294 (2021).
3. Fujita Y, Nakanishi T, Ueno M, Itohara S & Yamashita T. Netrin-G1 regulates microglial accumulation along axons and supports the survival of layer V neurons in the postnatal mouse brain. Cell Rep. 10:107580 (2020).
4. Tanabe S & Yamashita T. B-1a lymphocytes promote oligodendrogenesis during brain development. Nat Neurosci. 21:506-516 (2018).
5. Kuroda M, Muramatsu R, Maedera N, Koyama Y, Hamaguchi M, Fujishima H, Yoshida M, Konishi M, Itoh N, Mochizuki H & Yamashita T. Promotion of central nervous system remyelination by peripheral FGF21. J Clin Invest. 127:3496-3509 (2017).
6. Fujita Y, Masuda K, Nakato R, Katou Y, Tanaka T, Nakayama M, Takao K, Miyakawa T, Shirahige K & Yamashita T. Cohesin regulates formation of neuronal networks in the brain. J Exp Med. 214:1431-1452 (2017).

Molecular Immunology

Sho Yamasaki, PhD

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Research Assistant	5
Visiting Scientist	6
Support Staff	5

Timing matters for dendritic cell signaling

The common Fc receptor γ (FcRγ) chain is a signaling subunit common to several immune receptors, but cellular responses induced by FcRγ-coupled receptors are diverse. We investigated the mechanisms by which FcRγ generates divergent signals when coupled to Dectin-2 and Mincle, structurally similar C-type lectin receptors that induce the release of different cytokines from dendritic cells. Chronological tracing of transcriptomic and epi- genetic changes upon stimulation revealed that Dectin-2 induced early and strong signaling, whereas Mincle- mediated signaling was delayed, which reflects their expression patterns. Generation of early and strong FcRγ-Syk signaling by engineered chimeric receptors was sufficient to recapitulate a Dectin-2–like gene expression profile. Early Syk signaling selectively stimulated the activity of the calcium ion–activated transcription factor NFAT, which rapidly altered the chromatin status and transcription of the Il2 gene. In contrast, proinflammatory cytokines, such as TNF, were induced regardless of FcRγ signaling kinetics. These results suggest that the strength and timing of FcRγ-Syk signaling can alter the quality of cellular responses through kinetics-sensing signaling machineries.

β-GlcCer-induced phagoptosis of neurons by microglia causes Gaucher disease

Gaucher disease (GD) is the most common lysosomal storage disease caused by recessive mutations in the degrading enzyme of β-glucosylceramide (β-GlcCer). However, it remains unclear how β-GlcCer causes severe neuronopathic symptoms, which are not fully treated by current therapies. We herein found that β-GlcCer accumulating in GD activated microglia through macrophage-inducible C-type lectin (Mincle) to induce phagocytosis of living neurons, which exacerbated Gaucher symptoms. This process was augmented by tumor necrosis factor (TNF) secreted from activated microglia that sensitized neurons for phagocytosis. This characteristic pathology was also observed in human neuronopathic GD. Blockade of these pathways in mice with a combination of FDA-approved drugs, minocycline (microglia activation inhibitor) and etanercept (TNF blocker), effectively protected neurons and ameliorated neuronopathic symptoms. In this study, we propose that limiting unrestrained microglia activation using drug repurposing provides a quickly applicable therapeutic option for fatal neuronopathic GD.

MR1-reactive T cells, as pathogenic T cells in autoimmune diseases

MHC class I-related protein 1 (MR1) is a metabolite-presenting molecule that restricts MR1-reactive T cells including mucosal-associated invariant T (MAIT) cells. In contrast to MAIT cells, the function of other MR1-restricted T cell subsets is largely unknown. Here, we report that mice in which a T cell-specific transcription factor, B-cell lymphoma/leukemia 11B (Bcl11b), was ablated in immature thymocytes ($Bcl11b^{\Delta iThy}$ mice) develop chronic inflammation. $Bcl11b^{\Delta iThy}$ mice lack conventional T cells and MAIT cells, whereas $CD4^{+}IL\text{-}18R^{+}$ αβ T cells expressing skewed Traj33 $(J\alpha33)^{+}$ T cell receptors (TCR) accumulate in the periphery, which are necessary and sufficient for the pathogenesis. The disorders observed in $Bcl11b^{\Delta iThy}$ mice are ameliorated by MR1-deficiency, transfer of conventional T cells, or germ-free conditions. We further show the crystal structure of the TCR expressed by $Traj33^{+}$ T cells expanded in $Bcl11b^{\Delta iThy}$ mice. Overall, we establish that MR1-reactive T cells have pathogenic potential.

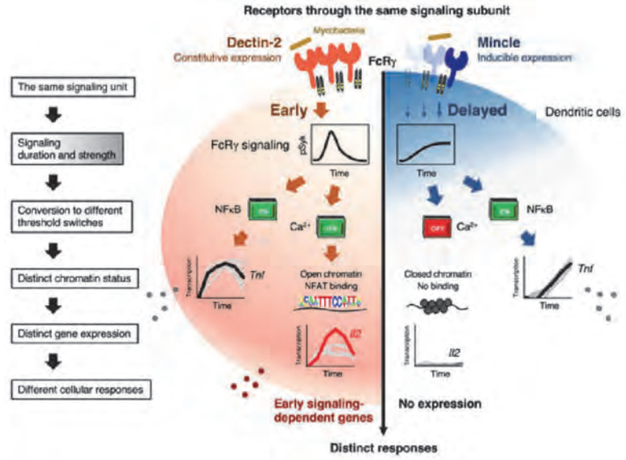


Figure 1.
A model for generating distinct dendritic responses through FcRγ signaling.

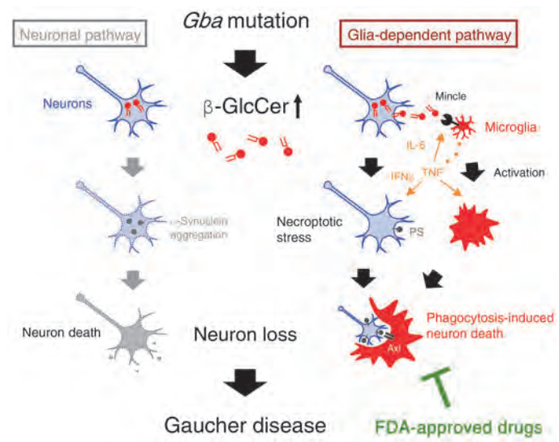
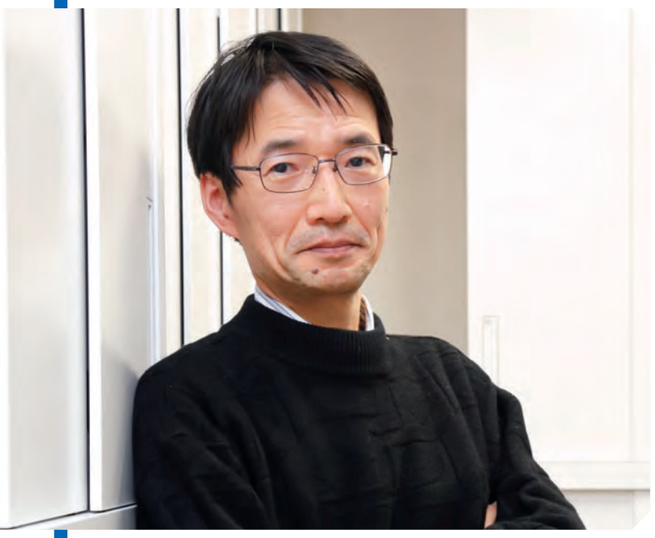


Figure 2.
Novel molecular mechanisms and therapeutic options for Gaucher disease.

Recent Publications

1. Watanabe M, Motooka D, Yamasaki S. The kinetics of signaling through the common Fc receptor γ (FcRγ) chain determine cytokine profiles in dendritic cells . Sci Signal. 16:eabn9909 (2023).
2. Shimizu T, Schutt CR, Izumi Y, Tomiyasu N, Omahdi Z, Kano K, Takamatsu H, Aoki J, Bamba T, Kumanogoh A, Takao M, Yamasaki S. Direct activation of microglia by β-glucosylceramide causes phagocytosis of neurons that exacerbates Gaucher disease. Immunity 56:307-319.e8 (2023).
3. Shibata K, Motozono C, Nagae M, Shimizu T, Ishikawa E, Motooka D, Okuzaki D, et al. Symbiotic bacteria-dependent expansion of MR1-reactive T cells causes autoimmunity in the absence of Bcl11b. Nat Commun. 13:6948 (2022).
4. Lu X, Hosono Y, Nagae M, Ishizuka S, Ishikawa E, Motooka D, et al. Identification of conserved SARS-CoV-2 spike epitopes that expand public cTfh clonotypes in mild COVID-19 patients. J Exp Med. 218:e20211327 (2021).
5. Nagata M, Toyonaga K, Ishikawa E, Haji S, et al. Helicobacter pylori metabolites exacerbate gastritis through C-type lectin receptors. J Exp Med. 218:e20200815 (2021).

Stem Cell Biology and Developmental Immunology



Takashi Nagasawa, MD/PhD

Professor	Takashi Nagasawa
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Support Staff	1

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 cavities. 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 colonization of bone marrow by HSCs during embryogenesis (Nagasawa et al. *Nature* 1996; Ara et al. *Immunity* 2003), maintenance of a pool of HSCs (Sugiyama et al. *Immunity* 2006), and development of immune cells, including B cells, plasmacytoid dendritic cells (pDCs) and NK cells in bone marrow 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 reticular cells expressing CXCL12 at high levels, termed CXCL12-abundant reticular (CAR) cells, within murine bone marrow (Tokoyoda et al. *Immunity* 2004; Sugiyama et al. *Immunity* 2006) and found that CAR cells are the major producer of CXCL12 and SCF (Omatsu at 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). Furthermore, we showed that numerous HSC niches remain empty and that all CAR cells create facultative niches for HSCs inconsistent with the classical concept (Shimoto et al. *Blood* 2017).

In addition, we showed that CAR cells are mesenchymal stem cells, which give rise to adipocytes and osteoblasts, and that transcription factors, Foxc1 and Ebf3 are preferentially expressed in CAR cells and play a critical role in the formation and maintenance of niches for HSCs and immune cells, inhibiting differentiation of CAR cells into adipocytes and osteoblasts, respectively (Omatsu et al. *Nature* 2014; Seike et al. *Genes Dev*. 2018). These studies clarified the nature and functions of CAR cells in murine bone marrow.

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

The results that Runx2, which is essential for generation of osteoblasts, is highly expressed in CAR cells prompted us to examine its role in the development and/or maintenance of CAR cells. We showed that Runx1, which is known to be essential for the establishment of definitive hematopoiesis in hemogenic endothelial cells during ontogeny, is predominantly expressed in CAR cells in adult bone marrow. Although CAR cells and HSC niches are normally formed and maintained in mice lacking Runx1 or Runx2 in CAR cells, mice lacking both Runx1 and Runx2 in CAR cells (tamoxifen-treated Ebf3-CreERT2;Runx1$^{f/f}$Runx2$^{f/f}$ mice) displayed an increase in fibrosis and bone formation with markedly reduced HSCs, hematopoietic progenitor cells, and immune cells in the bone marrow. Consistent with this, CAR cells from the mutants displayed markedly increased expression of fibrotic genes, including Col1a1, Col3a1, and Col6a3. *In vitro*, Runx1 was induced by Foxc1, and enforced expression of Runx1 decreased fibrotic gene expression in cultured CAR cells. Thus, CAR cells require Runx1 or Runx2 to prevent their fibrotic conversion and maintain HSCs and hematopoiesis in adults (Omatsu et al., *Nat Commun* 2022). Clinically, expression of Runx1 and Runx2 was reduced in CAR cells in a mouse model of marrow fibrosis, which affects up to 20% of myeloproliferative neoplasms (MPN) patients and is associated with a poor prognosis. These results strengthen the claim that CAR cells are the bone marrow specific fibroblasts, which express specific and critical transcription factors, including Foxc1, Ebf 1/3, and Runx1/2, providing HSC niches and bone (Omatsu et al., *Nat Commun* 2022).

Recently, Buechler et al. performed a meta-analysis of single-cell RNA sequencing (scRNA-seq) datasets from fibroblasts across 16 tissues and hypothesized that two clusters of fibroblasts they designated as universal fibroblasts present in all organs might give rise to distinct tissue-specific fibroblast subsets they designated as specialized fibroblasts, including CAR cells, splenic red pulp reticular fibroblasts, and Foxl1^{+} telocytes, which have been shown to create niches for intestinal stem cells (Buechler et al., *Nature* 593; 575, 2021). However, there is no direct evidence that universal fibroblasts have potential to give rise to specialized fibroblasts. Thus, we are trying to determine whether populations of universal fibroblasts have potential to give rise to CAR cells and clarify molecular mechanisms that regulate the development, maturation, and functions of CAR cells during development and homeostasis and in diseases.

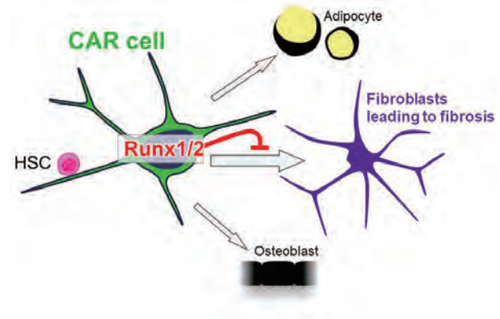

Figure 1.
The functions of Runx1/2 in CAR cell maintenance.
CAR cells require Runx1 or Runx2 to prevent their fibrotic conversion and maintain HSCs and lympho-hematopoiesis in adult bone marrow.

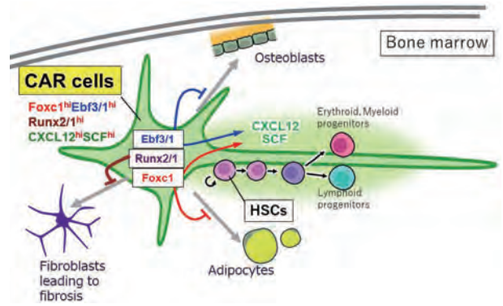

Figure 2.
The development and functions of CAR cells.
CAR cells are the major cellular component of non-hematopoietic cells in bone marrow characterized by several salient features in both mouse and human. The transcription factors, Foxc1, Ebf1/Ebf3, and Runx1/2 and cytokines, CXCL12 and SCF are preferentially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

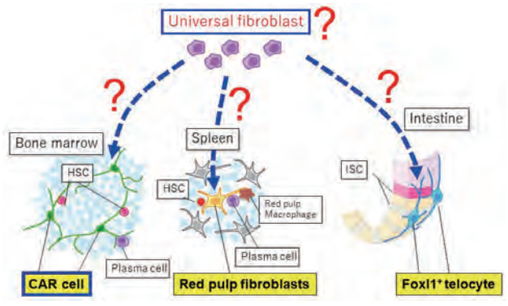

Figure 3.
Presumptive common fibroblast progenitors for CAR cells.
Universal fibroblasts might have potential to give rise to specialized fibroblasts creating niches for tissue stem cells, including CAR cells, splenic red pulp reticular fibroblasts, and Foxl1^{+} telocytes. ISC: intestinal stem cells.

Recent Publications

1. Omatsu Y, Aiba S, Maeta T, Higaki K, Aoki K, Watanabe H, Kondoh G, Nishimura R, Takeda S, Chung UI, Nagasawa T. Runx1 and Runx2 inhibit fibrotic conversion of cellular niches for hematopoietic stem cells. Nat Commun. 13:2654 (2022).
2. Seike M, Omatsu Y, Watanabe H, Kondoh G, Nagasawa T. Stem cell niche-specific Ebf3 maintains the bone marrow cavity. Genes Dev. 32:359-372 (2018).
3. Omatsu Y, Seike M, Sugiyama T, Kume T, Nagasawa T. Foxc1 is a critical regulator of hematopoietic stem/progenitor cell niche formation. Nature 508:536-540 (2014).
4. Omatsu Y, Sugiyama T, Kohara H, Kondoh G, Fujii N, Kohno K, Nagasawa T. The essential function of adipo-osteogenic progenitors as the hematopoietic stem and progenitor cell niche. Immunity 33:387-399 (2010).
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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	Tomonori Matsumoto
	Masahiro Wakita
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Visiting Scientist	2
Support Staff	2

Cellular senescence is a state of irreversible cell-cycle arrest induced by a variety of potentially oncogenic stimuli and is therefore thought to serve as an important tumour suppression mechanism. On the other hand, however, senescent cells also cause senescence-associated secretory phenotypes (SASP), which secrete a variety of pro-inflammatory factors (Fig.1). Therefore, the accumulation of senescent cells, which is often seen with aging and obesity, ultimately leads to harmful side effects, and there is currently a worldwide effort to develop drugs that selectively kill senescent cells. However, since senescent cells also play beneficial roles, for example, in promoting wound healing, maintaining the blood-tissue barrier, and activating the immune system, blindly removing senescent cells may be harmful. Therefore, identifying and preventing the causes of cellular senescence in vivo, rather than killing senescent cells, may be a safer and more efficient way to address the harmful side effects of senescent cells. However, to date, the triggers of cellular senescence *in vivo*, are poorly understood. Recently, we have discovered through studies using human clinical samples and mouse model that both butyrate-producing gut bacteria and severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) induce cellular senescence *in vivo*.

In the case of butyrate-producing bacteria, cellular senescence is induced directly in the affected cells via induction of $p16^{INK4a}$ and $p21^{Waf1}$ expression, while in the case of SARS-CoV-2, TNFα secreted from infected cells causes cellular senescence in surrounding uninfected cells. We also found that mice infected with a mouse-adapted strain of SARS-CoV-2 exhibit prolonged signs of cellular senescence and SASP in the lung at 14 days post-infection when the virus was no longer detectable, but this can be greatly reduced by administration of senolytic agents that specifically kill senescent cells. The sustained infection-induced senescence described here may be involved in the long-term inflammation caused by SARS-CoV-2 infection known as long-COVID. These results suggest that senolytic drug may be effective in alleviating long COVID. However, SASP has not only harmful but also beneficial effects, and it has recently been reported that the removal of accumulated senescent cells in mice results in severe liver dysfunction. In this regard, it is interesting to note that hamsters infected with SARS-CoV-2 showed resistance to superinfection with influenza virus A H1N1 (Fig. 2). Thus, it is also tempting to speculate that SARS-CoV-2-induced senescent cells may have some beneficial effects, depending on the biological context. Accordingly, a more rigorous analysis is needed to determine whether senolytic drug can serve as a preventive measure against long COVID. Nevertheless, our findings provide valuable new insights into the pathogenesis of long COVID and suggest new possibilities for its control.

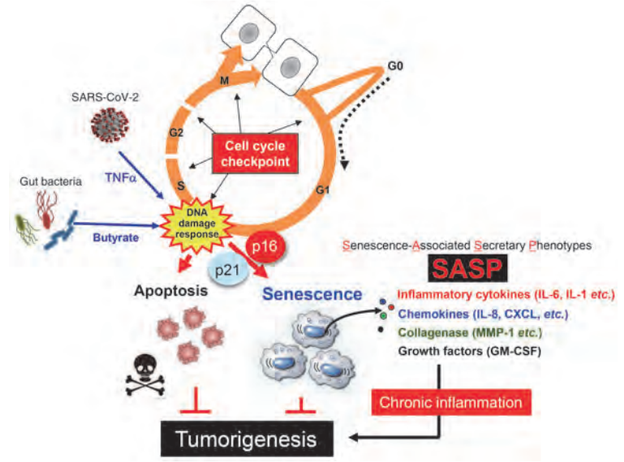

Figure 1.
Cellular senescence initially functions as a fail-safe mechanism by inhibiting the proliferation of damaged cells in response to various stimuli. In the long term, however, senescent cells eventually promote various inflammatory diseases such as tumorigenesis via SASP.

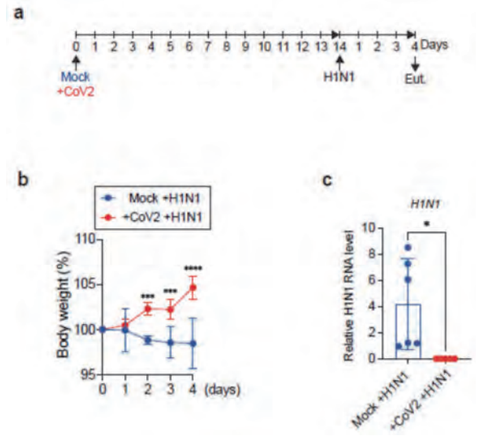

Figure 2.
a-c. Syrian hamsters were intranasally inoculated with SARS-CoV-2 or medium (mock). After 14 days post-infection of SARS-CoV-2, mock or SARS-CoV-2 infected hamsters were intranasally inoculated influenza virus A (H1N1). Hamsters infected with mock followed by H1N1 (mock+H1N1; n=6) and SARS-CoV-2 followed by H1N1 (CoV2+H1N1; n=6). The timeline of the experiment was shown (a). The body weight was monitored until day 4 post H1N1 infection (b). These hamsters were euthanized on day 4 after H1N1 infection, and the expression of H1N1 genomic RNA was analyzed using RT-qPCR (c).

Recent Publications

1. Tsuji S, Minami S, Hashimoto R, Konishi Y, et al. SARS-CoV-2 infection triggers paracrine senescence and leads to a sustained senescence-associated inflammatory response. Nat Aging. 2:115-124 (2022). doi: 10.1038/s43587-022-00170-7.

2. Okumura S, Konishi Y, Narukawa M, et al. Gut bacteria identified in colorectal cancer patients promote tumourigenesis via butyrate secretion. Nat Commun. 12:5674 (2021). doi: 10.1038/s41467-021-25965-x.

3. Wakita M, Takahashi A, Sano O, et al. A BET family protein degrader provokes senolysis by targeting NHEJ and autophagy in senescent cells. Nat Commun. 11:1935 (2020). doi: 10.1038/s41467-020-15719-6.

Oncogene Research

Masato Okada, PhD

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	(S.A.) Tetsuya Kimura
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Research Assistant	1

Role of Src tyrosine kinase in tumor progression

We have investigated the role of Src tyrosine kinase in tumor progression. Src is the first-identified oncogenic tyrosine kinase, but no significant mutation of the *SRC* gene occurs in any type of human cancer. Nonetheless, the function of Src is frequently upregulated in various malignant cancers, and it is appreciated that upregulated Src plays a crucial role in tumor progression, particularly in the acquisition of invasive and metastatic features. To elucidate the molecular mechanisms underlying upregulation of Src, we investigated the regulatory mechanism of *SRC* gene expression and searched for Src-activating factors. We found that TGF-β treatment directly induced *SRC* gene expression via the Smad pathway in coupled with additional transcription factors, and determined the promoter and enhancer regions located in the *SRC* gene. The upregulation of Src contributes to the progression of cell motility accompanied by TGF-β–induced epithelial-mesenchymal transition. On the other hand, we identified CUB-domain containing protein 1 (CDCP1) as a Src-activating membrane glycoprotein in lipid rafts of epithelial cells. Upregulation of CDCP1 induces prominent activation of Src and the STAT3 pathway, which promotes the invasive activity of epithelial cells. We also showed that ablation of CDCP1 inhibits HGF-induced morphological changes and cell growth, and attenuates membrane presentation of MET, resulting in inhibition of invasive activity induced by HGF (Kawase et al. *J Biol Chem*. 2022). These findings suggest that CDCP1 is a co-receptor of MET and thereby stabilizes its signaling (Fig. 1). Furthermore, we revealed that ablation of CDCP1 suppresses the compensatory renal hypertrophy, indicating that CDCP1 is required for the HGF-MET signaling even in vivo (Kajiwara et al. *Life Sci Alliance*. 2021). Analysis of the CDCP1-Src axis in the epithelial cells also showed that Src activation in lipid rafts confers epithelial cells with invasive potential to escape from apical extrusion during cell competition (Fig. 2. Kajiwara et al. *Curr Biol*. 2022). These findings underscore the crucial role of the CDCP1-Src axis in the tissue regeneration and the initial phase of tumor progression.

Role of p18 in the regulation of mTORC1 nutrient signaling

We previously identified a new Src substrate termed p18/Lamtor1, which exclusively localizes to lipid rafts of lysosomes. p18 functions by forming a hetero-heptamer complex (Ragulator), which is required for activation of mTORC1 on lysosomes. Subsequent in vivo analyses confirmed that p18-Ragulator is tightly associated with the regulation of mTORC1 nutrient signaling. The crystal structure of p18-Ragulator provided significant insights into the role of p18-mediated regulation of mTORC1 on lysosomes. Recent in vitro analysis also showed that p18-Ragulator complex provides a regulatory platform that is indispensable for amino acid-dependent regulation of mTORC1 (Fig. 3). Mathematical modeling of the mTORC1 signaling further supported our prediction. On the other hand, we found an additional function of the p18-Ragulator complex as a substrate-specific mTORC1 scaffold in regulating the nuclear translocation of transcription factor EB (Kimura et al. *J Biol Chem*. 2022). Collaborative studies with a member of IFReC (Prof. Kumanogoh) further revealed that the lysosomal Ragulator complex plays an essential role in leukocyte trafficking by activating myosin II (Nakatani et al. *Nature Commun*. 2021) and that the lysosomal Ragulator complex activates NLRP3 inflammasome *in vivo* via HDAC6 (Tsujimoto et al. *EMBO J*. 2022). These studies unraveled unique roles of the p18-Ragulator complex in the immune system, and provided new insights in the function of the p18-Ragulator complex on the lysosome membrane.

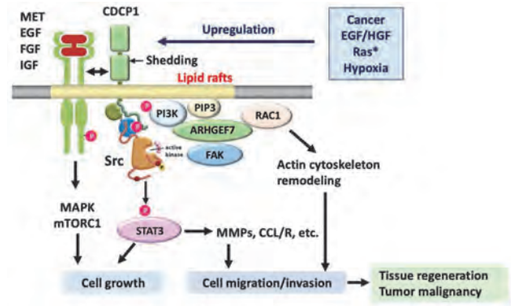

Figure 1.
Role of CDCP1-Src signaling in the regulation of tissue regeneration and tumor malignancy.

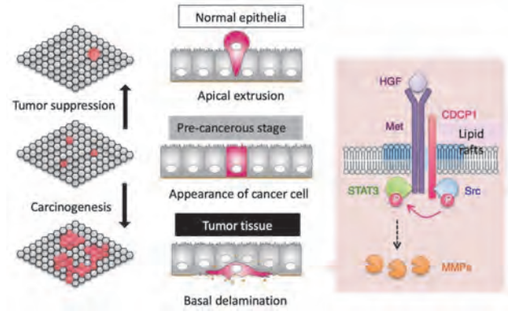

Figure 2.
Role of CDCP1-Src in the regulation of the fate of pre-cancerous cells.

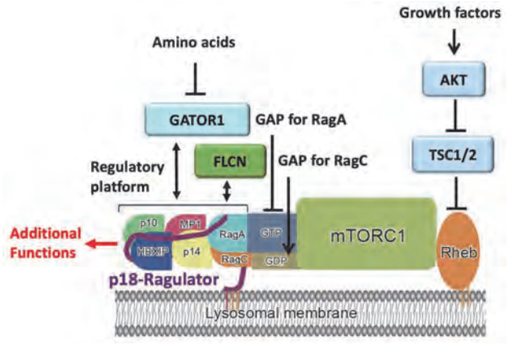

Figure 3.
Structural basis for the regulation of mTORC1 signaling via the p18-Ragulator complex.

Recent Publications

1. Tsujimoto K, Jo T, Nagira D, Konaka H, et al. The lysosomal Ragulator complex activates NLRP3 inflammasome in vivo via HDAC6. EMBO J. e111389. doi.org/10.15252/embj.2022111389 (2022).
2. Kajiwara K, Chen PK, Abe Y, et al. Src activation in lipid rafts confers epithelial cells with invasive potential to escape from apical extrusion during cell competition. Curr Biol. 32(16):3460-3476 (2022).
3. Kimura T, Hayama Y, Okuzaki D, Nada S, Okada M. The Ragulator complex serves as a substrate-specific mTORC1 scaffold in regulating the nuclear translocation of transcription factor EB. J Biol Chem. 298(3):101744 (2022).
4. Kawase N, Sugihara A, Kajiwara K, et al. SRC kinase activator CDCP1 promotes hepatocyte growth factor-induced cell migration/invasion of a subset of breast cancer cells. J Biol Chem. Mar;298(3):101630 (2022).
5. Kajiwara K, Yamano S, Aoki K, Okuzaki D, Matsumoto K, Okada M. CDCP1 promotes compensatory renal growth by integrating Src and Met signaling. Life Sci Alliance. 4(4):e202000832 (2021).

Signal Transduction

Nobuyuki Takakura, MD/PhD

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Support Staff	3

Vasculogenesis and angiogenesis are processes in the formation of blood vessels where the formation of a primary vascular plexus is induced by vascular endothelial growth factor (VEGF), which binds to its cognate receptor VEGFR2 on endothelial cells (ECs), and the formation process involves the development, proliferation, migration and tube formation of ECs. Subsequently, mural cells are recruited near ECs and adhere to them, inducing the blood vessels to mature. Platelet-derived growth factor secreted from ECs promotes the recruitment of mural cells near ECs, and angiopoietin-1 (Ang1) secreted from mural cells via its specific receptor Tie2 on ECs induces the adhesion of mural cells to ECs.

We analyzed the function of Tie2 activation during the maturation process of blood vessels and discovered the critical roles of apelin. Apelin is a ligand for APJ, which is a G protein-coupled receptor with 7 transmembrane domains expressed on ECs. We found that apelin is produced from ECs upon stimulation with Ang1 and stimulates APJ through an autocrine process to induce cell-cell aggregation of ECs, thereby resulting in the formation of larger blood vessels. Blood vessels become larger in caliber when a higher number of mural cells adhere to ECs, and we identified how blood vessels sense caliber size depending on the number of mural cells through Ang1/Tie2-apelin/APJ axis (EMBO J 2008). Moreover, we found that activation of APJ on ECs stabilizes membrane expression of VE-Cadherin in ECs, inducing tight adhesion of ECs and non-leaky blood vessels (Blood 2010). We also found that apelin/APJ plays a critical role in the alignment between arteries and veins. After blood vessels commit to forming into arteries and veins, apelin from arterial ECs significantly promotes migration of venous ECs expressing APJ. Furthermore, upon stimulation with apelin, venous ECs start to express sFRP1, promoting the production of matrix metalloproteinases from neutrophils around veins, and thereby enhancing of movement of veins to arteries (Dev Cell 2015).

We applied the function of vascular maturation by apelin to regulate tumor angiogenesis. When apelin expression is enhanced in tumor microenvironment, natural killer cells and CD8 T cells penetrate the tumor parenchyma to create immune hot spots that inhibit tumor growth (Oncogene 2012, Sci Rep 2021).

On the other hand, we found that APJ is not only expressed on ECs but also in cancer cells in tumor microenvironment, and its roles on cancer cells are unclear at the moment. Recently, we discovered that APJ in cancer cells is involved in vascular mimicry, in which cancer cells themselves form vascular tube like structures (Pathol Oncol Res 2023). In cancer cells, APJ usually negatively regulates epithelial mesenchymal transition (EMT), although in the absence of APJ, cancer cells exhibit vascular mimicry to supply nutrients and support tumor growth. Deficiency of APJ in cancer cells induces Zeb1 expression, which is involved in EMT. However, we found that Zeb1 expression is not regulated by the TGF□/smad pathway. Further analysis is required to understand the underlying mechanism of this vascular mimicry regulated by apelin/APJ.

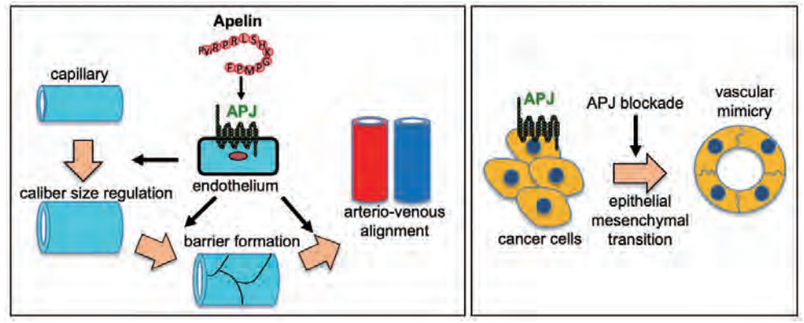

Figure.
Diverse function of apelin/APJ in vascular formation.
APJ is a receptor for apelin and is not only expressed on endothelial cells but also on cancer cells. Apelin/APJ is involved in the maturation process of blood vessels through APJ on ECs, i.e., caliber size regulation, barrier formation between ECs, and juxta-positional alignment between arteries and veins. Therefore, when APJ activation is induced on ECs in the tumor microenvironment, the blood vessels in tumors maturate, improving cancer drug delivery and immune cell infiltration. Moreover, APJ on cancer cells inhibits vascular mimicry. Taken together, APJ should be activated in the tumor microenvironment to suppress tumor growth. However, some controversial opinions suggest APJ suppression benefits tumor growth. Therefore, the function of APJ in tumors may be diverse depending on tumor cell type, and further analysis is required for the development of effective tumor therapies associated with apelin/APJ.

Recent Publications

1. Jia W, Kong L, Kidoya H, Naito H, Muramatsu F, Hayashi Y, Hsieh HY, Yamakawa D, Hsu DK, Liu F-T, and Takakura N. Indispensable role of Galectin-3 in promoting quiescence of hematopoietic stem cells. Nature Commun. 12:2118 (2021).
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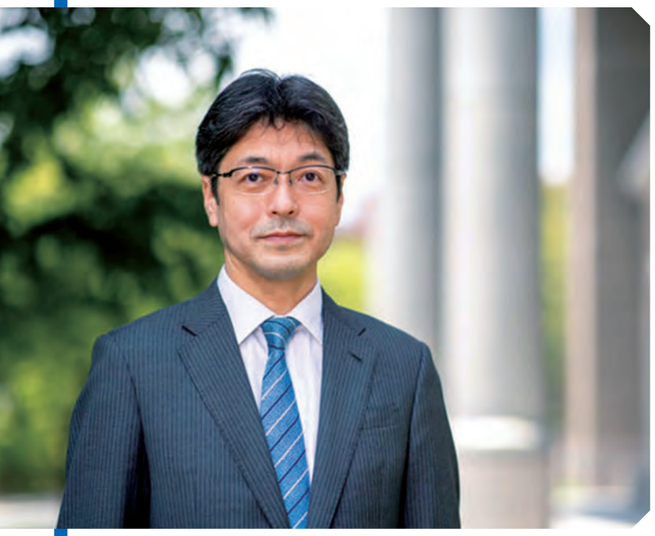
3. Kidoya H, Muramatsu F, Shimamura T, Jia W, Satoh T, Hayashi Y, Naito H, Kunisaki Y, Arai F, Seki M, Suzuki Y, Osawa T, Akira S, Takakura N. Regnase-1-mediated post-transcriptional regulation is essential for hematopoietic stem and progenitor cell homeostasis. Nat Commun. 10:1072 (2019).
4. Naito H, Iba T, Wakabayashi T, Tai-Nagara I, Suehiro JI, Jia W, Eino D, Sakimoto S, Muramatsu F, Kidoya H, Sakurai H, Satoh T, Akira S, Kubota Y, Takakura N. TAK1 prevents endothelial apoptosis and maintains vascular integrity. Dev Cell 48:151-166 (2019).
5. Wakabayashi T, Naito H, Suehiro JI, Lin Y, Kawaji H, Iba T, Kouno T, Ishikawa-Kato S, Furuno M, Takara K, Muramatsu F, Weizhen J, Kidoya H, Ishihara K, Hayashizaki Y, Nishida K, Yoder MC, Takakura N. CD157 marks tissue-resident endothelial stem cells with homeostatic and regenerative properties. Cell Stem Cell 22:384-397 (2018).

Cutaneous Immunology



Manabu Fujimoto, MD/PhD

Professor	Manabu Fujimoto
Research Assistant	4
Support Staff	1

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.

Possible Plasticity of Cytotoxic Resident Memory T Cells in Fixed Drug Eruption

We examined the phenotypical characteristics of T cells from fixed drug eruption (FDE) lesions and analyzed the origin of CD49a+CCR7+ T cells in FDE lesions. This study showed that the ratio of central memory T (TCM) cells was increased in recurrent FDE lesions, and the ratio of T cells expressing resident memory T (TRM) cell markers was limited. Both CD4 and CD8 T cells from recurrent FDE lesions highly expressed CD49a, a marker for IFN-g–producing cytotoxic TRM (cTRM) cells. The T-cell receptor (TCR) clones were also examined, and the commonality of TCR clones was the highest between skin cTRM and skin TCM cells, suggesting that TCM cells in recur are not originated from blood TCM cells.

Epidermal clearance of Candida albicans is mediated by IL-17 but independent of fungal innate immune receptors

We examined the role of IL-17 in host defense against Candida albicans in the epidermis. Using a murine model of epicutaneous candidiasis, we found that group 3 innate lymphoid cells (ILC3s) and γδT cells were the major IL-17 producers in the epicutaneous candidiasis model. Analyses of $Rag2^{-/-}$ mice and $Rag2^{-/-}$ $Il2rg^{-/-}$ mice revealed that production of IL-17A and IL-17F by ILC3s was sufficient for C. albicans clearance. These findings indicate a critical and redundant function of IL-17A and IL-17F produced by ILC3s in host defense against C. albicans in the epidermis. The results suggest that epidermal C. albicans clearance is independent of innate immune receptors.

Sirolimus relieves seizures and neuropsychiatric symptoms via changes of microglial polarity in tuberous sclerosis complex model mice

Tuberous sclerosis complex (TSC) is a genetic disorder associated with epilepsy and serious neuropsychiatric symptoms, collectively known as TSC-associated neuropsychiatric disorders (TAND). The overactivation of mTORC1 by mutations in TSC1 or TSC2 is thought to cause TSC, and mTORC1 inhibitors such as sirolimus are effective against various tumor types of TSC. We showed TSC2 conditional knockout mice exhibited phenotypes of TAND as well as epileptic seizures. Gene expression analysis revealed that changes to M1 in microglial polarity were involved in the onset of TSC epilepsy and neuropsychiatric symptoms and sirolimus treated epilepsy by shifting microglial polarity to M2.

Distinct transcriptional profiles in the different phenotypes of neurofibroma from the same neurofibromatosis 1 subject

Neurofibromatosis 1 (NF1) is a hereditary neurocutaneous disorder with different clinical phenotypes, including cutaneous neurofibroma (cNF) and plexiform neurofibroma (pNF). This study using single-cell RNA sequencing and immunohistochemical analysis found that cNF and pNF have distinct transcriptional profiles and microenvironments. pNF had more Schwann cells, fibroblasts with cancer-associated fibroblast-like phenotypes, angiogenic endothelial cells, and M2-like macrophages, while cNF had more CD8 T cells with tissue residency markers. This study provides new insights into the different features of cNF and pNF, which could be useful in developing targeted therapies for NF1 patients.

Collagen homeostasis paradoxically maintains in vitiligo lesion

The lesional skin of vitiligo would be more resilient than uninvolved lesion even in sun-exposed areas. Therefore, we hypothesized that a collagen homeostasis might paradoxically maintain in vitiligo lesion irrespective of substantial excessive oxidative stress condition without melanin protection. Single-cell RNA sequencing analysis showed that the expressions of collagen-related and anti-oxidative enzyme genes were up-regulated in the fibroblasts of lesional skin compared to those of uninvolved skin. Our results suggest that the dermal fibroblasts in vitiligo may play a protective role against the locally-exposed oxidative stress.

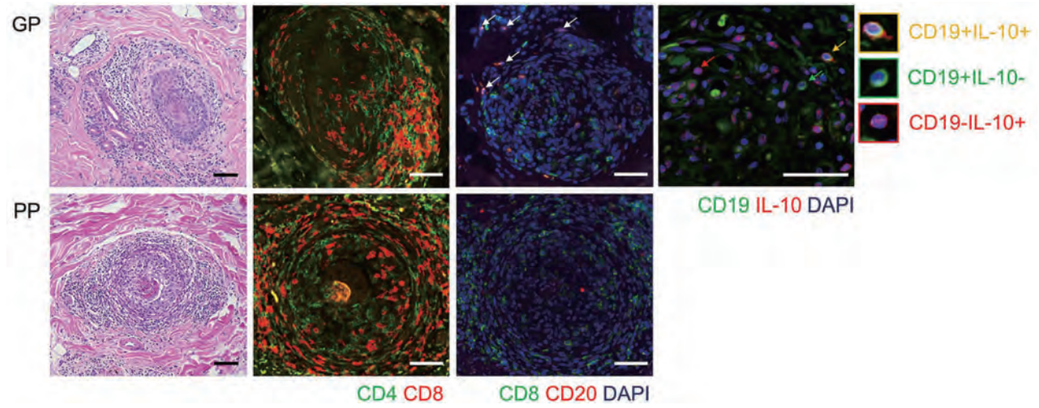


Figure.
Representative H&E and immunofluorescence results showing CD4/CD8 and CD8/CD20 from subjects with good prognosis (GP) and poor prognosis (PP). White arrows indicate B cells. In subjects with GP, a representative immunofluorescence result of CD19/IL-10 is also shown. Bars = 50 mm.

Recent Publications

1. Matsumura Y, Watanabe R, Koguchi-Yoshioka H, Kume M, Nakai S, Furuta J, Azukizawa H, Ishitsuka Y, Tanemura A, Taminato M, Tashima H, Otani N, Tomita K, Kubo T, Fujimoto M. Possible Plasticity of Cytotoxic Resident Memory T Cells in Fixed Drug Eruption. J Invest Dermatol. S0022-202X(22)02895-0 (2022).
2. Matsumura Y, Watanabe R, Koguchi-Yoshioka H, Nakamura Y, Saito A, Kume M, Nakai S, Ishitsuka Y, Furuta J, Fujimoto M. IL-10-Producing Potency from Blood B Cells Correlates with the Prognosis of Alopecia Areata. J Invest Dermatol. 143(5):871-874.e5 (2023).
3. Matsumura Y, Watanabe R, Fujimoto M. Suppressive mechanisms of regulatory B cells in mice and humans. Int Immunol. 35(2):55-65 (2023).
4. Koike-Kumagai M, Fujimoto M, Wataya-Kaneda M. Sirolimus relieves seizures and neuropsychiatric symptoms via changes of microglial polarity in tuberous sclerosis complex model mice. Neuropharmacology. 218:109203 (2022).
5. Iwasawa MT, Miyachi H, Wakabayashi S, et al. Epidermal clearance of Candida albicans is mediated by IL-17 but independent of fungal innate immune receptors. Int Immunol. 34(8):409-420 (2022).

Innate Immune Systems

Kazuyo Moro, DDS/PhD

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	Satoshi Koga
Postdoctoral Fellow	1
Research Assistant	2
Support Staff	1

1. Innate IgE exacerbates human allergic disease via amplification of ILC2 activation

ILC2s induce allergies by interacting with various immune and non-immune cells; however, their relationship with B cells is unclear. In this study, we found that IL-33 markedly promoted B1 cell proliferation via ILC2s and induced the production of "innate IgE," which recognizes autoantigens but not foreign antigens. B1 cells are innate type B cells that contribute to the clearance of microorganisms and apoptotic cells by producing low-affinity IgM that recognizes T cell-independent antigens, such as lipids and polysaccharides. Most research on B1 cells has been conducted using B1 cells in the peritoneal cavity of mice, and some researchers still believe that B1 cells do not exist in humans. Our results demonstrated that the class switch of IgE in B1 cells occurs in peripheral tissues by IRF4^{+}ILC2-derived IL-4 in a CysLT-dependent manner. Experiments using IRF4-deficient mice showed that IRF4 was essential for IL-4 production from ILC2s, and innate IgE was virtually undetected in ILC2-deficient mice. Innate IgE did not induce granulocyte degranulation but directly promoted the survival and lipid production of FcεR^{+} mast cells and basophils, resulting in amplification of ILC2/eosinophil-dependent allergic inflammation.

We focused on eosinophilic chronic rhinosinusitis (ECRS) to confirm the importance of innate IgE in humans. We found that ILC2s accumulate in the nasal polyps of ECRS patients and are predominantly correlated with IgE-bearing mast cells as well as eosinophils. The significant correlation between ILC2s and IgE production in nasal polyps suggests that ILC2s induce innate IgE production in B1 cells, leading to eosinophilic inflammation. Single-cell RNA-sequencing analysis demonstrated that B1 cells are also present in the nasal polyps of patients with ECRS and that ILC2s are major IL-4 producing cells. A comparison of B1 and B2 cells isolated from nasal polyps revealed that most IgE was produced by B1 cells. Taken together, our results indicate that innate IgE contributes to the pathogenesis of ECRS by forming a circuit that amplifies ILC2 function by promoting mast cell and basophil survival, and CysLT production.

2. Identification of appendectomy-related factors that inhibit development of ulcerative colitis

Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) that affects a growing number of people worldwide. In recent years, the advent of biologics, such as anti-TNF-α antibodies, has expanded the treatment options for UC, but no curative therapy has yet to be established. One cohort study reported that patients who underwent appendectomy by the age of 20 had a lower lifetime risk of developing UC. Subsequently, similar results have been obtained in cohort studies conducted in various countries. Although this phenomenon is well known to clinicians, no basic studies have examined this mechanism over the past 20 years. Therefore, we initiated this project with the aim of developing a new therapeutic approach by elucidating the mechanism by which appendectomy reduces the risk of colitis.

We first generated an appendectomy (APX) mouse modeland induced colitis by dextran sulfate sodium (DSS). Similar to the cohort study, pathologies such as IL-1β and IL-6 production, intestinal atrophy, and weight loss induced by DSS were reduced in APX mice compared to those in sham-operated mice. To understand the immunological changes in the colon after APX, we examined the immune cell profiles and found that ILC2s were significantly increased after APX. Consistent with this result, we found that type 2 cytokines, such as IL-5 and IL-13, increased after APX. In addition, measurements of IL-25 and IL-33, known activators of ILC2s, showed that IL-25 was elevated in the colonic epithelial fraction, whereas IL-33 was not altered. IL-25 is known to be produced by chemosensory epithelial cells called tuft cells.

The most significant finding of this study was the identification of APX-induced hyperplasia of tuft cells in the colon. To understand the role of IL-25 in UC, we performed APX in IL-25 knockout mice and found that suppression of DSS-induced colitis was abolished in these mice. In contrast, IL-25 administration produced the opposite result. Many studies on IBD have focused on factors that cause colitis; however, by focusing on factors that inhibit the development of colitis, as in this project, we hope that new methods of preventing IBD can be established.

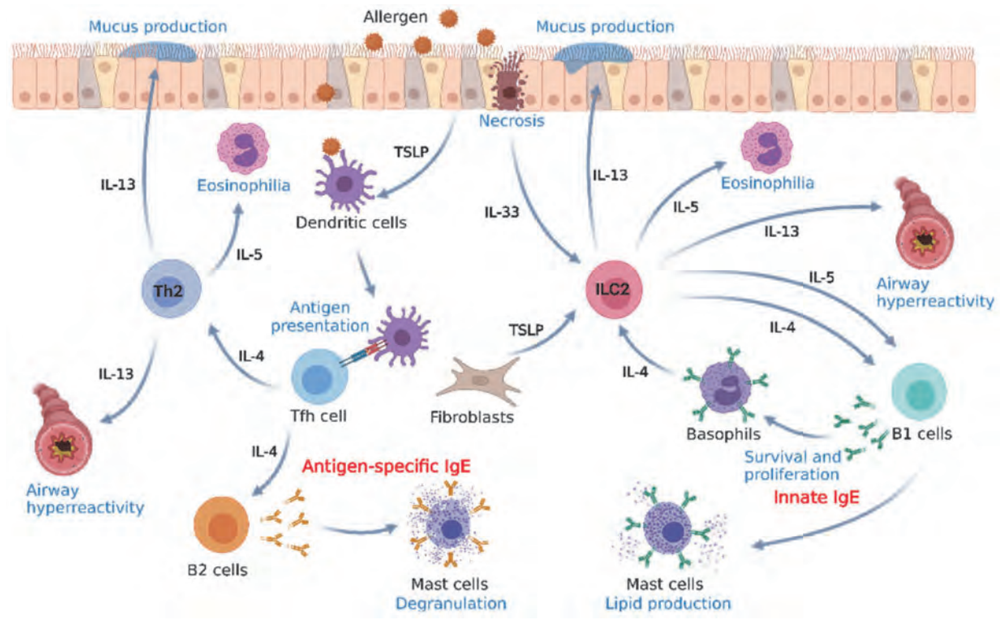

Figure.
IL-4 produced from T cells promotes antigen-specific IgE production from B2 cells in lymph nodes and induces mast cell degranulation. On the other hand, IL-4 produced from ILC2s induces the production of Innate IgE from B1 cells and supports survival, proliferation and lipid production of basophils and mast cells.

Recent Publications

1. Kobayashi T, Moro K. A hairy situation for ILC2s. Immunity 55:1756-1758 (2022).Kabata H, Motomura Y, Kiniwa T, Kobayashi T, Moro K. ILCs and Allergy. Advances in experimental medicine and biology 1365:75-95 (2022).
2. Kobayashi T, Moro K. Tissue-Specific Diversity of Group 2 Innate Lymphoid Cells in the Skin. Frontiers in immunology 13:885642 (2022).
3. Momiuchi Y, Motomura Y, Suga E, et al. Group 2 innate lymphoid cells in bone marrow regulate osteoclastogenesis in a reciprocal manner via RANKL, GM-CSF and IL-13. International immunology 33:573-585 (2021).
4. Hikichi Y, Motomura Y, Takeuchi O, Moro K. Posttranscriptional regulation of ILC2 homeostatic function via tristetraprolin. The Journal of experimental medicine 218 (2021).

Human Single Cell Immunology

James Badger Wing, PhD

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Assistant Professor	Jonas N. Søndergaard
Postdoctoral Fellow	3
Research Assistant	1
Visiting Scientist	1
Support Staff	1

The antibody response to COVID-19 is a key part of our defense against infection. Broadly speaking there are two main routes of antibody production; the follicular route, typified by the interaction between T-follicular helper (Tfh) T cells and germinal center B-cells, and the extrafollicular route typified by the interaction between T-peripheral helper (Tph) cells and extrafollicular B-cells. A further complication is that antibody production is controlled by a population of regulatory T-cells named T-follicular regulatory cells (Tfr). However, since the complex interaction of these rare cell types are difficult to resolve by common techniques our understanding of these processes is incomplete.

We used single cell analysis of proteomics by mass cytometry (CyTOF) to examine the status of rare cells of immune system in high detail in a cohort of COVID-19 patients with severe disease. Using this approach; we were able to find that there was a sex biased loss of circulating Tfr cells (cTfr) in all patients. In addition, we found that SARS-CoV2 neutralizing antibody concentrations were strongly associated with a network of extrafollicular cells related to antibody production. T-peripheral helper cells (Tph), proliferating memory B-cells and atypical/extrafollicular B-cells, while cTfr were negatively correlated with this network. This trend is stronger in male patients who also show increased numbers of antibody producing T and B-cells and fewer Tfr in comparison to female patients (Fig. 1, Søndergaard et al. PNAS, 2023). These results suggest that sex specific differences to the balance of cTfr and a network of extrafollicular antibody-production associated cell types may be a key factor in the altered humoral immune responses between male and female COVID-19 patients. A better understanding of the cellular interactions controlling antibody production in COVID-19 may allow the development of new treatments to control the disease. Additionally, the finding that male patients have strong, but dysregulated, antibody production may give us information that we must treat male and female COVID-19 patients differently.

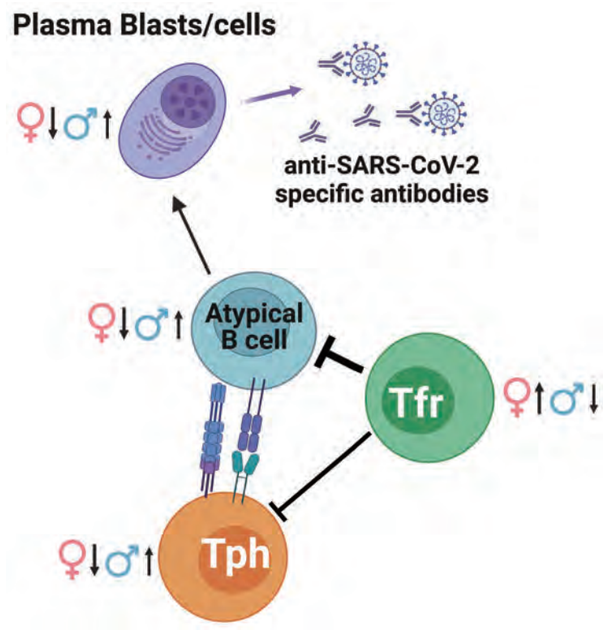

Figure 1.
Sex biased imbalance of cells that inhibit or boost antibody production during COVID-19 infection. T-follicular regulatory cells (Tfr) inhibit antibody production. T-peripheral helper cells (Tph) stimulate atypical B-cells to form antibody producing plasma cells.

Recent Publications

1. Søndergaard JN, Tulyeu J, et al. 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).
2. Yokoi T, et al. Identification of a unique subset of tissue-resident memory CD4+ T cells in Crohn's disease. Proc Natl Acad Sci USA. 120(1) e2204269120 (2023).
3. Shibata K, et al. Symbiotic bacteria-dependent expansion of MR1-reactive T cells causes autoimmunity in the absence of Bcl11b. Nat Commun. 13(1):1-15 (2022).
4. Yamaguchi Y, Kato Y, Edahiro R, Søndergaard JN, et al. Consecutive BNT162b2 mRNA vaccination induces short-term epigenetic memory in innate immune cells. JCI Insight. (2022) 10.1172/jci.insight.163347.
5. Namkoong H, Edahiro R, et al. DOCK2 is involved in the host genetics and biology of severe COVID-19. Nature. 609:754-760 (2022).

Human Immunology (Single Cell Genomics)

Daisuke Okuzaki, PhD

Associate Professor	Daisuke Okuzaki
Postdoctoral Fellow	3
Visiting Scientist	2
Support Staff	1

The Human Immunology Laboratory was established in November 2019 with the aim of accelerating the application of single-cell sequencing technology and advancing clinical research. As part of the largest single-cell RNA analysis initiative in Japan, our laboratory collaborated with the "Team Osaka University COVID-19 Research Group" to conduct whole blood single-cell RNA analysis of samples obtained from patients admitted to Osaka University Hospital. This effort was led by Professors Yukinori Okada and Kumanogoh from the School of Medicine, who oversaw the storage and management of specimens until the sixth wave of the pandemic. Our laboratory played a vital role in this project, contributing to the analysis of more than 200 specimens. The data was later utilized by the "Corona Control Task Force," a collaborative project involving Osaka University (led by Professor Okada), as well as over 100 hospitals and major universities in Japan. The project's findings, including single-cell RNA analysis, were published in Nature in August 2022. The study identified a correlation between genetic polymorphisms in the DOCK2 gene region and COVID-19 aggravation, representing the most extensive genome-wide association analysis of COVID-19 patients in Asia. Furthermore, combined with single-cell analysis, a COVID-19-associated risk variant at the IFNAR2 locus (rs13050728) had context-specific and monocyte-specific expression quantitative trait locus effects. This discovery was published in Nature Genetics.

We discovered a novel public antibody clonotype, PA-N-CoV1804, within the data 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 like the antiviral antibodies derived from pre-existing memory for seasonal human coronaviruses. However, these clonotypes were strongly reactive to the N protein of SARS-CoV-2 but not to that of seasonal coronaviruses. Therefore, these clones seem to be originated from naive B cells and developed through de novo clonal expansion after SARS-CoV-2 infection (Figure 1).

On the other hand, in a joint research project conducted with Critical Care Medicine at Osaka University, a platform serving as a guide for determining whether mechanical ventilation would be needed and for how long was established for severe COVID-19 patients who are transported to the ICU in 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.

Interactions between microRNA and mRNAs were also studied through simultaneous measurements 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.

In terms of proteomics research, blood protein analysis of COVID-19 patients was conducted both in the US and at Osaka University in Japan, and the analysis identified five proteins related to COVID-19 aggravation. Some COVID-19 patients became severely ill and required treatments, such as mechanical ventilation. We used a technology called Explore 1536 from Olink to identify the proteins in serum. To further identify effective diagnostic biomarkers, ROC analysis of four plasma proteins was conducted. Based on an analysis of each marker alone, we found a combination of three to four proteins that had higher prognostic accuracy than other predictors, such as CRP and D-dimer. Ultimately, we found that the four proteins related to aggravation were elevated in the blood of deceased patients. The utilization of single-cell data in collaborative efforts such as the "Corona Control Task Force" has led to important insights into the mechanisms of COVID-19 and has the potential to inform future approaches to managing the pandemic.

Our laboratory has been able to build a single-cell platform at IFReC while concurrently serving as an NGS core facility. It is now the leading single-cell analysis facility in Japan. Three years of collaborative research on COVID-19 with multiple institutions have also steadily produced results. Original studies such as the development of a method for simultaneous detection of bacteria and eukaryotic cells at the single-cell level, and the development of a method for detecting circRNA using long-read sequencers will advance research in many fields.

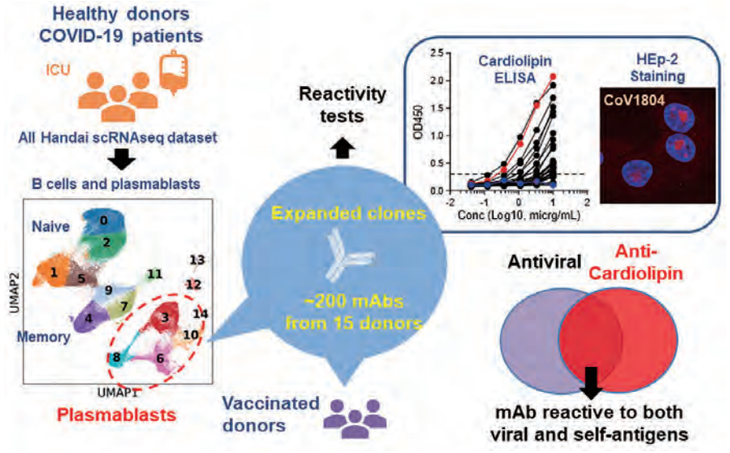

Figure 1.
Clonal landscape of viral infection-induced autoantibody production.

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1. 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).
2. 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).
3. Ishikawa M, Shimada Y, Ozono T, et al. Single-cell RNA-seq analysis identifies distinct myeloid cells in a case with encephalitis temporally associated with COVID-19 vaccination. Front Immunol. 14:998233 (2023).
4. Ito H, Ishikawa M, Matsumoto H, Sugihara F, Okuzaki D, Hirata H, Ogura H. Transcriptional differences between coronavirus disease 2019 and bacterial sepsis. Virol J. 19(1):198 (2022).
5. Yamaguchi Y, Kato Y, Edahiro R,et al. Consecutive BNT162b2 mRNA vaccination induces short-term epigenetic memory in innate immune cells. JCI Insight. Nov 22;7(22):e163347 (2022).

Immune Homeostasis

Yasutaka Okabe, PhD

Associate Professor	Yasutaka Okabe
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Research Assistant	2
Support Staff	1

Peritoneal cavity, the largest fluid-filled abdominal cavity in mammals, contains visceral organs such as stomach, spleen, liver, and intestine. Although peritoneal cavity is normally sterile, the infection, which can occur by a pathological or traumatic loss of intestinal wall integrity, cirrhosis, pancreatitis, abdominal surgery, or peritoneal dialysis, can cause life-threatening sepsis since the peritoneal cavity serves as a conduit to other vital organs for pathogen spreads.

Omental milky spots in the peritoneal cavity are lymphoid tissues exhibiting hybrid nature between secondary lymph organs and ectopic lymphoid tissues, although the mechanism of their development and maturation is poorly understood. We previously reported that retinoic acid, which controls the functional specialization of peritoneal macrophages, is abundantly present in omentum (Okabe & Medzhitov, 157, 832-844, Cell). Whereas retinoic acid is important for the development of secondary lymphoid organs such as lymph nodes and Peyer's patches, its role in milky spot formation remains under-explored.

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.

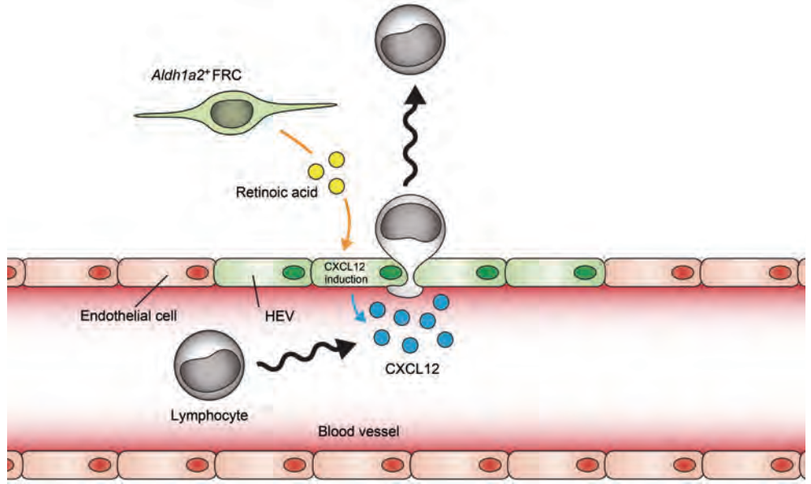

Figure.

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1. Yoshihara T & Okabe Y. Aldh1a2+ fibroblastic reticular cells regulate lymphocyte recruitment in omental milky spots. J Exp Med. 220(5), e20221813 (2023).
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3. Okabe Y. Molecular Control of the Identity of Tissue-Resident Macrophages. Int Immunol. 30:485-491 (2018).
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5. Okabe Y & Medzhitov R. Tissue-specific signals control reversible program of localization and functional polarization of macrophages. Cell. 157:832-844 (2014).

Cellular Immunotherapy

Naoki Hosen, MD/PhD

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Visiting Scientist	1
Support Staff	3

We are focusing on cellular immunotherapy, especially chimeric antigen receptor (CAR)-T cell therapy for cancers. CAR-T cells specifically recognize cancer cells using the cancer-specific mAb-derived antigen-recognition domain, and are activated. Activated CAR-T cells kill tumor cells and also proliferate extensively. CD19 CAR-T cells showed surprisingly high effectiveness against acute lymphocytic leukemia and malignant lymphoma. We discovered that the active conformer of an integrin could serve as a specific therapeutic target for multiple myeloma (MM), which is an incurable hematological cancer characterized by the accumulation of neoplastic plasma cells in the bone marrow (BM). Clinical trial of the CAR T-cell targeting the active conformation of integrin β7 for MM is now on-going.

Identification of a novel target antigen for multiple myeloma and development of CAR-T cell therapy targeting them

Cancer-specific cell surface antigens are ideal therapeutic targets for monoclonal antibody (mAb)-based therapy. However, most transcripts or proteins highly specific for cancer cells have already been identified after extensive efforts using transcriptome or proteome analyses. We screened more than 10,000 mAb clones raised against MM cells, and identified R8H283 as a 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 abundantly expressed CD98 heterodimers to intake amino acids for constitutive production of immunoglobulin. 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 be a cause for lack of R8H283 reactivity in normal leukocytes. R8H283 exerted significant anti-MM effects without damaging normal hematopoietic cells. These findings not only suggest that R8H283 is a new source for mAb-based therapies such as CAR-T cell therapy against MM, but also that a cancer-specific conformational epitope in a ubiquitous protein, which cannot be identified by transcriptome or proteome analyses, can be found by extensive screening with primary human tumor samples. We are now developing R8H283-derived CAR T-cells.

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

We applied the same strategy to various types of cancers. For hematological cancers such as acute myeloid leukemia (AML), the only hurdle for developing CAR-T cell therapy is lack of an appropriate cancer-specific cell surface antigen. We have already established huge numbers of mAbs reacting with AML cells and found ones recognizing antigen structures highly specific for AML cells. We also applied the same strategy to several types of solid tumors in collaboration with several departments treating cancers in Osaka University Hospital. We have recently showed that our screening method also works in glioblastoma.

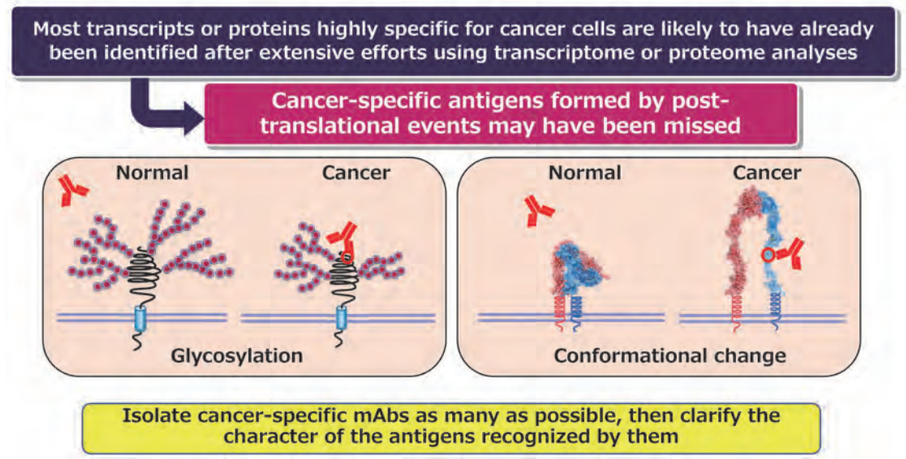

Figure 1.
The strategy for identifying novel cancer-specific cell surface targets for CAR-T cell therapy.

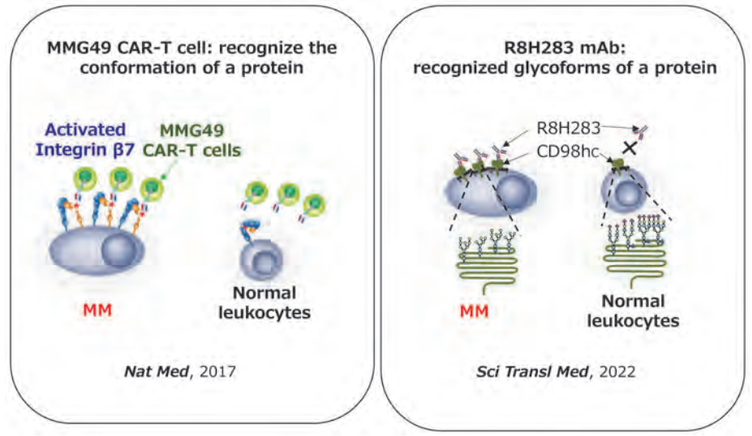

Figure 2.
Novel target antigen structures that we have identified for CAR-T cell therapy against multiple myeloma.

Recent Publications

1. Nakagawa T, Kijima N, Hasegawa K, 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).
2. Uchihara Y, Permata TBM, Sato H, 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).
3. Hino A, Fukushima K, Kusakabe S, et al. Prolonged gut microbial alterations in post-transplant survivors of allogeneic haematopoietic stem cell transplantation. Br J Haematol. (2022).
4. Hasegawa K, Ikeda S, Yaga M, et al. Selective targeting of multiple myeloma cells with a monoclonal antibody recognizing the ubiquitous protein CD98 heavy chain. Sci Transl Med. 14:eaax7706 (2022).

Microbiology and Immunology

Nobuhiko Kamada, PhD

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Associate Professor	Sho Kitamoto
	Hiroko Kitamoto
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 recently 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 inflammation T cells of oral origin are activated by ectopically colonized oral pathobionts and contribute to inflammation in the gut mucosa.

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

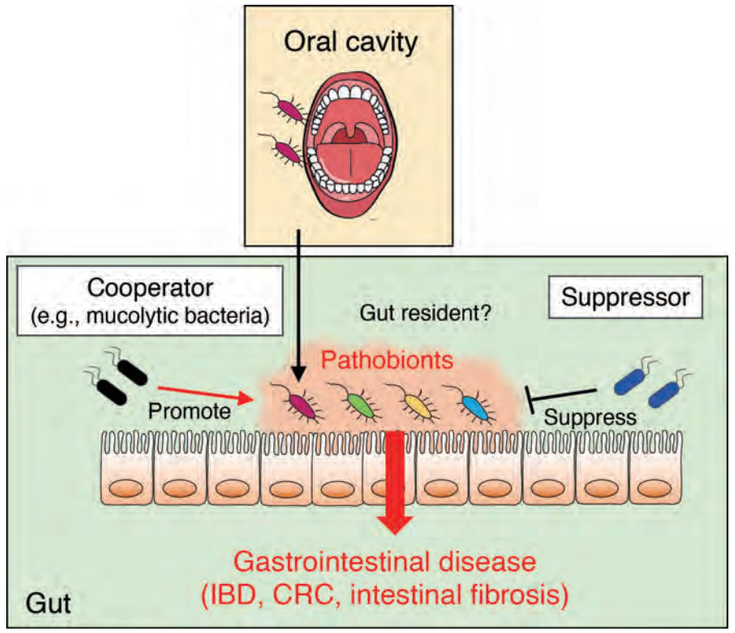

Figure.
Pathobionts promote various gastrointestinal diseases, including IBD, CRC, and intestinal fibrosis. Some pathobionts are oral origins. Certain symbiotic bacteria, such as mucolytic bacteria, act as 'cooperator' and help pathobionts acquire required nutrients. Some symbiotic bacteria may serve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

Recent Publications

1. Sugihara K, Kitamoto S, Saraithong P, Nagao-Kitamoto H, et al. Mucolytic bacteria license pathobionts to acquire host-derived nutrients during dietary nutrient restriction. Cell Reports. 40(3):111093 (2022). doi: 10.1016/j.celrep.2022.111093.

2. Imai J, Ichikawa H, Kitamoto S, Golob JL, Kaneko M, Nagata J, Takahashi M, Gillilland MG, Tanaka R, Nagao-Kitamoto H, Hayashi A, Sugihara K, Bishu S, Tsuda S, Ito H, Kojima S, Karakida K, Matsushima M, Suzuki T, Hozumi K, Watanabe N, Giannobile WV, Shirai T, Suzuki H, Kamada N. A potential pathogenic association between periodontal disease and Crohn's disease. JCI Insight. e148543 (2021). doi: 10.1172/jci.insight.148543.

3. Kitamoto S, Nagao-Kitamoto H, et al. 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, et al. 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).

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Cutaneous Allergy and Host Defense

Yumi Matsuoka-Nakamura, MD/PhD

Professor Yumi Matsuoka-Nakamura

Our laboratory team was established in November 2023. We are interested in understanding the pathogenesis of inflammatory skin diseases, such as atopic dermatitis (AD) and acne vulgaris. We also aim to unravel the mechanism by which Methicillin-resistant *Staphylococcus aureus* (MRSA) develops multi-drug resistance and instigates intractable infections in hospitals. In particular, our research projects are focused on the interaction between the *S. aureus* accessory gene regulator (Agr) quorum sensing (QS) and host innate immune responses. QS refers to the ability of bacteria to sense their population density. As bacterial populations grow, they risk nutrient scarcity. Consequently, they alter their behavior by regulating the production of various genes and substances, aiming to acquire more nutrients from the host or eliminate bacteria with similar nutritional requirements.

The expression of Staphylococcus Agr is crucial for epidermal colonization and is associated with the development of AD, which often correlates with colonization by *S. aureus* on the affected skin. AD is the most prevalent allergic skin disease, affecting 15-20% of children and 2-10% of adults worldwide. Both intrinsic genetic factors, such as susceptibility to type 2 inflammation or skin barrier dysfunction, and extrinsic environmental factors, like air pollen and skin microbiota, contribute to AD. *S. aureus*, which does not typically colonize the skin of healthy individuals, is often found in the lesional skin of patients with AD, correlating with disease flares. However, the role of *S. aureus* in the pathogenesis of AD has not been fully elucidated. We previously discovered that Th2-type dermatitis can be induced through mast cell degranulation triggered by δ-toxin under Agr-QS expression. We also found that PSMα under Agr-QS expression induces skin barrier disruption and IL-17-dependent dermatitis. In our study using whole-genome sequencing of *S. aureus* strains isolated from the skin of Japanese infants, we observed that infants who developed AD early in life were more likely to have cheek skin colonized by *S. aureus*. Interestingly, infants harboring *S. aureus* with spontaneous mutations in *agr* were more likely to remain healthy, despite the presence of this bacterium on their skin. This work suggests that *S. aureus* and functional QS may play a role in the onset of AD in children.

In a separate infant cohort study in collaboration with pediatricians at Chiba University, we monitored patients who received skincare interventions, and obtained longitudinal skin microbiome samples from the presymptomatic stage of infantile AD and food allergies (FA). We are currently analyzing the association between presymptomatic changes in the infant skin microbiome and the onset of AD and FA.

In our ongoing project, we are investigating how Agr-QS in *S. aureus* contributes to MRSA's adaptation in hospital environments and its acquisition of multidrug resistance. MRSA is a significant health problem worldwide, not only due to its high pathogenicity but also its propensity to cause infection outbreaks. The mechanisms by which bacteria adapt to hospital environments to cause recurrent disease remain unknown. To understand the bacterial factors that enable *S. aureus* to survive and persist in the hospital environment, we are analyzing MRSA strains isolated during an infection outbreak in a neonatal intensive care unit (NICU) in a hospital in Japan.

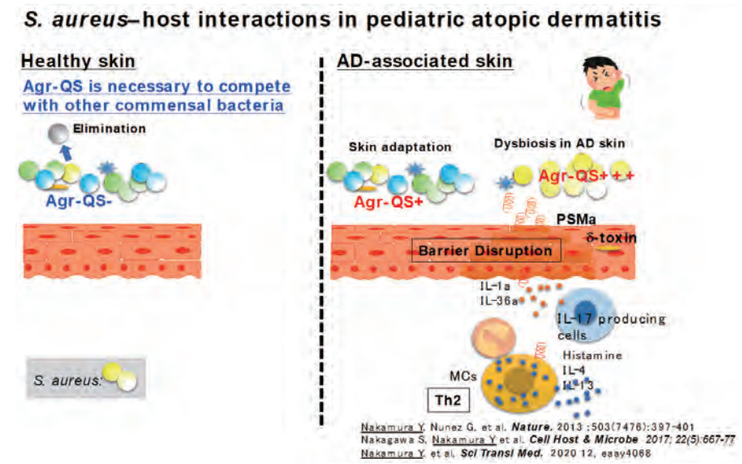

Figure.
The role of the *Staphylococcus aureus* accessory gene regulator quorum sensing system in the development of atopic dermatitis.

Recent Publications

1. Iwasawa MT, Miyachi H, Wakabayashi S, et al. Epidermal clearance of Candida albicans is mediated by IL-17 but independent of fungal innate immune receptors . Int Immunol. 34(8):409-420 (2022).

2. 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).

3. Nakamura Y, Takahashi H, Takaya A, et al. Staphylococcus Agr virulence is critical for epidermal colonization and associates with atopic dermatitis development . Sci Transl Med. 12(551): eaay4068 (2020).

4. Miyachi H, Wakabayashi S, Sugihira T, et al. Keratinocyte IL-36 Receptor/MyD88 Signaling Mediates Malassezia-Induced IL-17-Dependent Skin Inflammation . J Infect Dis. 223(10):1753-1765 (2021).

5. Nakagawa S, Matsumoto M, Katayama Y, Oguma R, Wakabayashi S, Nygaard T, Saijo S, Inohara N, Otto M, Matsue H, Núñez G, Nakamura Y. Staphylococcus aureus Virulent PSMα Peptides Induce Keratinocyte Alarmin Release to Orchestrate IL-17-Dependent Skin Inflammation Cell Host Microbe. 22(5):667-677 (2017).

Single Molecule Imaging

Toshio Yanagida, PhD
Ben Seymour, MD/PhD

Professor	Toshio Yanagida
	Ben Seymour

Illness, infection and injury are an inevitable consequence of life in a hazardous and competitive world, and animals' ubiquitous capability for tissue healing provides a passage to recovery that has been an essential feature of life across species throughout evolution. Alongside immune mechanisms, illness is associated with a pattern of recuperative behaviour that allows safe and effective recovery, typically including pain, fatigue, and changes in sleep, appetite, and mood. These behaviours are considered adaptive in an evolutionary context, as they help promote optimal recovery and maximise survival chance. But they remain poorly understood and frequently appear in clinical contexts to be exaggerated. Thus they reflect a major component of symptomatic states and quality of life in many patients, spanning immune disease to musculoskeletal injury, and often do not respond to treatment of the tissue event itself. A major goal of our lab is to try and understand the central, brain process that reflect the highest level of control of illness and injury states, directing physiological, immune, and behavioural changes.

At the heart of our approach is conceptualising illness/injury homeostasis as control-theoretic problem, and building models of brain information processing that optimize some quantitative function of recovery. We have proposed that this process approximates a hierarchical Bayesian control process, which the brain uses multimodal sensory information to infer injury and illness states, represented in cortical-subcortical networks, spanning insula and hypothalamic hubs in particular. These then control multiple effector processing, including modulation of autonomic, immune and endocrinological processes, alongside behavioural control processes (Seymour et al, 2023).

We have explored several aspects of this homeostatic control model. On the sensor arm of the process, one key question is how the brain monitors the temporal evolution of injury through the constant stream of nociceptive (pain) information. We have recently shown, using fMRI, how this feeds into a statistical model of the pain-causing process, and relies on a pathway through somatosensory cortices in the brain (Mancini et al, 2022). Critically, and consistent with the Bayesian model, higher (top-down) cortical processing modulate the incoming sensory signals according to their uncertainty (Mulders et al, 2023).

On the effector side, another question is how behaviour is controlled: in particular, whether opiidergic or dopaminergic signalling pathways modulate the perception of pain in this control loop. We have recently shown that dopaminergic pathways appear most important, and discovered a highly specific modulation of pain perception according to information-specific components of control (Dersch et al, 2023).

This ultimately leads to the question as to whether these putative insula-centric neural control hubs are relevant to our understanding of symptom generation – pain, fatigue, and mood changes – in immunological disease. Our ongoing clinical research in rheumatoid arthritis suggests that it is, and using brain network modelling we've been able to show that insula centrality (a graph-theoretic measure of brain networks) appears central to symptom states.

Overall, this suggests that optimal treatment of immune disease may require targeting both immune process peripherally, and brain processes centrally. This highlights the potential application of our currently research developing novel interventional technologies that could achieve this.

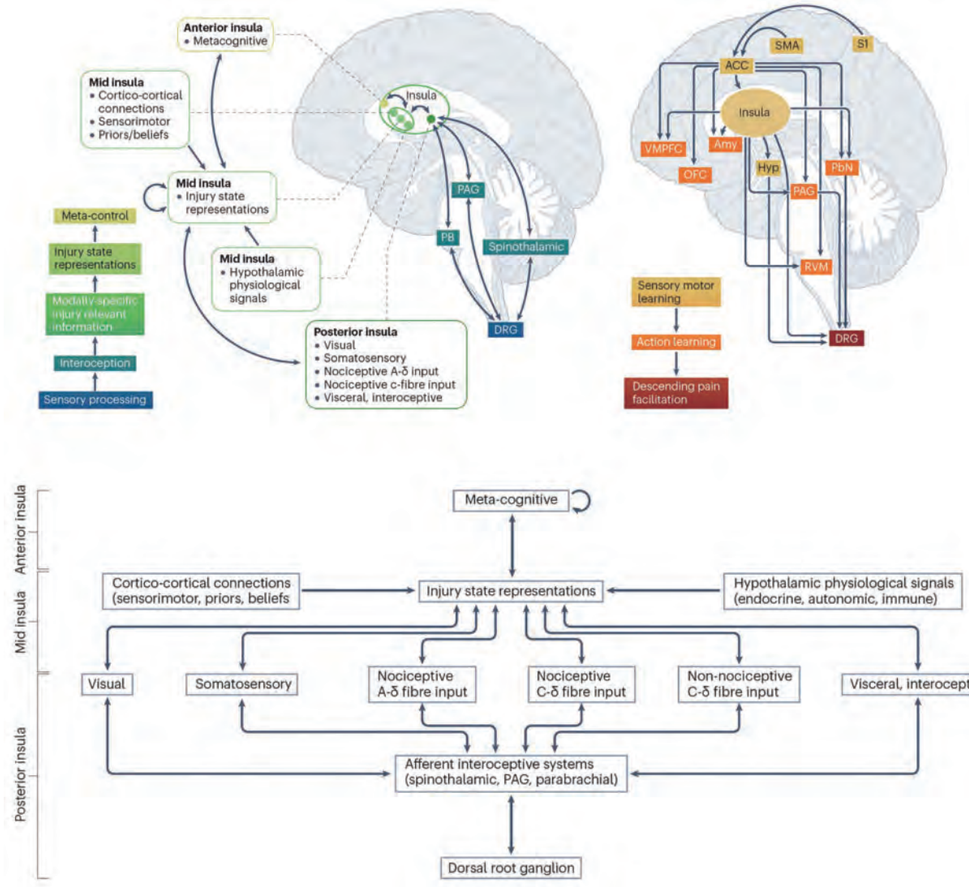

Figure.
Information flows and afferent (*left*) and efferent (*right*) pathways for insula-centered injury-state inference and effector control. At the heart of this is an insula-centered hierarchy with successively higher latent abstractions of the injury state. Afferent pathways feed various inputs to the hub, from subcortical and cortico-cortical projections; and efferent routes can implement different types of responses. This includes the multiple afferent pathways that ascend the spinal cord to various brainstem nuclei, such as the parabrachial, periaqueductal gray (PAG), dorsal respiratory group (DRG), locus coeruleus (LC) and others, forming the bidirectional brainstem-subcortical network. Below this is a schematic illustration of an insula-hub perspective for injury state representations in more details, including the different types of sensory information important for inference. The anterior, mid, and posterior segments of the insula have distinct and complementary functional roles. Note that the injury state inference may be shared with broader cortical areas, including the ACC and VMPFC, which are omitted here.

Recent Publications

1. Seymour B, Crook RJ & Chen ZS. Post-injury pain and behaviour: a control theory perspective. Nat Rev Neurosci. 1-15 (2023).
2. Mancini F, Zhang S, & Seymour B. Computational and neural mechanisms of statistical pain learning. Nat Commun. 13(1):6613 (2022).
3. Mulders D, Seymour B, Mouraux A, & Mancini F. Confidence of probabilistic predictions modulates the cortical response to pain. Proc Natl Aca Sci USA. 120(4):e2212252120 (2023).
4. Desch S, Schweinhardt P, Seymour B, Flor H, & Becker S. Evidence for dopaminergic involvement in endogenous modulation of pain relief. Elife. 12:e81436 (2023).

Immunology and Cell Biology

Masaru Ishii, MD/PhD

Professor	Masaru Ishii
Associate Professor	Junichi Kikuta
	Yasuhito Yahara
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	Kentaro Fujii
	Maki Uenaka
Postdoctoral Fellow	5
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).

The ability to observe live, moving cells has led to the identification of cell populations with novel functions that would not have been discovered using conventional methods. For example, macrophages are chameleon-like cells that change their properties according to their specific location in tissue. Using conventional analytical methods to isolate macrophages from tissue or organs of interest would result in changes to their phenotypes; thus, cell populations specifically inhabiting local tissue would not be recognized with these methods. To date, we have successfully identified novel cell fractions that can only be found in live tissue by using unique imaging technologies under a variety of pathological conditions. For example, osteoclasts are macrophage lineage cells that digest old bone and facilitate the turnover of bone tissue; it is believed that overactivation of osteoclasts in rheumatoid arthritis and bone metastasis can cause pathological bone destruction. We captured images showing the process of inflammation-induced bone/joint destruction and discovered a novel type of “bad” osteoclast in the diseased bone tissue, in contrast to the “good” osteoclasts observed in normal tissue (Figure 2). Similarly, we utilized intravital imaging techniques to identify the specific pathogenic macrophage that induces fibrogenesis in chronic and refractory interstitial pneumonia. These achievements in discovering novel cell fractions were based on the observation of live, moving cells in tissue. Imaging analysis of the dynamic cellular society has proven effective for identifying a variety of new members.

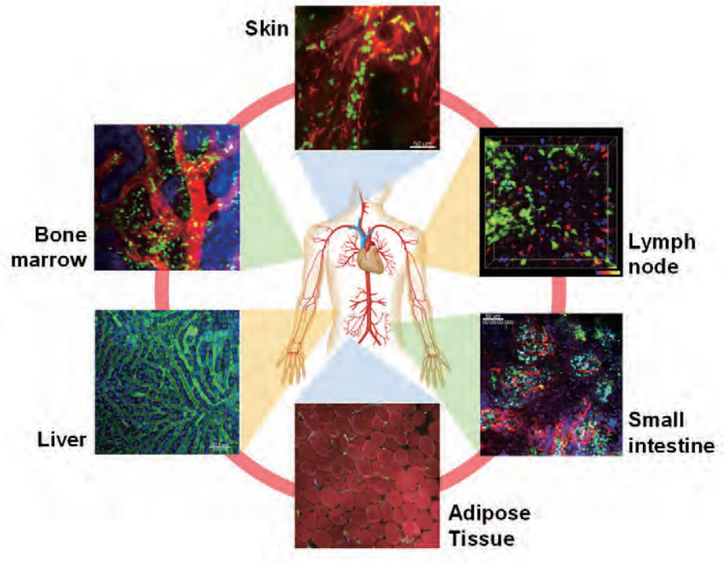

Figure 1.
A dynamic cellular system inside the living body.

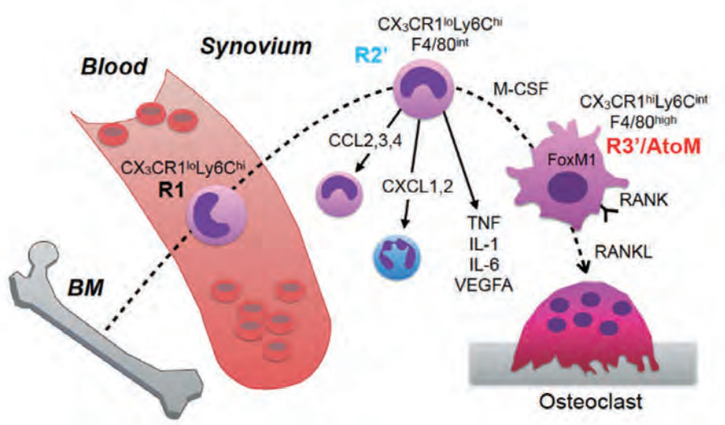

Figure 2.
Schematic illustration of ‘bad’ osteoclast differentiation axis in arthritic inflammation.

Recent Publications

1. 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).

2. Uenaka M, Yamashita E, Kikuta J, et al. Osteoblast-derived vesicles induce a switch from bone-formation to bone-resorption in vivo. Nat Commun. 13:1066 (2022).

3. Sudo T, Motomura Y, Okuzaki D, Hasegawa T, et al. Group 2 innate lymphoid cells support hematopoietic recovery under stress conditions. J Exp Med., 218(5):e20200817 (2021).

4. Morimoto A, Kikuta J, Nishikawa K, et al. SLPI is a critical mediator that controls PTH-induced bone formation. Nat Commun. 2(1):2136 (2021).

5. Hasegawa T, Kikuta J, Sudo T, et al. Identification of a novel arthritis-associated osteoclast precursor macrophage regulated by FoxM1. Nat Immunol, 20(12):1631-1643 (2019).

Chemical Imaging Techniques

Kazuya Kikuchi, PhD

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Research Assistant	1
Support Staff	1

'OFF–ON–OFF' fluorescence protein-labeling probe for real-time visualization of the degradation of short-lived proteins in cellular systems

Short-lived proteins play critical roles in various cellular processes, but their rapid turnover poses a challenge for researchers studying their functions and dynamics. Traditional methods for monitoring protein degradation, such as pulse-chase experiments or Western blotting, provide only static snapshots of protein levels, making it difficult to capture the dynamic changes in protein levels that occur in real time. Several covalent self-labeling fluorescent tags for imaging protein constructs in cell systems have been developed. However, these labeling systems are not well-suited to imaging cellular protein degradation, because the fluorophore remains fluorescent after the protein-probe conjugate has been degraded.

We here present a new approach for tracking the degradation of short-lived proteins in living cells through use of new trifunctional PYP-tag labeling 'OFF–ON–OFF' probes that contain distinct xanthene fluorophore, coumarin ligand binding and dinitroaryl quenching units. Xanthene-based fluorophore fluoresce in the green to near-infrared (NIR) region with high quantum yields and good molar absorptions that are well suited for cellular imaging studies. The fluorophore of the unbound probe is turned'OFF' via intramolecular association between the xanthene fluorophore and the dinitroaryl quenching unit. Covalent binding of the probe's coumarin ligand to the hydrophobic pocket of a PYP-tag protein results in a trans-thioesterification reaction with the thiol unit of Cys69, which cleaves the probe's quenching unit to produce a PYP-tag–probe conjugate whose fluorescence is turned 'ON'. Subsequent proteolytic degradation of the fluorescent PYP-tag–probe conjugate then generates probe-labeled fragments whose fluorescence are turned 'OFF' through intramolecular quenching interactions between the probe's xanthene fluorophore and its coumarin ligand unit (Fig. 1, top). Therefore, the fluorescence signal emitted by the probe is an "OFF–ON–OFF" pattern, with the signal initially off, turning on upon degradation of the target protein, and then turning off again as the fluorescence protein is degraded.

In vitro treatment of PYP-tag with F5-DNB2 resulted in rapid formation of fluorescent PYP-tag–probe conjugates that gave maximal fluorescence intensities after 60 min. These fluorescent conjugates were then treated with trypsin to induce proteolytic digestion, with the fluorescence intensities of both PYP-tag–probe conjugates decreasing gradually over time, reaching stable fluorescence minima after 130 min. These results demonstrate that proteolysis of the fluorescent PYP-tag–probe conjugates produces the desired 'ON–OFF' switch in fluorescence in probe cleavage products. Next, real-time imaging of the proteolytic degradation of short-lived proteins in cellular systems using an OFF–ON–OFF fluorescence switch was performed. Mouse ornithine decarboxylase (MODC) was chosen as a potential short-lived protein to explore degradation studies because its C-terminal domain is rich in Pro, Glu, Ser and Thr (PEST sequence) residues that are known to induce rapid proteasomal degradation. F5-DNB2 probe was then used to visualize the expression (fluorescence 'ON') and proteolytic degradation (fluorescence 'OFF') of a PYP-tag-MODC$^{422\text{-}461}$ in cell nuclei in real time. Time-lapse imaging of the transfected cells using confocal microscopy revealed that the green fluorescence intensity of the cell nuclei decreased steadily over time (Fig. 1, bottom).

The "OFF–ON–OFF" fluorescence protein-labeling probe represents a significant improvement over traditional methods for monitoring protein degradation. It provides a real-time, dynamic readout of protein degradation that can be used to study the functions and dynamics of short-lived proteins in living cells. The probe can be adapted to target other short-lived proteins of interest and can be combined with other imaging techniques, such as confocal microscopy, to provide more detailed insights into protein degradation pathways.The probe has broad applications in drug discovery, basic research, and biomedical imaging, and has the potential to open up new avenues of investigation into the mechanisms that regulate protein turnover in living cells.

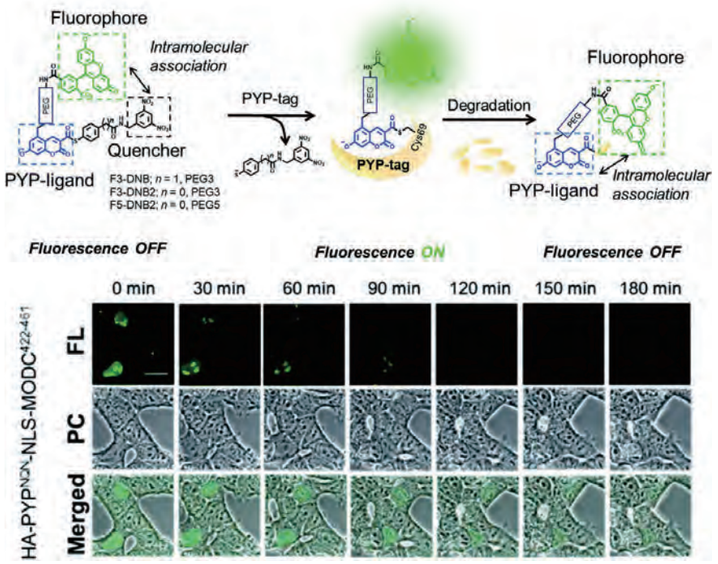

Figure 1.
Schematic illustration of OFF-ON-OFF imaging for protein degradation using fluorogenic probes (top), Live cell imaging of degradation of short-lived protein using F5-DNB2 (bottom).

Recent Publications

1. Minoshima M, Umeno T, Kadooka K, Roux M, Yamada N, Kikuchi K. Development of a Versatile Protein Labeling Tool for Live-Cell Imaging Using Fluorescent β-Lactamase Inhibitors. Angew Chem Int. Ed. 2023, in press. doi: 10.1002/anie.202301704.
2. Yari S, Kikuta J, et al. JAK Inhibition Ameliorates Bone Destruction by Simultaneously Targeting Mature Osteoclasts and Their Precursors. Inflamm Regen., 43:1-12 (2023).
3. Salaam J, Minoshima M, Kikuchi K. Recent Advances in Activatable ^{19}F Magnetic Resonance Imaging Nano-Probes for the Detection of Biomarkers. Anal Sens. 2023, in press. doi: 10.1002/anse.202200081.
4. Hashimoto R, Minoshima M, et al. Efficient Visible/NIR Light-Driven Uncaging of Hydroxylated Thiazole Orange-Based Caged Compounds in Aqueous Media. Chem Sci. 13:7462-7467 (2022).
5. Reja SI, Hori Y, et al. An "OFF-ON-OFF" fluorescence protein-labeling probe for real-time visualization of the degradation of short-lived proteins in cellular systems. Chem Sci. 13:1419-1427 (2022).

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 β_2-adrenergic receptor expressed on lymphocytes enhance the responsiveness of a specific set of 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 β_2-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 suggested that the COMMD3/8 complex may 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).

Having established the involvement of the COMMD3/8 complex in a mouse model of rheumatoid arthritis, we demonstrated that celastrol exerts anti-inflammatory activity by targeting the COMMD3/8 complex. These findings provide a proof of concept that disrupting the interaction between COMMD3 and COMMD8 may be a useful strategy for the treatment of autoimmune diseases and supports the consideration of celastrol as a lead candidate in that endeavor.

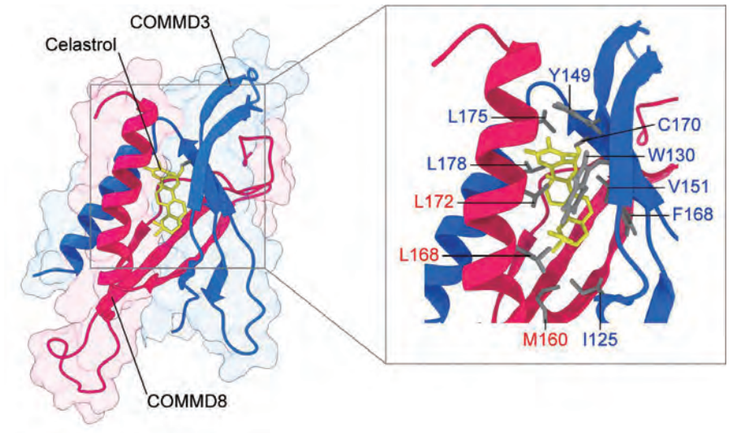


Figure.
A model of the celastrol-bound COMMD3/8 complex.

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).

4. Nakai A, Hayano Y, Furuta F, Noda M and Suzuki K. Control of lymphocyte egress from lymph nodes through β_2-adrenergic receptors. J Exp Med. 211:2583-2598 (2014).

Biophotonics

Nicholas Isaac Smith, PhD

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

The Biophotonics laboratory develops tools for label-free analysis of single cells. Single-cell analysis is a popular target for a large number of researchers, 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 made several important advances. Extending our previous project focused on macrophage uptake of fatty acids, we looked at metabolic and spatiotemporal changes in macrophages in a fatty acid-specific manner. Of high importance for artherosclerosis and lifestyle diseases, HDL and LDL cholesterol uptake in macrophages plays a critical role in plaque formation. Therefore we imaged HDL and LDL uptake in macrophages and found evidence that the form of cholesterol may be changing (i.e. in terms of degree of crystallization) after the uptake occurs, and found a specific Raman biomarker that can be used to identify crystallized cholesterol within the cell.

Methods for rapid and non-invasive detection of cell phenotype continue to be one of our primary interests, especially at higher throughputs than typically used in Raman analysis. Accordingly, we completed a Raman-based study to monitor T-cell activation/differentiation states. Murine naïve T-cells undergo activation and then differentiate into effector cells when co-cultured with artificial antigen-presenting cells (aAPCs), and Raman spectra were collected from groups of between 2000-2500 cells per group, per day. Statistical models were developed to assess the degree of changes occurring on a single-cell level, and these label-free metrics were further validated by additionally using gold-standard T-cell analytic methods such as CD25/CD69 and CD62L/CD44 surface marker measurements. The use of machine learning methods can also be used to assist in the visualization of the transitory single-cell states across the whole experiment. See Pavillon et al 2023, and figure 1 for details.

We completed several works with collaborating groups including in microscopy techniques with the Fujita group in Applied Physics, where we share a common interest in higher throughput label-free imaging. Dr Lelliot also completed a study with collaborators in the Kumanogoh group, where we evaluated a long-standing goal in the lab to determine whether label-free Raman analysis could be implemented to reliably identify the presence of the neutrophil extracellular trap (NET) response in a non-invasive manner. Neutrophils produce extracellular debris, which is a loosely aggregated gel-like structure that contains various biomolecules, forming in response to a wide variety of disease conditions, including COVID-19. While it is known that NETs are implicated in many diseases, there is still considerable debate on how they should be defined, and whether apparent subtypes should be considered as separate processes. We used Raman analysis on NETs from human donors, and even though the NETs themselves are less dense than our typical cell targets, the Raman analysis could successfully determine the presence of NETs, could discriminate clearly between NETs and other types of cell debris (such as occurs in necrosis) and could even determine which pathway had been used to activate the NET response.

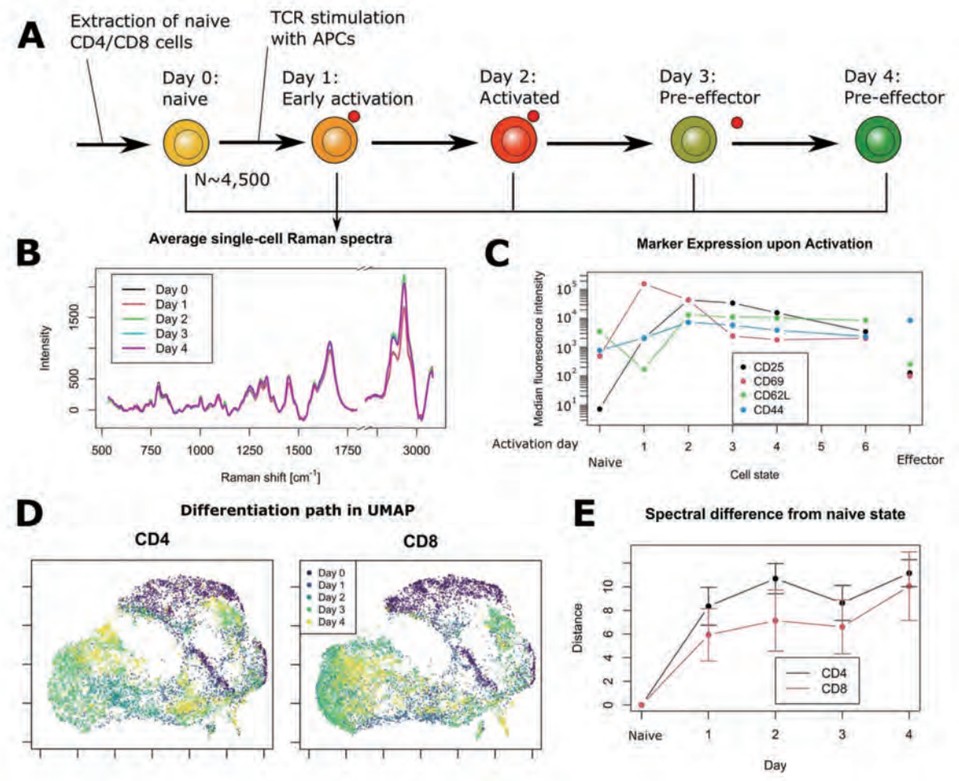


Figure.
Early T cell differentiation monitored with Raman spectroscopy. (A) Experimental protocol, where murine T cells are stimulated with artificial APCs and measured every day during 5 days. (B) Average single-cell Raman spectra (approximately 2000 cells per day and per type), showing that only small changes can be observed during the onset of activation. (C) Median fluorescence intensity signal derived from the expression of surface markers of CD4 cells over time, representative of T cell activation and differentiation. (D) UMAP decomposition of Raman data, where the gradual evolution of the signal upon activation can be readily observed. Differences between phenotypes (CD4/CD8) are also clearly visible. All results are representative of at least 3 experiments. (E) Quantification of the daily signal changes with the Mahalanobis-like distance. Curves are the average of 3 experiments, error bars indicate standard deviation. See Pavillon et al 2023 for details.

Recent Publications

1. Pavillon N, and Smith NI. Non-invasive monitoring of T cell differentiation through Raman spectroscopy. Scientific Reports 13(1):3129 (2023).

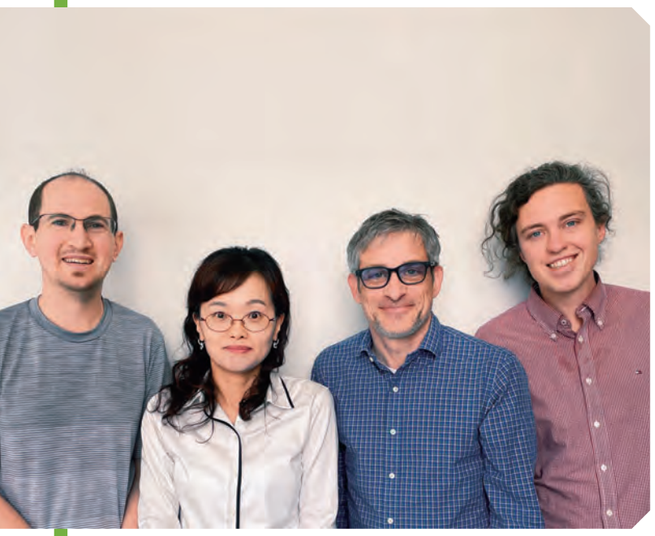
2. Lelliott PM, et al. Cellular Adhesion Is a Controlling Factor in Neutrophil Extracellular Trap Formation Induced by Anti-Neutrophil Cytoplasmic Antibodies. ImmunoHorizons 6(2):170-183 (2022).

3. Pavillon N, and Smith NI. Deriving accurate molecular indicators of protein synthesis through Raman-based sparse classification. Analyst 146:3633-3641 (2021).

4. Sugiyama T, Hobro AJ, Pavillon N, Umakoshi T, Verma P, and Smith N. 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 M. Standley, PhD

Professor	Daron M. Standley
Associate Professor	Kazutaka Kato
	Li Songling
	Soyoung Park
Assistant Professor	Shuhei Sakakibara
	Chao-Yuan Tsai
Postdoctoral Fellow	3
Research Assistant	2
Support Staff	1

Classification of health/disease from Adaptive Immune Receptors (AIRs)

We are developing methods to predict health/disease status from AIRs. In particular, we are interested in identifying signatures of past or present antigen engagement that are accessible in blood. One of our goals is to represent such signatures as machine learnable features that generalize well to new donors (i.e. ,not seen by the classifier). We have found that the most widely used AIR features (clonotype clusters) do not generalize well to new donors, but that our proposed paratope network features do generalize well. Our preliminary data for COVID-19, autoimmune hepatitis (AIH), and breast cancer suggests that these features are robust and have the potential to be novel disease biomarkers (Fig.1).

Drawbridge model for T cell receptor triggering

T cell receptors (TCRs) play a critical role in adaptive immunity. Despite decades of research, the mechanism that transmits peptide-MHC (pMHC) binding to intracellular signaling – TCR triggering – remains enigmatic. TCR-CD3 complexes both with and without a bound peptide-MHC (pMHC) exhibit little variation by single-particle cryo-EM microscopy, which makes it difficult to understand how changes in TCR structure trigger signaling across the plasma membrane. To visualize TCR dynamics, we performed molecular dynamics simulations of TCR-CD3 and TCR-CD3-pMHC complexes in which the TCR-CD3 and pMHC components were embedded in separate membranes. CD3 protein dynamics were qualitatively different in the two systems, and we found that conformational changes in the TCR directly regulated CD3 dynamics. Specifically, in the TCR-CD3-pMHC system, the TCR acted as a "drawbridge" that allowed the CD3 proteins to move freely, thus transmitting the signal from outside to inside the T cell (Fig. 2).

Structure/function analysis and development of RNA inhibitors of Regnase-1

Regnase-1 is an important RNA binding protein that controls inflammatory and immune responses. Regnase-1-deficient CD8+ T cells markedly improve therapeutic outcomes in mouse models of melanoma (J. Wei et al. Nature, 2019). We are investigating RNA-bound structures of Regnase-1 and developing nucleic acid therapeutics to inhibit Regnase-1 using chemically modified oligonucleotides. We have designed amino acid-conjugated oligonucleotides based on structural analysis of Regnase-1 and are investigating their binding efficacy and inhibitory activity using biophysical techniques such as electrophoretic mobility shift assays and microscale thermophoresis (Fig. 3).

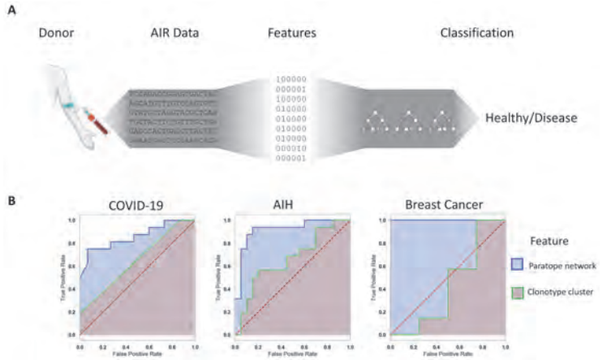


Figure 1.
Classification of health/disease from Adaptive Immune Receptors (AIRs). A, AIRs can be easily acquired from a routine blood sample, sequenced and transformed into feature vectors for use in machine-learning based classifiers.
B, We have tested the performance of classifiers trained using the popular clonotype cluster features and on our newly proposed paratope network features. In a wide range of diseases, including COVID-19, autoimmune hepatitis, and breast cancer, the paratope network features were more robust than clonotype cluster features.

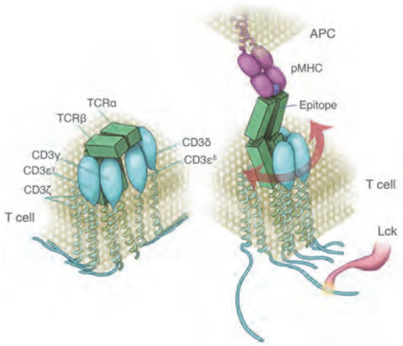


Figure 2.
Drawbridge model for T cell receptor triggering. At rest, the T cell receptor adopts a bent conformation in which it retains the CD3 proteins (left). Binding to a pMHC involves the elongation of the T cell receptor. This breaks the interactions between the T cell receptor and the CD3 proteins, and the CD3 proteins are subsequently free to diffuse around the T cell receptor (right).

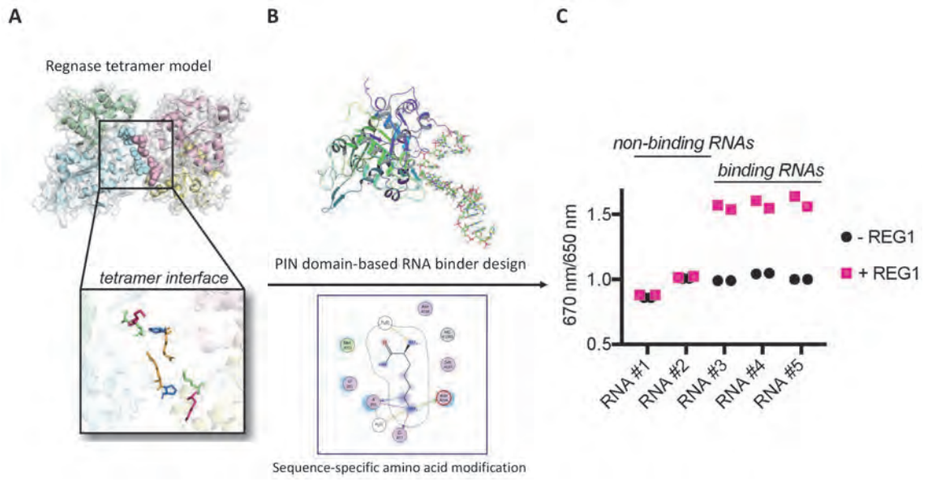


Figure 3.
Structure/function analysis and development of RNA inhibitors of Regnase-1. A, A model of the Regnase-1 tetramer showing critical residues at the dimer-dimer interface (inset). **B,** Design of modified RNA inhibitors based critical residues involved in RNA binding and cleavage. **C,** Microscale thermophoresis showing the spectral shift of Cy5-labeled RNAs binding to recombinant Regnase-1 protein.

Recent Publications

1. Tanaka A, Maeda S, Nomura T.et al. Construction of a T cell receptor signaling range for spontaneous development of autoimmune disease. J Exp Med. 220 (2023) 10.1084/jem.20220386.
2. Jiravejchakul N, Abe GL, Loza M, Park S, Matangkasombut P, Sasaki JI, Imazato S, Diez D & Standley DM. Intercellular crosstalk in adult dental pulp is mediated by heparin-binding growth factors Pleiotrophin and Midkine. BMC Genomics 24:184 (2023) 10.1186/s12864-023-09265-w.
3. Eerden FJv, Sherif AA, et al. TCR-pMHC complex formation triggers CD3 dynamics. eLife 2023 in press.
4. Xu Z, Davila A, Wilamowski J, Teraguchi S. & Standley DM. Improved Antibody-Specific Epitope Prediction Using AlphaFold and AbAdapt. Chembiochem 23:e202200303 (2022) 10.1002/cbic.202200303.
5. Shirai T, Nakai A, et al. Celastrol suppresses humoral immune responses and autoimmunity by targeting the COMMD3/8 complex. Sci Immunol. 8 (2023), eadc932410.1126/sciimmunol.adc9324.

Statistical Immunology

Yukinori Okada, MD/PhD

Professor	Yukinori Okada
Associate Professor	Qingbo S Wang
Assistant Professor	Kenichi Yamamoto
Research Assistant	6
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.

Metagenome-wide association study of gut microbiome and virome revealed disease-specific features

Microbiomes play substantial roles in homeostasis and biology of a variety of human diseases through interaction with host. Metagenome-wide association studies (MWAS) utilizing whole-genome shotgun sequencing is a promising tool to elucidate microbiome etiology. To construct gut microbiome catalogue of Japanese, we assembled 19,084 prokaryotic and 31,395 viral genomes from 787 Japanese gut metagenome shotgun sequencing data; JMAG and JVD (Figure 1). Traditional Japanese food-related features were observed in Japanese microbial genome, such as natto (fermented soybeans) and nori (laver). Dietary-related Enterococcus_B lactis and Streptococcus thermophilus were nominally associated with the East Asian-specific missense variant rs671:G>A in ALDH2, which was associated with dairy consumption. We annotated subtypes of crAss-like phages and identified their associations with disease status and microbiome diversity. In particular, crAss-like phage dosages were decreased in autoimmune diseases including rheumatoid arthritis (RA), systemic lupus erythematosus, and inflammatory blow diseases (Tomofuji Y et al. Cell Genom 2022).

A cross-population atlas of human genotype-phenotype associations.

Construction of human genotype-phenotype catalog is essentially important to elucidate genetic backgrounds and underlying biology of diseases. Through leading international collaboration partnerships, we reported the cross-population GWAS meta-analysis integrating diverse human ancestry of RA (RACI consortium; Ishigaki K et al. Nat Genet 2022) and adult height (GIANT consortium; Yengo L et al. Nature 2022). These results highlighted the value of cross-population GWAS to improve disease risk prediction model and showed saturated map of common variant impacts on phenotype heritability. As for our current challenges in the COVID-19 pandemic, we conducted the COVID-19 GWAS of Japanese, which identified a risk variant close to the dedicator of cytokinesis 2 gene (DOCK2). Single-cell RNA-seq (scRNA-seq) identified cell-type-specific DOCK2downregulation and a COVID-19-specific decreasing effect of the risk allele in non-classical monocytes (Figure 2). DOCK2 inhibition increased the severity of pneumonia in a Syrian hamster model of SARS-CoV-2 infection. DOCK2 has an important role in severe COVID-19, and could be further explored as a potential biomarker and/or therapeutic target (NamKoong H et al. Nature 2022).

In silico drug repositioning based on human disease genomics

Utilization of human disease genetics for novel drug discovery is essential. Biological dynamics in disease status provides directional dosage effect on drug targets, which facilitates efficient high-throughput screening of chemical compounds library. We introduced a practical guideline for genomics-driven drug discovery as lessons from the Global Biobank Meta-analysis Initiative (GBMI). Integration of the three methods (GREP, Trans-Phar, endophenotype Mendelian randomization) provided a catalog of candidate compounds and target genes for drug repositioning (Namba S et al. Cell Genom 2022). We applied these drug discovery methods to the cross-population GWAS results of stroke and reported multiple promising targets (Mishra A et al. Nature 2022).

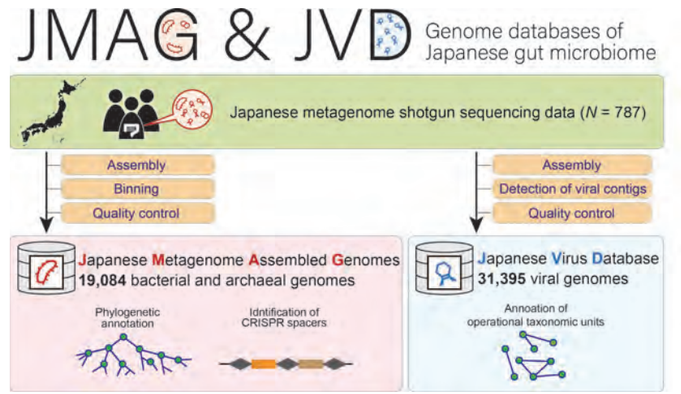


Figure 1.
JMAG (Japanese Metagenome Assembled Genomes Platform) and JVD (Japanese Virus Database) database for Japanese gut microbiome catalogue.

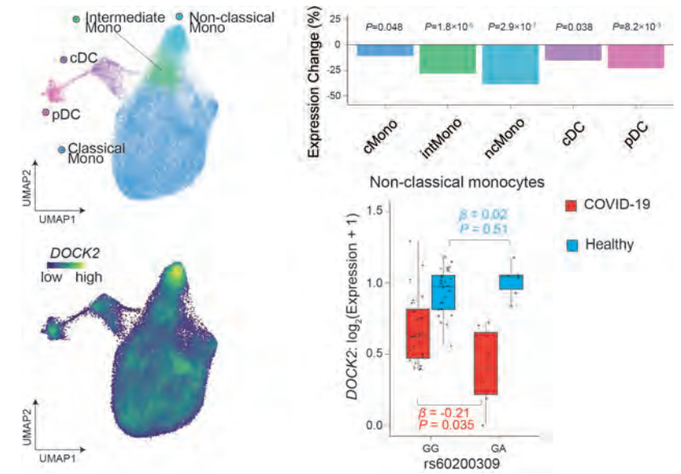


Figure 2.
Non-classical monocyte-specific and COVID-19 specific eQTL effect of the *DOCK2* variant.

Recent Publications

1. Namkoong H, et al. DOCK2 is involved in the host genetics and biology of severe COVID-19. Nature 609:754-760 (2022).
2. Yengo L, et al. A saturated map of common genetic variants associated with human height. Nature 610:704-712 (2022).
3. Namba S, et al. A practical guideline of genomics-driven drug discovery in the era of global biobank meta-analysis. Cell Genom 2:100190 (2022).
4. Tomofuji Y, et al. Prokaryotic and viral genomes recovered from 787 Japanese gut metagenomes revealed microbial features linked to diets, populations, and diseases. Cell Genom 2:100219 (2022).
5. Ishigaki K, et al. Multi-ancestry genome-wide association analyses identify novel genetic mechanisms in rheumatoid arthritis. Nat Gen. 54:1640-1651 (2022).

Quantitative Immunology

Associate Professor	Diego Diez
Postdoctoral Fellow	1

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

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). 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. 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 study the 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. With the Experimental Immunology laboratory and the Systems Immunology laboratories, we study how TCR signaling impacts T cell repertoire in SKG mice. With the Immune Regulation laboratory, we study the role of CD4 T cells in human Eosinophilic Chronic Rhinosinusitis. With the Host Defense laboratory, we study the role of the RNase Reg-1 in immune cell development.

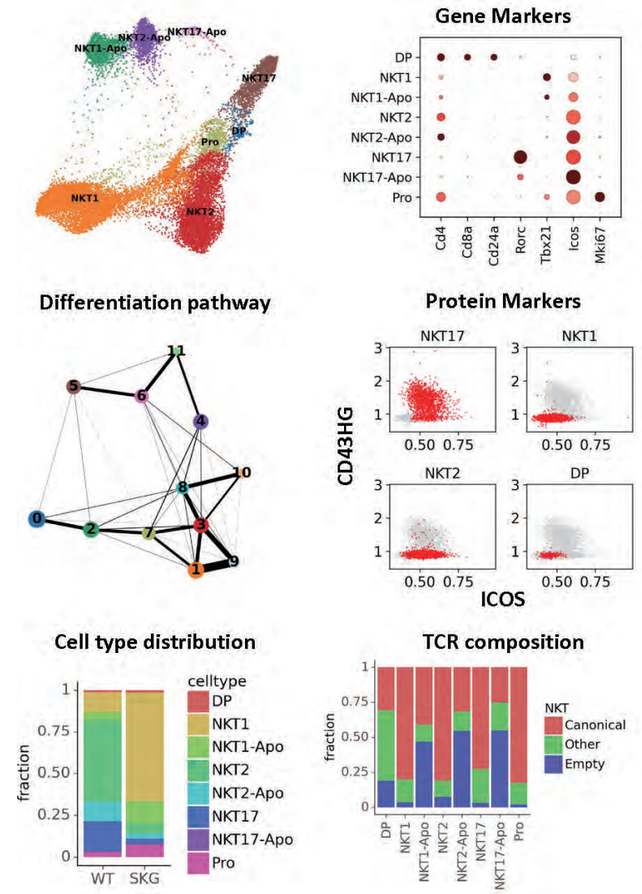


Figure.
Single cell genomics identifies RNA and TCR features of differentiating NKT cells.

Recent Publications

1. Loza M, Teraguchi S, Standley DM & Diez D. Unbiased integration of single cell transcriptome replicates. NAR Genom Bioinform 4, lqac022, doi:10.1093/nargab/lqac022 (2022).
2. 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).
3. Vandenbon A and Diez D. A clustering-independent method for finding differentially expressed genes in single-cell transcriptome data. Nat Commun 11(1):4318, doi:10.1038/s41467-020-17900-3 (2020).
4. Teraguchi S, Saputri DS, Llamas-Covarrubias MA, Davila A, Diez D, et al. Methods for sequence and structural analysis of B and T cell receptor repertoires. Comput Struct Biotechnol J 18, 2000-2011, doi:10.1016/j.csbj.2020.07.008 (2020).
5. Diez D, Agusti A & Wheelock CE. Network analysis in the investigation of chronic respiratory diseases. From basics to application. American journal of respiratory and critical care medicine 190:981-988, doi:10.1164/rccm.201403-0421PP (2014).

Events & Outreach Activities

International Symposium on Microbiology and Immunology

The 12th 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), and Osaka International Conference Center. Centering on basic research achievements relating to microbiology and immunology, this symposium consists of 11 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.

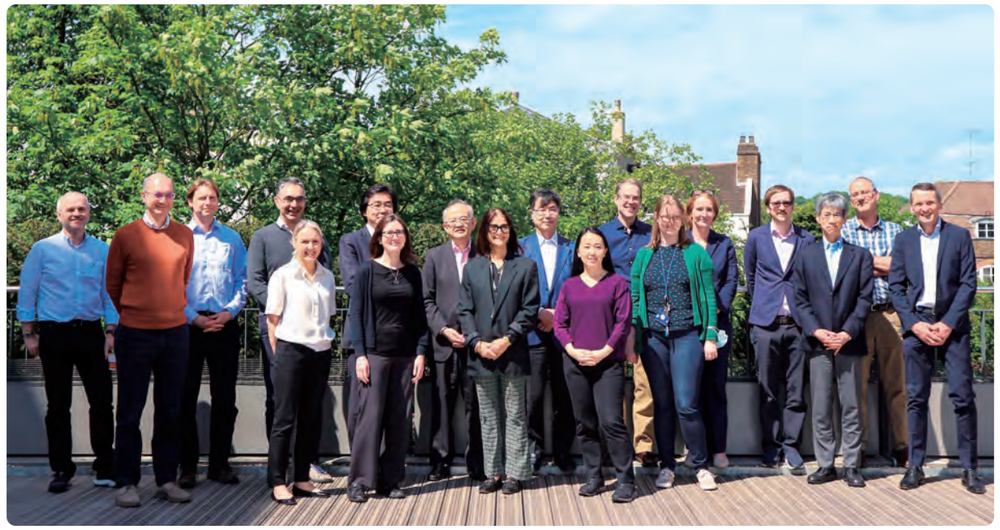
◆ Date : February 3, 2023
◆ Venue : Osaka International Convention Center (Grand Cube Osaka)

Speaker	Title
Keiji Itaka (CiDER, Osaka University/Tokyo Medical and Dental University, Japan)	"mRNA as a new drug modality"
Anna C. Aschenbrenner (DZNE, Bonn, Germany)	"From acute infection to chronification: What can transcriptomics teach us about a new disease?"
Yumi Matsuoka-Nakamura (IFReC, Osaka University, Japan)	"Impact of Staphylococcal Agr quorum-sensing system on atopic dermatitis and systemic infection"
Ron Geller (University of Valencia, Spain)	"Comprehensive profiling of polyclonal sera targeting a non-enveloped viral capsid"
Sidonia Eckle (University of Melbourne, Australia)	"Probing the universe of MAIT cell antigens and associated immune functions by MAIT cells"
Nobuhiko Kamada (IFReC, Osaka University, Japan)	"Intermucosal innate and adaptive immune networks in inflammatory disease"
Alison Simmons (University of Oxford, UK.)	"Human intestinal cellular anatomy through development, in health and inflammatory bowel disease"
Koji Yasutomo (Tokushima University, Japan)	"AFF genes as regulators for B and T cells"
Pavel Tolar (University College London, UK)	"Regulation of antigen persistence in germinal centres"
Hisashi Arase (IFReC, Osaka University, Japan)	"Autoimmunity by aberrant MHC class II self-antigen presentation associated with viral infection"
Alexander Rudensky (Memorial Sloan Kettering Cancer Center, USA)	"Regulatory T cells as purveyors of immune tolerance"

UCL - IFReC Osaka University Immunology Symposium

◆ Date : May 13, 2022
◆ Venue : Conference room at the University College of London

Speakers (order of talk)
1. Shimon Sakaguchi (IFReC)
2. Anne Pesenacker (UCL)
3. James Badger Wing (IFReC)
4. Dave Sansom (UCL)
5. Kiyoshi Takeda (IFReC)
6. Benedict Seddon (UCL)
7. Kazuyo Moro (IFReC)
8. Masaru Ishii (IFReC)
9. Lucy Walker (UCL)
10. Tomohiro Kurosaki (IFReC)
11. Claudia Mauri (UCL)
12. Emma Morris (UCL)
13. Steve Ley (UCL)
14. Maddi Noursadeghi (UCL)



IFReC - Doherty Institute & Partners Immunology Symposium

◆ Date : March 20-21, 2023
◆ Venue : Melbourne Connect, Forum 3 & University House, Matthaei Room

Speakers (order of talk)
1. Kiyoshi Takeda (IFReC)
2. Andrew Brooks (UoM/Doherty Institute)
3. Tomohiro Kurosaki (IFReC)
4. Sammy Bedoui (UoM/Doherty Institute)
5. Sho Yamasaki (IFReC)
6. Katherine Kedzierska (UoM/Doherty Institute)
7. Hisashi Arase (IFReC)
8. Alexandra Corbett (UoM/Doherty Institute)
9. Masaru Ishii (IFReC)
10. Michelle Boyle (Burnet Institute)
11. Kazuyo Moro (IFReC)
12. Stephen Nutt (WEHI)
13. Yumi Matsuoka (IFReC)
14. Amy Chung (UoM/Doherty Institute)
15. James Badger Wing (IFReC)
16. Axel Kallies (UoM/Doherty Institute)

The 1st ImmunoSensation² - IFReC International School on Advanced Immunology

The International School on Advanced Immunology started in 2022 and is jointly organized by IFReC and ImmunoSensation², Germany. The purpose of the school is to foster young immunologists into leaders of the next generation to lead to breakthroughs in the field of immunology. In the four-day intensive course, the school provided an opportunity for 49 international PhD students and young postdocs to meet with a faculty of 15 world-class experts to push the frontiers of immunology and network with peers of the same generation.

◆ **Date :** November 7-10, 2022
◆ **Venue : Awaji Yumebutai International Conference Center, Awaji-city, Hyogo, Japan**

Lecturers
Shizuo Akira (IFReC, Osaka University)
Ido Amit (Weizmann Institute of Science)
Eva Bartok (University Hospital Bonn, ImmunoSensation²)
Keishi Fujio (The University of Tokyo)
Florent Ginhoux (Singapore Immunology Network)
Koji Hase (Keio University)
Axel Kallies (The University of Melbourne)
Eicke Latz (University Hospital Bonn, ImmunoSensation²)
Elvira Mass (University of Bonn, ImmunoSensation²)
Gabriel Núñez (University of Michigan)
Marion Pepper (University of Washington)
Marco Prinz (University of Freiburg)
Michel Sadelain (Memorial Sloan Kettering Cancer Center)
Shimon Sakaguchi (IFReC, Osaka University)
Ziv Shulman (Weizmann Institute of Science)

NGS Expo 2022

The "NGS Expo" is a new academic event for users and potential users of next generation sequencer, and organized by IFReC and RIMD, Osaka University. About 500 people participated in this symposium, both on-site and online. We experimentally incorporated "online salon", a communication tool between participants, and nearly 40,000 page views were obtained.

◆ **Date :** October 18-19, 2022
◆ **Venue : Osaka International Convention Center (Grand Cube Osaka)**

Colloquia and Seminars

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

Date	Speakers
May 26, 2022	**Yukinori Okada** (PI, Statistical Immunology Lab) **Kazuhiro Suzuki** (PI, Immune Response Dynamics Lab)
July 21, 2022	**Sho Yamasaki** (PI, Molecular Immunology Lab) **Masahiro Yamamoto** (PI, Immunoparasitology Lab)
September 15, 2022	**Yasuharu Nagahama** (Host Defense Lab) **Bo Li** (Mucosal Immunology Lab)
November 17, 2022	**Shunsuke Mori** (Immunochemistry Lab) **Motonao Osaki** (Experimental Immunology Lab)
March 16, 2023	**Takashi Saito** (PI, Cell signaling Lab) **Michelle Lee** (Malaria Immunology Lab)

◆**IFReC ImmunoSeminar :** Inviting world-class immunologists mainly online.

Date	Speakers
April 28, 2022	**Henrique Veiga-Fernandes** (Champalimaud Center for the Unknown, Lisboa, Portugal)
June 24, 2022	**James McCluskey** (The University of Melbourne, Australia)
January 23, 2023	**Laurent Rénia** (Lee Kong Chian School of Medicine/ A*STAR Infectious Diseases Labs, Singapore)

◆**Other Seminars :** Inviting wide variety of scientists as speakers.

Date	Speakers
November 4, 2022	**Noboru Mizushima** (Graduate School of Medicine, University of Tokyo, Japan)
December 15, 2022	**Ryo Morimoto** (Thomas Boehm Lab, Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany)
January 31, 2023	**Anna C. Aschenbrenner** (Systems Medicine, DZNE, Bonn, Germany)
February 14, 2023	**Samuel Liegeois** (Host-Pathogen Interactions and Resilience, Insect Models of Innate Immunity, Strasbourg University, France)

Outreach Activities

In 2022, face-to-face outreach events were revived while implementing COVID-19 preventive measures.

The 11th WPI Science Symposium

◆ Date : November 23, 2022
◆ Venue : Ito Memorial Hall, The University of Tokyo
◆ Speakers : Kazuyo Moro (IFReC), et al.

Kagaku Zam-mai in Aichi

◆ Date : December 27, 2022
◆ Venue : Okazaki Conference Hall
◆ Speakers : Students from Super Science High Schools

SpringX Super School - Online Lecture series for protecting our lives from infectious diseases -

◆ Speakers :
- Yumi Matsuoka : "Staphylococcus aureus, an old but new bacteria" (Jun. 24, 2022)
- Daisuke Okuzaki : "Data analysis for COVID-19" (Aug. 26, 2022)
- Sho Yamasaki : "Immune system for eliminating pathogens" (Nov. 25, 2022)
- Masahiro Yamamoto : "Science of Toxoplasma" (Feb. 24, 2023)

実践的な学び X スタートアップ
SpringX
KNOWLEDGE CAPITAL

Osaka University Co-Creation Day at EXPOCITY

◆ Date : June 13, 2022
◆ Venue : LaLaport EXPOCITY

Life Science Seminar for High School Students

◆ Date : August 8, 2022
◆ Venue : Taniguchi Memorial Hall, RIMD and IFReC
◆ Speakers : Yumi Matsuoka (IFReC), et al.

Science Café at the Nakanoshima Festival

◆ Date : December 4, 2022
◆ Venue : Lecture Hall A, Osaka University School of Medicine
◆ Speaker : Yoko Fukushima (Department of Ophthalmology, Osaka University School of Medicine)

Japanese Language Class

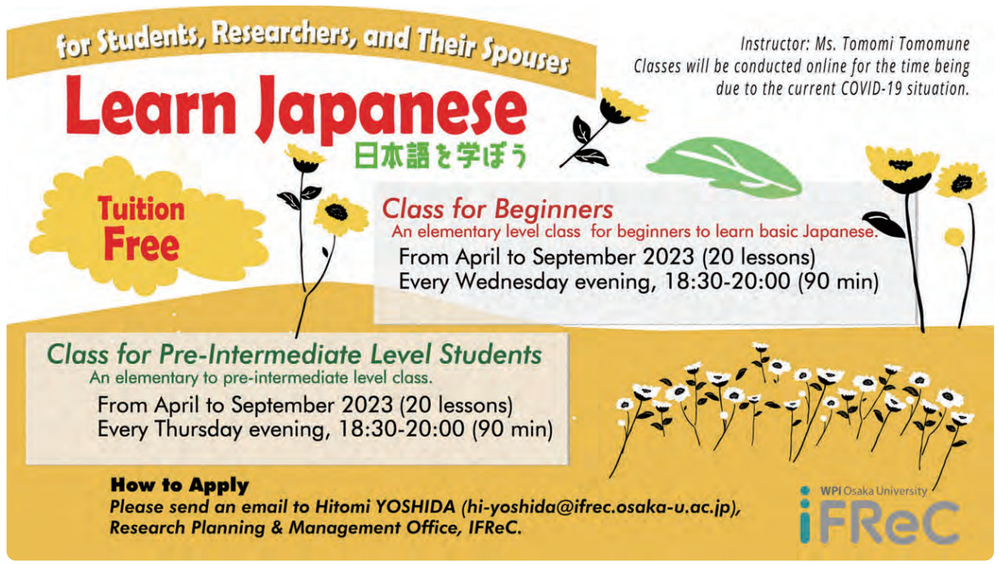
Message from Ms. Tomomi Tomomune, class instructor for 2023.

"I would like to support you by providing effective lessons to make your life more enjoyable. As we learn, we use Japanese to communicate and learn about each other. Through interaction with others, you can gain insight into unique, fascinating cultures and broaden your perspective. I am looking forward to meeting you all at IFReC."

Message from the students of the Japanese class

"It has been essential in my Japanese language development. I am very thankful to have this course and I appreciate all of the effort put in to make this happen."

"Tajima-sensei has been an absolutely fantastic instructor. She has done a wonderful job engaging the students and creating a fun/educational environment."



Information

Major Awards

Clarivate Highly Cited Researchers (HCR) 2022

Shizuo Akira
Nobuhiko Kamada
Shimon Sakaguchi

Commended by the Minister of Internal Affairs and Communications

Toshio Yanagida

The Takeda Prize for Medical Science 2022

Kiyoshi Takeda

The Osaka Science Prize 2022

Yukinori Okada

The Hideyo Noguchi Memorial Prize 2022

Sho Yamasaki

The Order of the Sacred Treasure, Gold and Silver Star

Shigekazu Nagata

The Japanese Society for Immunology Prize 2022

Masaru Ishii

The Toyoichi Ohtawara Award 2022

Hisashi Arase

The 13th Ikushi Prize by Japan Society for the Promotion of Science

Yoshihiko Tomofuji
Yoshiaki Yasumizu

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.

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 2022.

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, over 140 researchers have received these grants.

Support for Paper Submission

Due to the influence of COVID-19, it has been difficult for researchers to find opportunities to present their research results at overseas conferences. This program aims to support the dissemination of research results by the researchers of IFReC.

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

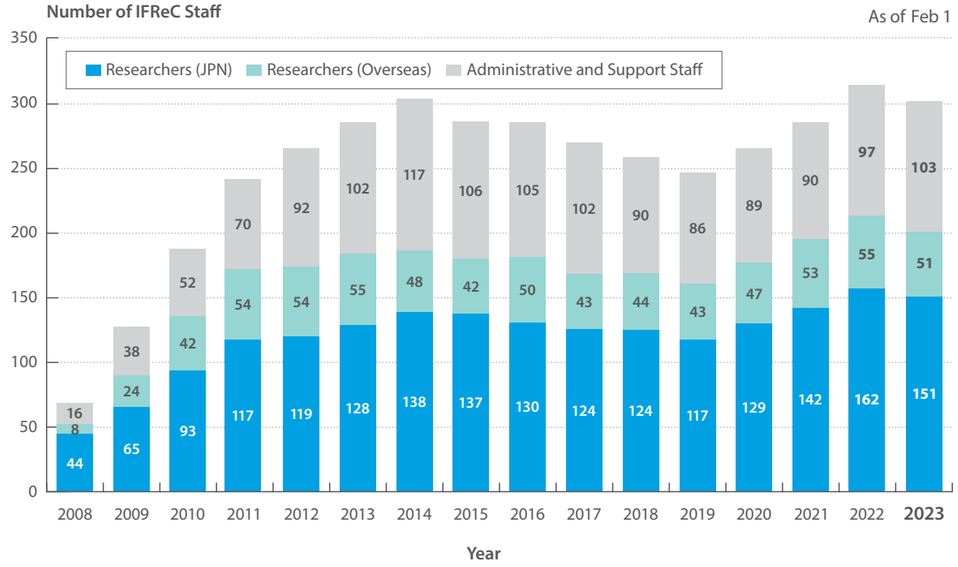
- Provision and maintenance of network infrastructure: LAN system and servers (web, mail, mailing lists, etc.)

Core Instrumentation Facility

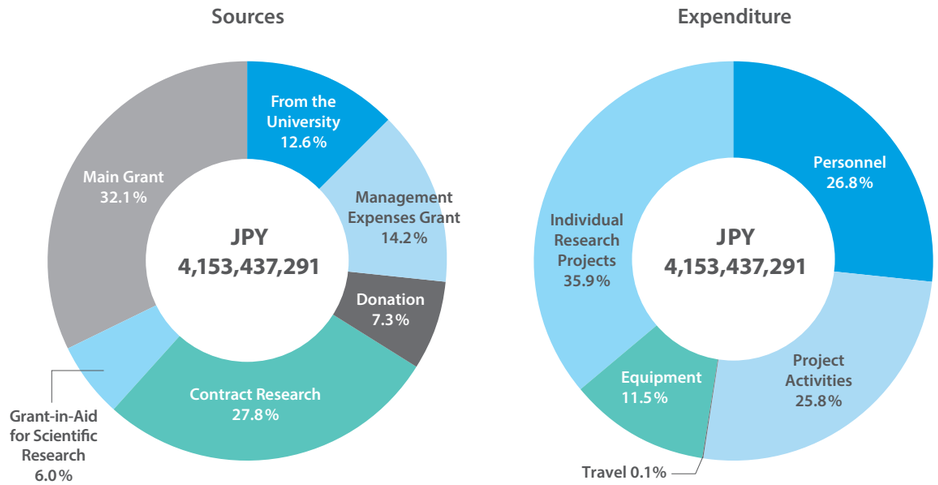
- Basic and advanced instruments
- In-house service
- DNA sequencing, cell sorting, electron microscopy, mass spectrometry and next-generation sequencing analysis
- Radio isotope facility

Composition & Finance

Composition



Finance



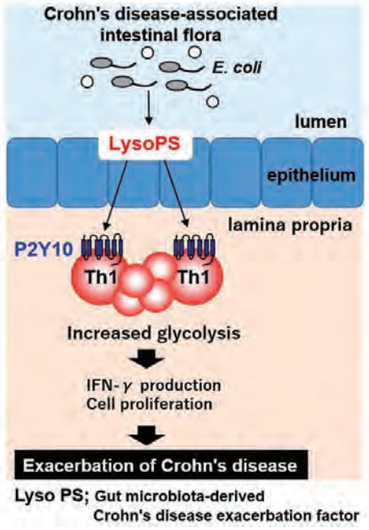
Selected Articles

Lysophosphatidylserines derived from microbiota in Crohn's disease elicit pathological Th1 response.

Otake-Kasamoto Y, Kayama H, et al.

J Exp Med. 219(7): e20211291 (2022).

Microbiota alteration and IFN-γ–producing CD4+ T cell overactivation are implicated in Crohn's disease (CD) pathogenesis. However, it remains unclear how dysbiosis enhances Th1 responses, leading to intestinal inflammation. The research group of Yuriko Otake-Kasamoto, Hisako Kayama, and Kiyoshi Takeda identified key metabolites derived from dysbiotic microbiota that induce enhanced Th1 responses and exaggerate colitis in mouse models. Their findings elaborate on the mechanism by which metabolites elevated in patients with CD harboring dysbiotic microbiota promote Th1-mediated intestinal pathology.



Runx1 and Runx2 inhibit fibrotic conversion of cellular niches for hematopoietic stem cells.

Omatsu Y, Aiba S, et al.

Nat Commun. 13:2654 (2022).

In bone marrow, special microenvironments, known as niches, are essential for the maintenance of hematopoietic stem cells (HSCs). Yoshiki Omatsu, Takashi Nagasawa, and the research group showed HSC cellular niches require Runx1 or Runx2 to prevent their fibrotic conversion and maintain HSCs and hematopoiesis in adults.

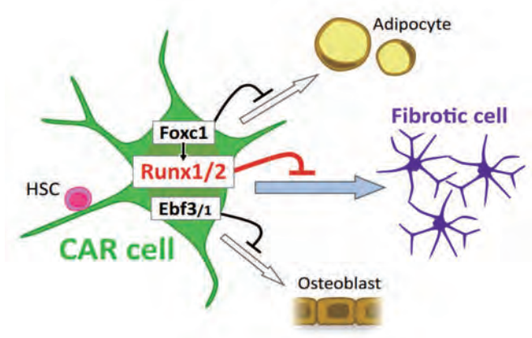


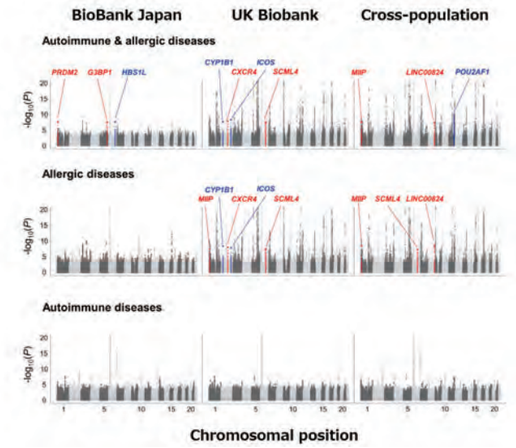
Figure : CAR cells are specialized mesenchymal stem cells, which express the specific transcription factors, including Runx1/2 as well as Foxc1 and Ebf1/3. Runx1/2 prevents fibrotic conversion of CAR cells to maintain HSC niches.

Multi-trait and cross-population genome-wide association studies across autoimmune and allergic diseases identify shared and distinct genetic component.

Shirai Y, Nakanishi Y, et al.

Ann Rheum Dis. 81(9):1301-1312 (2022).

Autoimmune and allergic diseases are outcomes of the dysregulation of the immune system. Yukinori Okada and his research group analyzed the human genome information of 840,000 individuals, and identified their genetic characteristics common to autoimmune and allergic diseases. Their multi-trait and cross-population study should elucidate complex pathogenesis shared components across autoimmune and allergic diseases.

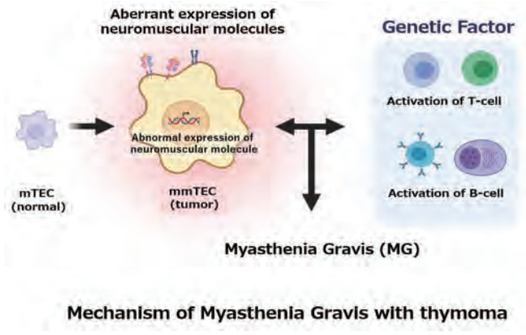


How myasthenia gravis develops in thymoma patients?

Yasumizu Y, Ohkura N, et al.

Nat Commun. 13:4230 (2022).

Although Myasthenia gravis (MG) frequently develops in thymoma patients, the etiologic factors for MG are not well understood. By constructing a comprehensive atlas of thymoma using bulk and single-cell RNA-sequencing. The group of Yoshiaki Yasumizu, Naganari Ohkura, and Shimon Sakaguchi identified ectopic expression of neuromuscular molecules in MG-type thymoma. These molecules are found within a distinct subpopulation of medullary thymic epithelial cells (mTECs), and named as "neuromuscular mTECs (nmTECs)" by the authors. This study suggests that nmTECs have a significant function in MG pathogenesis via ectopic expression of neuromuscular molecules.



Inefficient development of syncytiotrophoblasts in the Atp11a-deficient mouse placenta.

Ochiai Y, Suzuki C, et al.

Proc Natl Acad Sci USA. 119(18): e2200582119 (2022).

Yuki Ochiai, Katsumori Segawa (Tokyo Medical and Dental University), Shigekazu Nagata and the research group showed that a flippase Atp11a at the plasma membrane plays an important role in the formation of syncytiotrophoblasts in placental development.

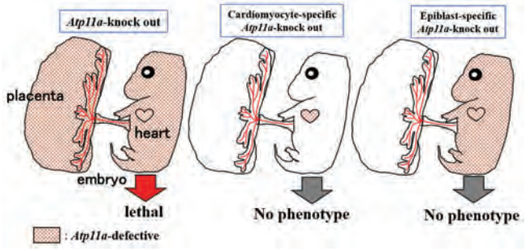


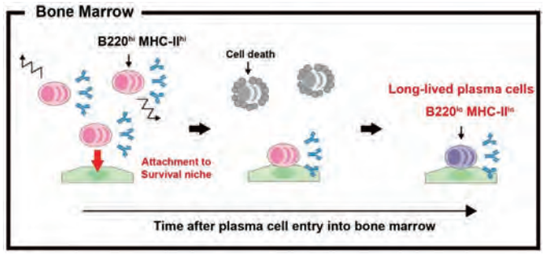
Figure : Embryonic lethality of Atp11a$^{-/-}$ mice. Atp11a$^{-/-}$ mouse embryos died at E14.5 with thin-walled heart ventricles. However, the cardiomyocyte- or epiblast-specific Atp11a deletion did not affect mouse development, suggesting the inability of Atp11a$^{-/-}$ placentas to support the embryos.

Progressive differentiation towards the long-lived plasma cell compartment in the bone marrow.

Koike T, Fujii K, et al.

J Exp Med. 220 (2): e20221717 (2023).

Many plasma cells die shortly after participating in an immune response, but a small population of plasma cells called long-lived plasma cells (LLPCs) can survive in the body for months or even years. The research group led by Wataru Ise and Tomohiro Kurosaki generated a time-stamping method to trace the development and survival of plasma cells in the bone marrow and spleen. They found that plasma cells were continuously replenished by new cells, a small portion of which differentiated into LLPCs. Their findings may aid in the development of new vaccines that efficiently induce LLPCs.

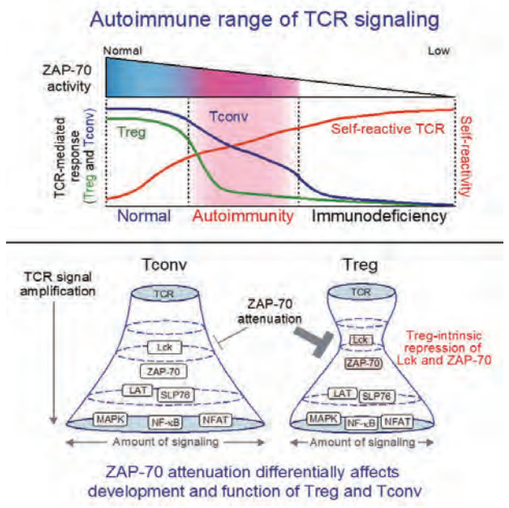


Construction of a T-cell receptor signaling range for spontaneous development of autoimmune disease.

Tanaka A, Maeda S, et al.

J Exp Med. 220 (2): e20220386 (2023).

"How an anomaly in a TCR signaling molecule leads to spontaneous autoimmunity over immunodeficiency?" is unclear. Atsushi Tanaka and Shimon Sakaguchi group 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. 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 results provide a general model of how altered TCR signaling evokes autoimmune disease.

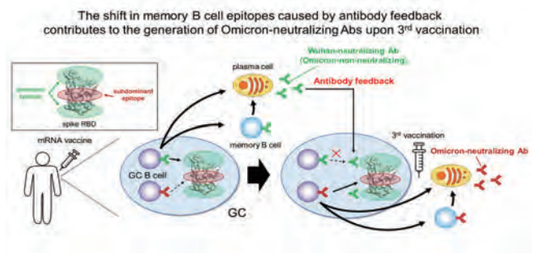


Antibody feedback contributes to the development of Omicron-reactive memory B cells.

Takeshi Inoue T, Shinnakasu R, et al.

J Exp Med. 220 (2): e20221786 (2023).

In contrast to a second dose of the SARS-CoV-2 vaccine, a third dose elicits potent neutralizing activity against the Omicron variant. Takeshi Inoue, Tomohiro Kurosaki, and the research group examined spike receptor binding domain-specific memory B cells in vaccinated individuals. They showed that pre-generated antibodies modulate the selection of GC and subsequent memory B cells after the second vaccine dose, accumulating more Omicron-reactive memory B cells over time, which contributes to the generation of Omicron-neutralizing antibodies elicited by the third vaccine dose.



Activin A-producing alveolar macrophage supports the progression of lung cell carcinoma.

Taniguchi S, Matsui T, et al.

Nat Commun. 14:143 (2023).

Alveolar macrophages (AMs) are crucial for maintaining normal lung function. They are abundant in lung cancer tissues, but their pathophysiological significance remains unknown. Using an orthotopic murine lung cancer model and human carcinoma samples, Masaru Ishii and his research group revealed that AMs support cancer cell proliferation and thus contribute to unfavorable outcome.

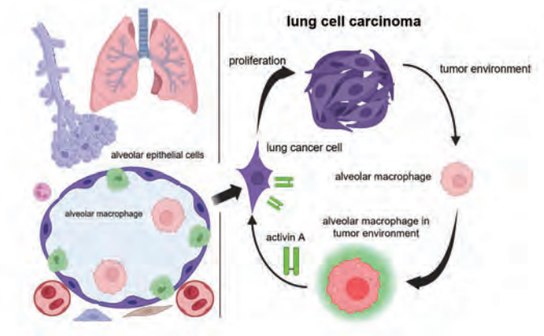


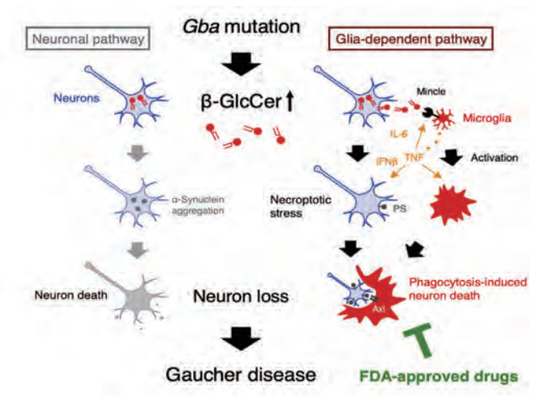
Figure : The findings demonstrate the critical pathological role of activin A-producing AMs in tumorigenesis, and provides means to clearly distinguish them from their healthy counterparts.

Direct activation of microglia by b-glucosylceramide causes phagocytosis of neurons that exacerbates Gaucher disease.

Shimizu T, Schutt CR, et al.

Immunity 56:307-319 (2023).

Gaucher's disease is a disease that develops due to the systemic accumulation of glucosylceramide. Although the disorders of central nervous system are particularly severe, the detailed molecular mechanisms are unknown and there are no effective treatments. The research group led by Sho Yamasaki revealed the direct activation of microglia by β-glucosylceramide causes phagocytosis of neurons that exacerbates Gaucher disease. The blockade of this pathway with FDA-approved drugs improves symptoms and survival.



A sex-biased imbalance between Tfr, Tph, and atypical B cells determines antibody responses in COVID-19 patients.

Søndergaard JN, Tulyeu J, et al.

Proc Natl Acad Sci USA. 120 (4): e2217902120 (2023).

Sex-biased humoral immune responses to COVID-19 patients have been observed. The identification of the cellular basis for the known sex-specific differences will be key in protecting everyone, especially those most at risk from COVID-19 infection. The research group of Jonas Søndergaard and James Wing uncovered the sex-specific differences in a type of regulatory T cells (Treg), and in the production of antibodies.

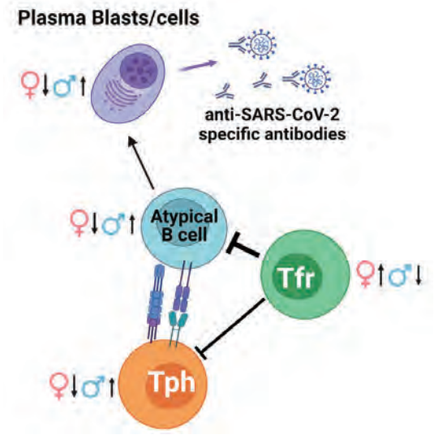


Figure : Sex skewed disruption between T-follicular regulatory cells (Tfr), T-peripheral helper cells (Tph) and antibody producing B-cells and plasmablasts during acute COVID-19.

Aldh1a2+ fibroblastic reticular cells regulate lymphocyte recruitment in omental milky spots.

Yoshihara T, and Okabe Y.

J Exp Med. 220 (5): e20221813 (2023).

Lymphoid clusters in visceral adipose tissue omentum, known as milky spots, play a central role in the immunological defense in the abdomen. However, their development and maturation mechanisms are poorly understood. Tomomi Yoshihara and Yasutaka Okabe identified a subset of fibroblastic reticular cells (FRCs) that are uniquely present in omental milky spots.

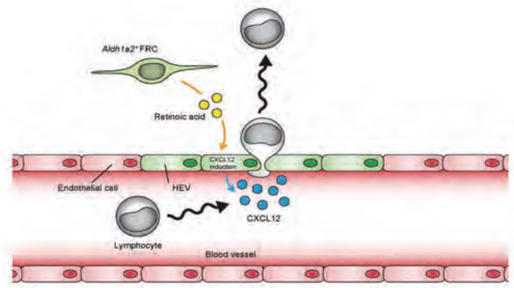


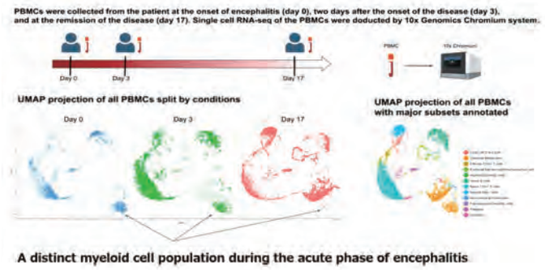
Figure : The proposed model for the recruitment of circulating lymphocytes into milky spots. Aldh1a2+ fibroblastic reticular cell (FRC)-derived retinoic acid induces CXCL12 expression in the endothelium, which regulates the constitutive recruitment of circulating lymphocytes to the milky spots.

Single-cell RNA-seq analysis identifies distinct myeloid cells temporally associated with COVID-19 vaccination.

Ishikawa M, Shimada Y, et al.

Front Immunol. 14: 998233 (2023).

Recently accumulating evidence has highlighted the rare occurrence of COVID-19 vaccination-induced inflammation in the central nervous system. However, the precise information on immune dysregulation related to the COVID-19 vaccination-associated autoimmunity remains elusive. Using scRNA-seq analysis, Daisuke Okuzaki and his group identified a distinct myeloid cell population during the acute phase of encephalitis. This specific myeloid population was detected neither in the remission phase of the disease nor in the healthy cohort.



A distinct myeloid cell population during the acute phase of encephalitis

Celastrol suppresses humoral immune responses and autoimmunity by targeting the COMMD3/8 complex

Shirai T, Nakai A, et al.

Sci Immunol. 8(81): eadc9324 (2023).

Celastrol, a bioactive molecule extracted from the Tripterygium wilfordii plant, has been shown to exhibit anti- inflammatory properties. However, its mechanism of action has not been fully elucidated. Taiichro Shirai, Kazuhiro Suzuki, and the research group showed celastrol suppresses humoral immune responses and autoimmunity by targeting the COM MD3/8 complex.

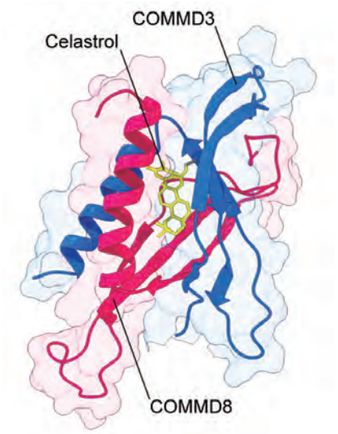


Figure : *In silico* modeling of the celastrol-bound COMMD3/8 complex Celastrol, an herbal medicinal ingredient, improves the pathology of autoimmune diseases by suppressing the COMMD3/8 complex.

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The articles were published between April, 2022 and March, 2023. The data were acquired using Web of ScienceTM Core Collection on May 29, 2023, and sorted by alphabetical order of the first authors.

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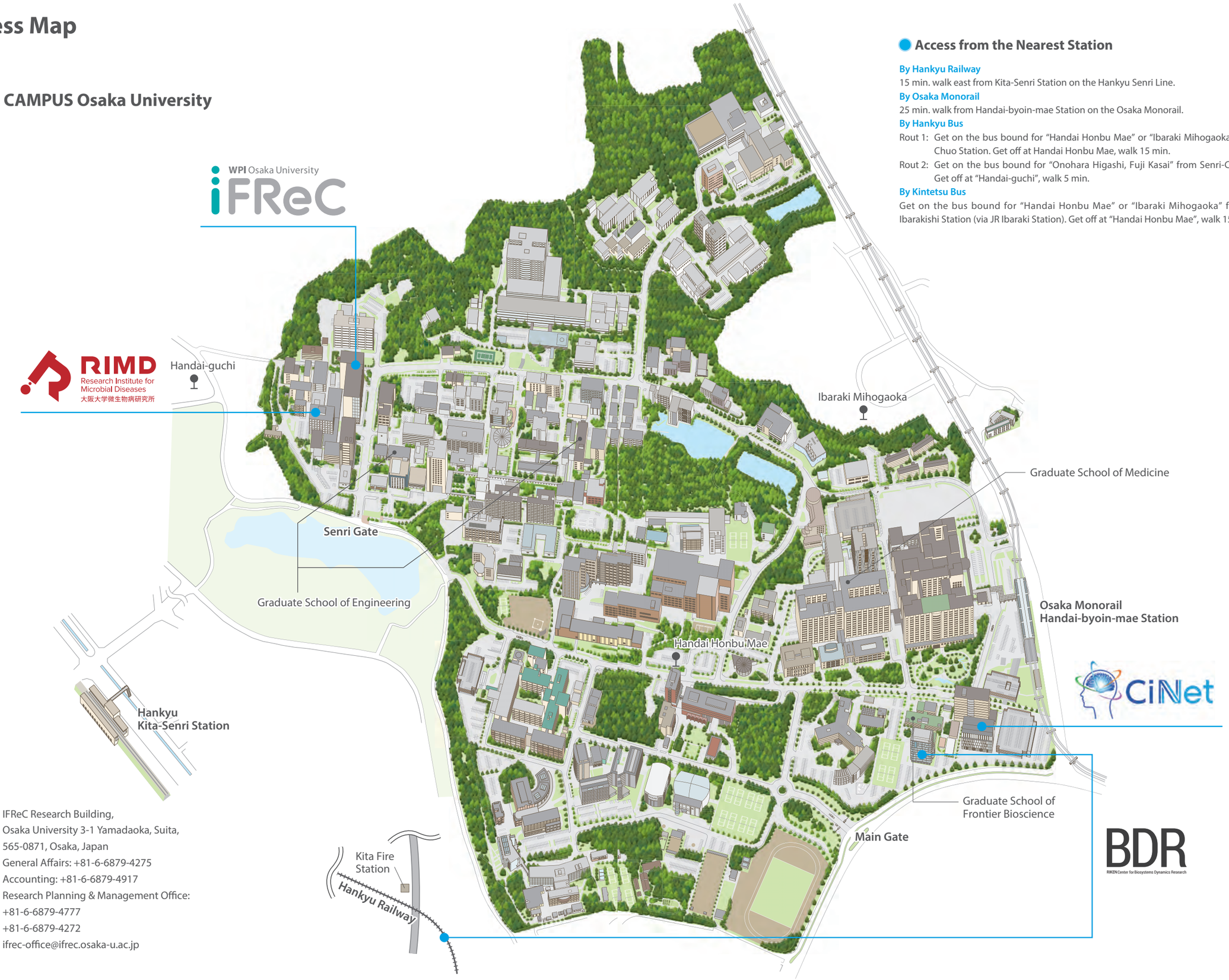

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