
WPI Immunology Frontier Research Center 2021-2022

Osaka University



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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 the year 2020-2021.

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 research center through a novel academic-industry partnership agreement.

More than two years have passed since COVID-19 pandemic began. After several major epidemic peaks, the total number of COVID-19 cases in Japan exceeded 6.5 million as of late March 2022. In other words, one out of every 19 Japanese persons has been infected.

Against this backdrop, the Center for Infectious Diseases Education and Research (CiDER) was established at Osaka University in response to the outbreak of COVID-19 and IFReC plays a central role in its research division.

Although the traditional role of scientists involves directly discussing their findings across national borders, the COVID-19 pandemic has forced the cancellation or postponement of many academic events that invite guest speakers from abroad. Similarly, many outreach activities such as science cafés have also been put on hold.

In response to these challenges, we launched the online "Immuno-Seminar Series" to virtually connect the continents and welcomed eight world-class researchers as invited speakers in FY2021. This seminar series was very well received and will continue in FY2022. We also held "The 2nd ImmunoSensation2-IFReC Joint Workshop" in an online format with the University of Bonn. Despite the time difference between the two countries, many researchers participated in this event. In 2022, we will jointly hold an on-site symposium with University College London in the UK. We also plan to co-host an on-site "International School on Advanced Immunology" with the University of Bonn on Awaji Island.

In FY2021, we welcomed Dr. Nobuhiko Kamada, who is actively engaged in research at Michigan University, as a new principal investigator. Dr. Kamada aims to elucidate how pathogenic commensal microbiota cause diseases and how the host immune system fights them.

In this era of global pandemics, we continue our efforts to conduct basic research in immunology and to seek ways to contribute to society. Through research and education, IFReC continues 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, 2022

**Osaka University**
International Advanced Research Institute (IARI)

**MEXT**
**WPI Initiative Program Committee**
**JSPS**

WPI Osaka University
iFReC

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

**Joint Research Chairs with the Pharmaceutical Companies**
- Innovative Drug Discovery in Immunology (Chugai Pharma)
- Immune-therapeutic Drug Discovery (Otsuka Pharma)
- Innate Immunity
- Innovative Drug Discovery in Host Defense

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

## Administrative Office

### General Affairs Section
- Employment and acceptance procedures for researchers and staff
- Social insurance (for part-time staff), employment insurance
- Various research supports (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], Clusters 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, 2021

| | |
|---|---|
| **Akira UKAWA** | WPI Program Director and Academy Director |

### Program Committee Members

| | |
|---|---|
| **Michinari HAMAGUCHI (Chairperson)** | President, Japan Science and Technology Agency (JST)<br>Chairperson, The Japanese National Commission for UNESCO |
| **Hiroshi AMANO** | Professor, Nagoya University, Tokai National Higher Education and Research System<br>Nobel laureate in Physics (2014) |
| **Mariko HASEGAWA** | President, The Graduate University for Advanced Studies, SOKENDAI |
| **Kazuhiko ISHIMURA** | President, National Institute of Advanced Industrial Science and Technology |
| **Maki KAWAI** | Director General, Institute for Molecular Science (IMS)<br>National Institutes of Natural Sciences (NINS) |
| **Kiyoshi KUROKAWA** | Professor Emeritus, National Graduate Institute for Policy Studies |
| **Hiroshi MATSUMOTO** | President, RIKEN |
| **Ryozo NAGAI** | President, Jichi Medical University |
| **Rita COLWELL** | Distinguished University Professor, University of Maryland |
| **Richard DASHER** | Director, US-Asia Technology Management Center, Stanford University |
| **Victor Joseph DZAU** | President, National Academy of Medicine |
| **Klaus von KLITZING** | Director, Max Planck Institute for Solid State Research<br>Nobel laureate in Physics (1985) |
| **Chuan Poh LIM** | Chairman, Singapore Food Agency (SFA) |
| **Harriet WALLBERG** | Professor, Karolinska Institutet |
| **Jean ZINN-JUSTIN** | Scientific adviser, Institute of Research into the Fundamental Laws of the Universe (IRFU/CEA) |

## 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, 2022

| | |
|---|---|
| **Lewis LANIER** | University of California, San Francisco |
| **Anne O'GARRA** | The Francis Crick Institute |
| **Jeffrey RAVETCH** | Rockefeller University |
| **Art WEISS** | University of California, San Francisco/Howard Hughes Medical Institute |
| **Joachim SCHULTZ** | DZNE/LIMES Institute, University of Bonn |

# Laboratories

As of March, 2022

# Host Defense

## Shizuo Akira, MD/PhD

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

We are focused on the innate immune system, which is an evolutionally conserved host defense mechanism against various pathogens. Innate immune responses are initiated by pattern recognition receptors, which recognize specific structures of microorganisms. Toll-like receptors (TLRs) are capable of sensing organisms ranging from bacteria to fungi, protozoa and viruses, and play a major role in innate immunity. Individual TLRs recognize different microbial components, and give rise to different patterns in gene expression.

### Molecular mechanism of endoribonuclease Regnase-1 in inflammation

Regnase-1 is a member of CCCH-type zinc finger proteins. Regnase-1 has the endonuclease activity and destabilizes a set of mRNAs through cleavage of their 3' UTRs such as *IL-6* and *IL-12 p40* in macrophages and *c-Rel*, *Ox40*, and *IL-2* in $CD4^+$ T cells. Recent studies reveal that both static and dynamic control of mRNA expression by Regnase-1 contributes to robustness of cellular functions in various types of cells including macrophages, T cells, intestinal epithelial cells, and etc. And Regnase-1 is thought to be a key contributor to maintenance of homeostasis in various types of tissues and cells. Based on these findings, we promote understanding of the precious roles of Regnase-1 in immune and non-immune cells by using tissue-specific *Regnase-1*-deficient mice and mutant mice.

Regnase-1 is inactivated in response to external stimuli through posttranslational modifications, however, yet the precise role of phosphorylation largely remains unknown. We showed that IL-17 induces the phosphorylation of Regnase-1 in an Act1-TBK1-IKKi-dependent manner, especially in non-hematopoietic cells. Phosphorylated Regnase-1 is released from the endoplasmic reticulum into the cytosol, thereby losing its mRNA degradation function, which leads to expression of IL-17 target genes (Fig.1). IL-17-induced Regnase-1 phosphorylation is completely blocked in two *Regnase-1-mutant* (*Regnase-1*$^{AA/AA}$ and *Regnase-1*$^{\Delta CTD/\Delta CTD}$) mice. Thus, Regnase-1 plays a critical role in the development of IL-17-mediated inflammatory diseases via the Act1-TBK1-IKKi axis. Blockade of Regnase-1 phosphorylation sites may be promising for treatment of T helper 17-associated diseases.

We recently found that Regnase-1 also play a critical role in cytotoxic activity of natural killer (NK) cells. NK cells are essential for immunesurveillance against tumor and viral infection by secretion of several substances associated with cytolysis, such as Perforin, Granzymes, and Interferons. *Regnase-1*-deficient in NK cells (*Regnase-1*$^{\Delta NK}$) mice were highly resistant to tumor progression and metastasis by hyprproduction of Perforin and Interferon-γ. Now we try to determine the mechanism how Regnase-1 gene deficiency results in strong anti-tumor activity.

We are further studying to achieve the goal of a comprehensive understanding of the innate immune system and to develop an effective treatment for immune-related inflammatory diseases.

### Heterogeneity of monocyte subsets comprise diverse macrophages in mouse and human lung fibrosis

Fibrosis is a life-threatening disease of unknown aetiology. Its pathogenesis is poorly understood, and there are few effective therapies. The development of fibrosis is associated with activation of monocytes and macrophages. We identified new macrophage subset that Ceacam1+Msr1+Ly6C-F4/80-Mac1+ monocytes, which we termed SatM (segregated-nucleus-containing atypical monocytes), share granulocyte characteristics, are regulated by C/EBPβ (CCAAT/enhancer binding protein beta), and are critical for fibrosis. To investigate the physiological role of SatM and related subsets, we recently identified an RNA-binding protein RBM7 that is a component of the NEXT (nuclear exosome targeting) complex. We found that the expression of Rbm7 is increased in the fibrotic phase. *Rbm7*-deletion in nonhematopoietic cells suppresses fibrosis (Fig.2). Dysregulated expression Rbm7 triggers apoptosis via nuclear degradation of noncoding RNA *Neat1*. Rbm7 in epithelial cells plays a critical role in the development of fibrosis by regulating ncRNA decay, thereby producing of chemokines that recruit SatMs. Inhibition of RBM7 would provide an effective treatment of fibrosis in patients. We further investigated the fibrotic lungs of mice and humans using spatial transcriptomics and single-cell RNA-seq. We found close interactions of pro-fibrotic macrophages derived from distinct monocyte subsets, and other immune and non-immune cells releasing the essential factors maintaining the fibrotic niche. Now, we are trying to unveil a comprehensive understanding of the mechanisms involved in the development and maintenance of fibrosis.

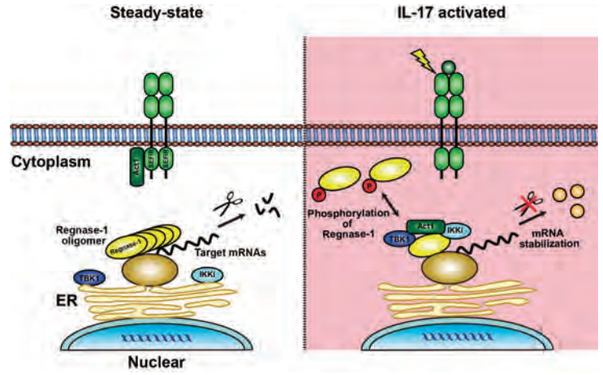



**Figure 1.**
IL-17 induces Regnase-1 phosphorylation in an Act-1/TBK1/IKKi –dependent manner. Phosphorylation of Regnase-1 is released from the endoplasmic reticulum into the cytosols, resulting in loss of the mRNA degradation function.

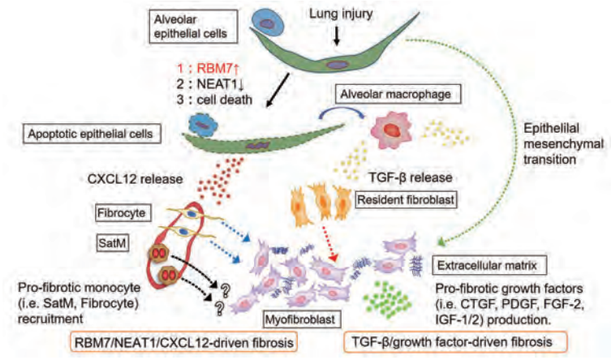
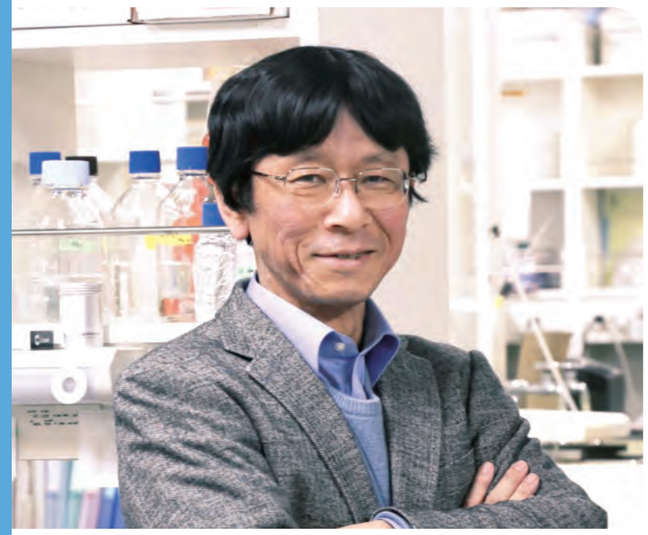



**Figure 2.**
RBM-7 regulates fibrosis formation through ncRNA decay and cell death. RBM-7 destabilizes ncRNA NEAT1 and induces cell death in lung epithelial cells, resulting in recruitment of SatM and fibrocytes into lung to promote lung fibrosis.

## Recent Publications


1. Kawasaki T. et al. Loss of FCHSD1 leads to amelioration of chronic obstructive pulmonary disease. Proc Natl Acad Sci U S A 118:e2019167118 (2021).
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, 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, et al. 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 U S A. 115:11036-11041 (2018).

# Immunoglycobiology




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


| | |
|---|---|
| Professor | Taroh Kinoshita<br>Yoshiko Murakami |
| Postdoctoral Fellow | 1 |
| Research Assistant | 1 |
| Support Staff | 3 |

The main topics of research in the Immunoglycobiology laboratory have been biology and medicine of glycosylphosphatidylinositol (GPI). In FY 2021, we made following progress.

**Studies on GPI biosynthesis.**

Biosynthesis pathway of GPI initiates on the cytoplasmic side of the endoplasmic reticulum (ER), flips into the luminal side at the third step, and continues on the luminal side beyond. How the second intermediate glucosaminyl-phosphatidylinositol (GlcN-PI) flips from the cytoplasmic side to the luminal side across the ER membrane has been unclear. We identified CLPTM1L, an ER membrane protein with eight transmembrane domains, as a major GlcN-PI scramblase in HEK293 cells. Thus, GPI-anchored protein (GPI-AP) levels were clearly reduced but not completely in CLPTM1L-KO HEK293 cells, suggesting the presence of yet another scramblase for GlcN-PI. CLPTM1L scrambled not only GlcN-PI but also PI, GlcNAc-PI, phosphatidylethanolamine and phosphatidylcholine in in vitro scrambling assays using poroteoliposomes (Figure 1). These results indicate that CLPTM1L is a new lipid scramblase critical for the GPI biosynthesis pathway (Wang Y et al, Proc. Natl. Acad. Sci. USA, 2022).

**Pathophysiological roles of side-chain modification of GPI-anchors.**

The conserved GPI core of mammalian GPI-APs are often modified by an N-acetylgalactosamine (GalNAc) side chain attached to the first mannose. We previously reported a Golgi-resident GalNAc transferase (PGAP4/TMEM246) that mediates this modification, however, roles of the side chain have been unclear. To clarify this, we generated PGAP4-knockout (KO) mice. PGAP4-KO mice showed various phenotypes, including a high blood alkaline phosphatase level, impaired bone formation, lowered locomotion activity, and impaired memory, despite normal expression levels and raft-association of various GPI-APs. Therefore, the GalNAc side chain is required for *in vivo* functions of GPI-APs in mammals, especially in bone and brain. Moreover, PGAP4-KO mice were more vulnerable to prion diseases and died earlier after intracerebral inoculation of the pathogenic prion strains than wild-type mice, highlighting the protective roles of the GalNAc-side chain against prion diseases (Hirata T et al, J. Biol. Chem., 2022).

**Free, un-protein-linked GPIs as the Emm blood group antigen.**

We previously reported presence of free, un-protein-linked GPI in the various tissues (Wang Y et al, J. Biol. Chem., 2018). In collaboration with Drs. Peyrard and Azouzi group in Paris, we demonstrated that free, un-protein-linked GPI, bearing ethanolamine-phosphate side chain linked to the second mannose, is expressed on the surface of red blood cells, platelets and neutrophils. Patients with inherited GPI deficiency caused by null mutations of PIGG gene did not have this GPI structure and generated natural antibody against this type of free GPI. Thus, the free GPI bearing ethanolamine-phosphate side chain linked to the second mannose represents the Emm blood group antigen known as a GPI-related blood group. (Duval R et al, Blood, 137:3660, 2021)

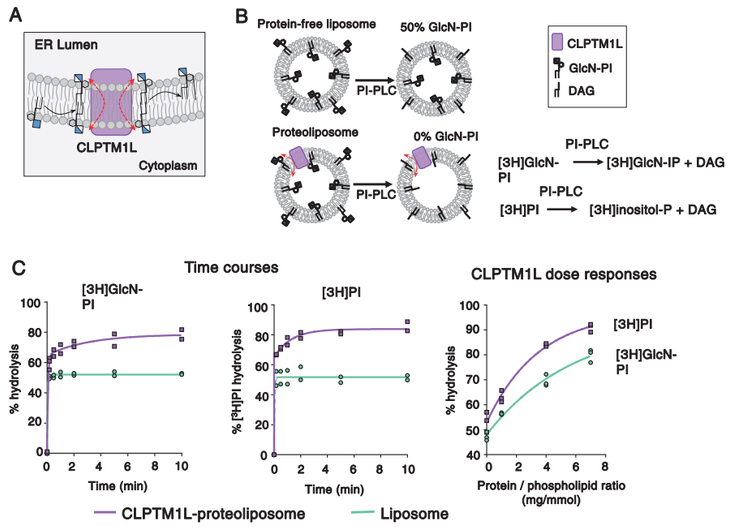



**Figure 1.**
CLPTM1L scrambled GlcN-PI and PI in vitro. A. A schematic of CLPTM1L-mediated scrambling of GlcN-PI across the endoplasmic reticulum (ER) membrane during biosynthesis of GPI-anchor. B. Principles of in vitro scrambling assay using liposomes and proteoliposomes. Phosphatidylinositol-specific phospholipase C (PI-PLC) cleaves radiolabeled GlcN-PI and PI in the outer leaflet, releasing radioactive GlcN-inositolcyclicphosphate and inositolcyclicphosphate, respectively from liposomes. C. Time course and CLPTM1L-dose response of hydrolysis of GlcN-PI and PI in liposomes. (Adopted from Wang Y et al, Proc. Natl. Acad. Sci. USA, 2022)

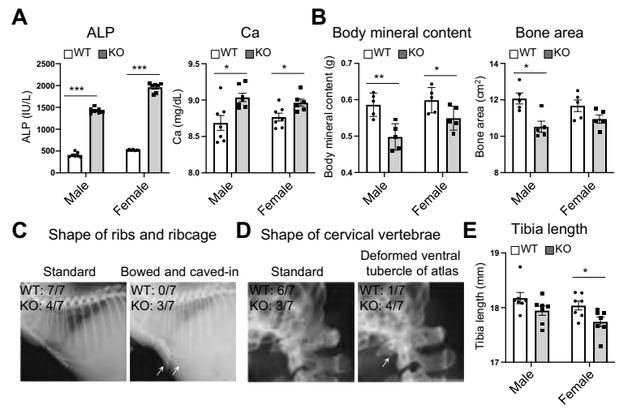



**Figure 2.**
Abnormal bone formation in PGAP4-knockout mice. A. Blood alkaline phosphatase level (ALP) and blood calcium level (Ca). B. Body mineral content and bone area. C. Normal (left) and abnormal (right) shapes of ribs and ribcage. The number of male mice showing the phenotypes are indicated. D. Normal (left) and abnormal (right) shapes of cervical vertebrae. The number of female mice showing the phenotypes are indicated. E. Tibia length. (Adopted from Hirata T et al, J. Biol. Chem., 2022)

## Recent Publications


1. Wang Y, Menon AK, Maki Y, Liu Y-S, Iwasaki Y, Fujita M, Guerrero PA, Varón Silva D, Seeberger PH, Murakami Y and Kinoshita T. Genome-wide CRISPR screen reveals CLPTM1L as a lipid scramblase required for efficient glycosylphosphatidylinositol biosynthesis. Proc Natl Acad Sci USA in press (2022).

2. Hirata T, Kobayashi A, Furuse T, Yamada I, Tamura M, Tomita H, Tokoro Y, Ninomiya A, Fujihara Y, Ikawa M, Maeda Y, Murakami Y, Kizuka Y and Kinoshita T. 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. in press (2022).

3. Langemeijer S, Schaap C, Preijers F, Jansen JH, Blijlevens N, Inoue N, Muus P, Kinoshita T and Murakami Y. Paroxysmal nocturnal hemoglobinuria caused by CN-LOH of constitutional *PIGB* mutation and 70-kb microdeletion on 15q. Blood Adv. 4:5755-5761 (2020).

4. Wang Y, Maeda Y, Liu YS, Takada Y, Ninomiya A, Hirata T, Fujita M, Murakami M & Kinoshita T. Cross-talks of glycosylphosphatidylinositol biosynthesis with glycosphingolipid biosynthesis and ER-associated degradation. Nat Commun. 11:860 (2020).

5. Hochsmann B, Murakami Y, Osato M, Knaus A, Kawamoto M, Inoue N, Hirata T, Murata S, et al. Complement and inflammasome overactivation mediates paroxysmal nocturnal hemoglobinuria with autoinflammation. J. Clin. Invest. 129:5123-5136 (2019).

# Immunopathology

## Atsushi Kumanogoh, MD/PhD

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

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

Functional coupling of the neural, metabolic, and immune systems is essential for organ development and maintenance, but the molecular mechanisms controlling this network remain unclear. Here, we demonstrate that an axonal guidance cue Semaphorin 6D (Sema6D) combines systemic metabolism and myelopoiesis by tuning sympathetic innervations. *Sema6d*$^{-/-}$ mice fed a high-fat diet (HFD) exhibited attenuated obesity and enhanced myelopoiesis. Combinational analysis of bone marrow (BM) chimeric and conditional knockout mice revealed that non-hematopoietic and neuron-derived Sema6D is responsible for these phenotypes through Plexin-A4. Notably, *Sema6d* deficiency led to increased sympathetic innervations in the BM. Consistently, loss of β3-adrenergic receptor signaling resolved metabolic and hematopoietic defects in HFD-fed *Sema6d*$^{-/-}$ mice. Collectively, these data demonstrate that Sema6D orchestrates systemic metabolism and hematopoiesis by tuning sympathetic innervations. Thus, Sema6D functions as a hub for neural–metabolic–immune circuits by repelling/pruning sympathetic innervations. Our findings provide mechanistic and clinical insights into the hierarchical regulation of the immune and metabolic diseases.

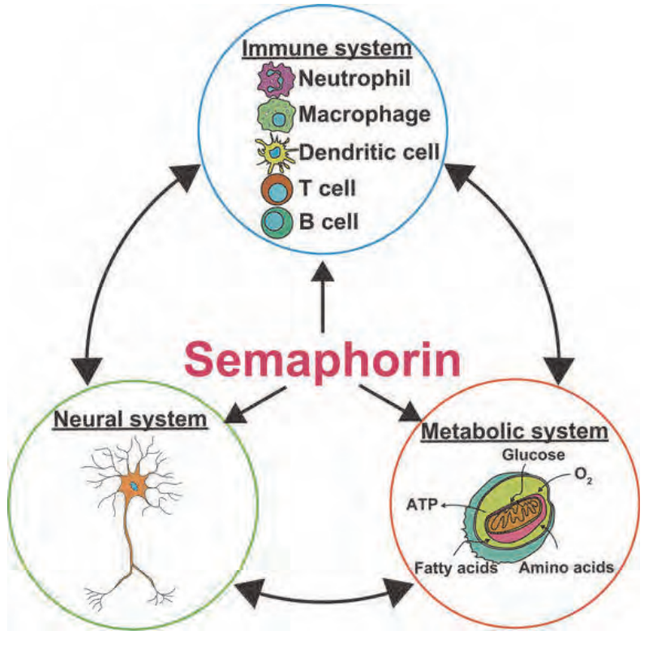



**Figure.**
**Sema6D guides sympathetic neurons to regulate systemic metabolism and myelopoiesis.**
A schematic view of the neural–metabolic–immune connections regulated by Sema6D. The loss of Sema6D in neurons causes increased sympathetic innervations in the BM, resulting in enhanced systemic metabolism and myelopoiesis under stress conditions. These metabolic and hematopoietic defects are resolved by blocking β3-adrenergic receptor signaling. Thus, neuron-derived Sema6D fine-tunes sympathetic innervations in the BM, leading to coupling of metabolic and hematopoietic adaptations under metabolic stress.

## Recent Publications


1. 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).

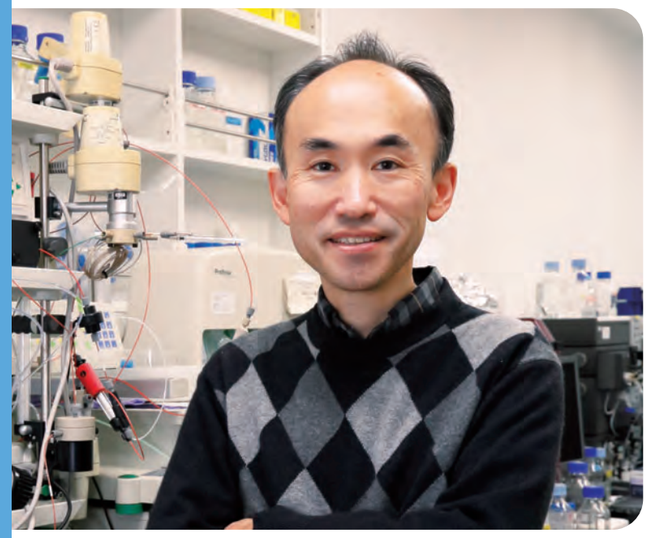
2. Tsuda T, Nishide M, Maeda Y, Hayama Y, Koyama S, Nojima S, Takamatsu H, Okuzaki D, Morita T, Nakatani T, Kato Y, Nakanishi Y, Futami Y, Suga Y, Naito Y, Konaka H, Satoh S, Naito M, Izumi M, Obata S, Nakatani A, Shikina T, Takeda K, Hayama M, Inohara H, and Kumanogoh A. Pathological and Therapeutic Implications of Eosinophil-Derived Semaphorin 4D in Eosinophilic Chronic Rhinosinusitis. *J Allergy Clin Immunol*. 145(3):843-854 (2020).

3. Kang S, Nakanishi Y, Kioi Y, Okuzaki D, Kimura T, Takamatsu H, Koyama S, Nojima S, Nishide M, Hayama Y, Kinehara Y, Kato Y, Nakatani T, Shimogori T, Junichi Takagi J, Toyofuku T. and Kumanogoh A. Semaphorin 6D reverse signaling controls macrophage lipid metabolism and anti-inflammatory polarization. *Nature Immunol*. 19:561-570 (2018).

4. Nishide M, and Kumanogoh A. The role of semaphorins in immune responses and autoimmune rheumatic diseases. *Nat Rev Rheumatol* 14:19-31 (2018).

5. Hosen N, Matsunaga Y, Hasegawa K, Matsuno H, Nakamura Y, Makita M, Watanabe K, Yoshida M, Satoh K, Morimoto S, Fujiki F, Nakajima H, et al. 2017. The activated conformation of integrin β. *Nat Med*. 23:1436-1443 (2017).

# Immunochemistry



## Hisashi Arase, MD/PhD

| Professor | Hisashi Arase |
|---|---|
| Associate Professor | Masako Kohyama |
| Assistant Professor | Wataru Nakai |
| Postdoctoral Fellow | 2 |
| Research Assistant | 5 |
| 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 cellular misfolded 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 disease. 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

Immune system has evolved with infectious diseases, indicating that studies on host-pathogen interaction is important to understand immune system. We have found that PILRα, one of paired 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 Sigle-7, one of paired receptors, are involved in VZV infection (*PNAS* 2010; *BBRC* 2022). LILR is another type of paired receptor family. We found that activating LILRA2 is a sensor to detect 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 the 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 the infectivity. In particular, anti-NTD antibodies that enhance the 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.

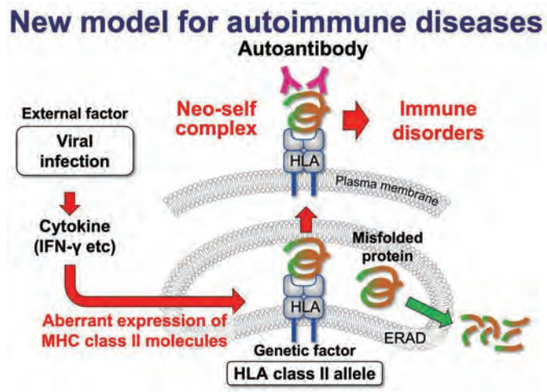



**Figure 1.**
**Misfolded proteins transported to the cell surface by MHC class II molecules are targets for autoantibodies.**
Cellular misfolded proteins are generally degraded in the cells and are not transported to outside the cells. Therefore, misfolded proteins transported to the cell surface by MHC class II molecules may be recognized as 'neo-self' antigens by immune system, which initiates 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).

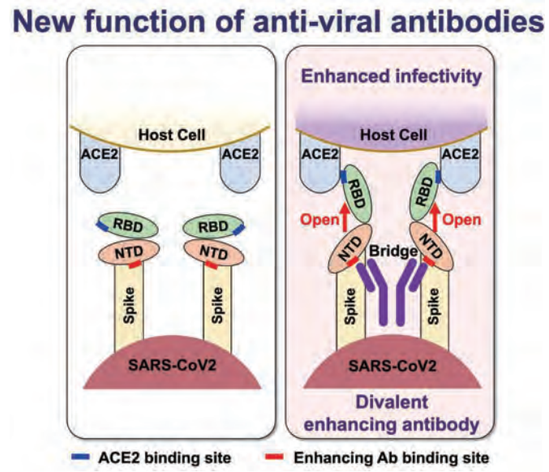



**Figure 2.**
**SARS-CoV-2 infectivity enhancing antibodies.**
Some antibodies against NTD of spike protein induces conformational change in spike protein to induce the open form of RBD and enhance the infectivity of SARS-CoV-2 (*Cell* 2021).

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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. 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, Matsuura Y, 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).
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# Immune Regulation


## Tadamitsu Kishimoto, MD/PhD

| | |
|---|---|
| Professor | Tadamitsu Kishimoto |
| Associate Professor | Sujin Kang |
| Postdoctoral Fellow | 2 |
| Research Assistant | 2 |
| Support Staff | 3 |

### A novel insight into endothelial injury at the onset of cytokine storm

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. Additionally, treatment with an anti-IL-6R antibody, tocilizumab inhibited endothelial activation and prevented the development of cytokine release syndrome (CRS). In accordance with our findings, tocilizumab was recently approved by the United States Food and Drug Administration for the treatment of SARS-CoV-2 infection-induced pneumonia. Nevertheless, the long-lasting and discriminate 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 several 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 plasminogen activation inhibitor-1 production, compared with the normal half-life anti-IL-6R antibody. Consequently, treatment with silent anti-IL-6R antibody showed significant efficacy in several acute inflammatory diseases by controlling endothelial injury (Fig. 1). However, the mechanisms involved in how the silent antibody affects immune cells and endothelial damage require further study. Additionally, the efficacy of the silent antibody should be tested in several infectious diseases.

### Arid5a promotes immune evasion by augmenting tryptophan metabolism and chemokine expression.

The acquisition of mesenchymal traits leads to immune evasion in various cancers, but the underlying molecular mechanisms remain unclear. In this study, we found that the expression levels of AT-rich interaction domain-containing protein 5a (Arid5a), an RNA-binding protein, were substantially increased in mesenchymal tumor subtypes. The deletion of Arid5a in tumor cell lines enhanced antitumor immunity in immunocompetent mice, but not in immunodeficient mice, suggesting a role for Arid5a in immune evasion. Furthermore, an Arid5a-deficient tumor microenvironment was shown to have robust antitumor immunity, as manifested by suppressed infiltration of granulocytic myeloid-derived suppressor cells and regulatory T cells. In addition, infiltrated T cells were more cytotoxic and less exhausted. Mechanistically, Arid5a stabilized Ido1 and Ccl2 mRNAs and augmented their expression, resulting in enhanced tryptophan catabolism and an immunosuppressive tumor microenvironment. Thus, our findings demonstrate the role of Arid5a beyond inflammatory diseases and suggest Arid5a as a promising target for the treatment of immunotolerant malignant tumors.

### Threonine Phosphorylation of STAT1 Controls the Host Inflammatory Response

The STAT1 working paradigm has focused on interferon (IFN) mediated JAK-Tyr$^{701}$ phosphorylation. However, IFN-JAK signaling does not explain the full spectrum of STAT1 functions against inflammatory stimuli. Our recent work identified the novel Thr$^{749}$ (Thr$^{748}$ in mouse) phosphorylation of STAT1, which triggered distinct gene-regulatory functions of STAT1 independent of the canonical Tyr$^{701}$ phosphorylation. **1) Disruption of Thr$^{748}$ phosphorylation of STAT1 protects the host against lipopolysaccharide (LPS)-induced septic shock:** We generated genetically engineered mice expressing a non-phosphomimic threonine748-to-alanine (T748A) mutant STAT1. We found a gradual trend of survival against LPS-induced septic shock where STAT1T748A and heterozygous mice exhibited higher survival compared with their wild-type littermates, suggesting that pThr STAT1 promoted the host inflammatory response in septic shock. Additionally, STAT1T748A mice were resistant to LPS-induced lethality, albeit to a lesser extent than their STAT1-deficient littermates. Of note, the expression of STAT1 and other STAT proteins was unperturbed in STAT1T748A mice. These findings showed that threonine748 phosphorylation is a major contributor to STAT1 functions in LPS-induced septic shock. **2) Disruption of Thr$^{748}$ phosphorylation of STAT1 promotes colitis:** A deficiency in STAT1 leads to severe DSS-induced colitis, a murine model which closely resembles human ulcerative colitis. However, colon mucosal samples from patients with ulcerative colitis exhibited the 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. In this study, we found that Stat1T748A mice exhibited severe intestinal inflammation compared with their wild-type mice. Intriguingly, the Stat1T748A mice phenotype completely mimicked the detrimental phenotype of their STAT1 knockout mice as shown by their weight loss, colon lengths, and histopathology analysis. Our data indicate that the threonine748 phosphorylation on STAT1 is a key driver for the 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. The simplest interpretation of our findings is that cell-dependent threonine kinase pathways may contribute to the specificity of STAT1 functions, especially those not driven by the canonical JAK pathway (Fig. 2).

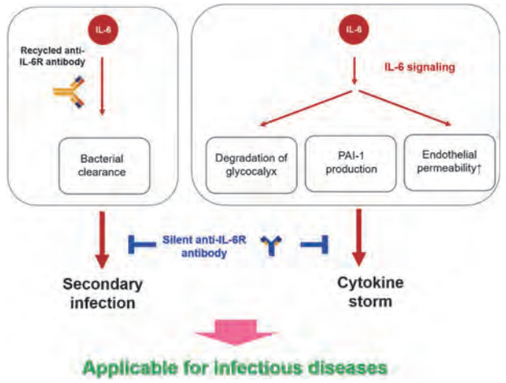


**Figure 1.**
Targeting of anti-IL-6R antibody for infectious diseases.

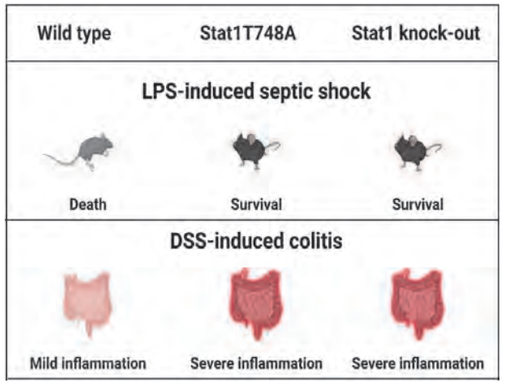


**Figure 2.**
Role of novel phosphorylation site of STAT1 *in vivo*.

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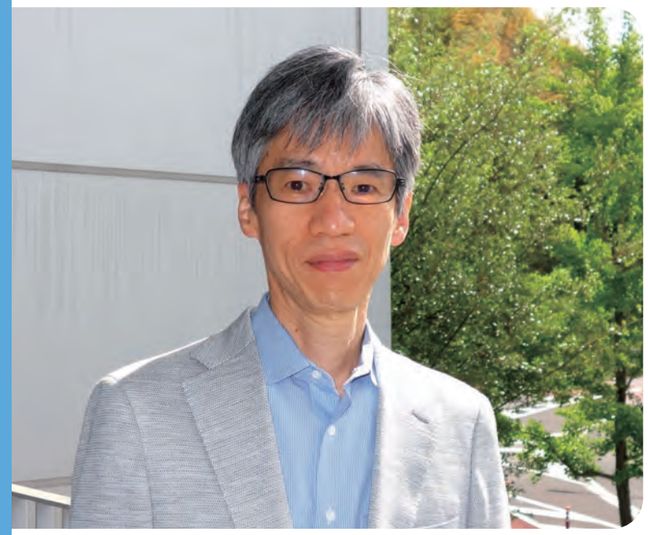
# Mucosal Immunology



## Kiyoshi Takeda, MD/PhD

| Professor | Kiyoshi Takeda |
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| Associate Professor | Hisako Kayama |
| Assistant Professor | Ryu Okumura |
| | Mari Murakami |
| | Taiki Sakaguchi |
| Postdoctoral Fellow | 1 |
| Research Assistant | 12 |
| Support Staff | 2 |

We previously demonstrated that microbial metabolites: adenosine triphosphate (ATP) and lactate/pyruvate exert their immunomodulatory activities through P2X/P2Y receptor and GPR31, respectively. Activation of the lactate/pyruvate-GPR31 signaling pathway facilitates uptake of lumina bacteria by $CX_3CR1$ phagocytes and thereby protects the host against pathogenic bacteria. Although induction of inflammatory responses by extracellular ATP is essential for host defense, aberrant ATP signaling results in disruption of intestinal immune system. Therefore, luminal ATP is tightly regulated by ATP-hydrolyzing ectoenzymes in epithelial cells, such as E-NPP3, E-NTPD7, and E-NTPD8, suggesting that microbial metabolites in intestinal lumen are strictly tuned to maintain the gut homeostasis.

Crohn's disease (CD), the main inflammatory bowel disease clinical phenotype, is a chronic gastrointestinal tract disorder. IFN-γ-producing $CD4^+$ T (Th1) cells is associated with CD severity. Along with increased number of immunopathological Th1 cells, alterations of intestinal metabolites caused by microbiota community alterations (dysbiosis) are a feature of CD. However, the physiological or pathological functions of dysbiotic microbiota-derived metabolites in the gut of patients with CD remain poorly understood.

### Dysbiotic microbiota promote lysophosphatidylserine generation in patients with CD

Some lysophospholipid species were identified to elevate in the plasma of patients with CD. To define whether plasma lipid profiles reflect intestinal lipid profiles, we performed a fecal lipidomic analysis. Fifteen lipid species were upregulated in fecal samples from patients with CD relative to those from healthy controls (HCs). Among these lipid species, 18:0 and 18:1 lysophosphatidylserine (LysoPS) were commonly augmented in both the fecal and plasma samples from patients with CD. Microbial phospholipase A (PLA) generates lysoglycerophospholipid species including LysoPS through the hydrolysis of host cell membrane phospholipids. Therefore, to define whether dysbiosis in patients with CD lead to the generation of more intestinal LysoPS by microbial PLA, we performed whole-genome shotgun sequencing of fecal DNA extracts. Lower fecal microbiota species diversity was observed in CD samples compared with HC samples. Additionally, a β-diversityanalysis demonstrated that the microbial community profiles of CD samples substantially separated from those of HC samples. Furthermore, ECSF_3660 gene predicted to encode PLA (mainly derived from *Escherichia coli*) was increased in the fecal samples of patients with CD compared with HCs. Gnotobiotic mice colonized with fecal microbiota from patients with CD showed increased fecal level of 18:0/18:1 LysoPS compared to those in mice transplanted healthy microbiota. Similarly, germ-free mice inoculated with *E. coli* with ECSF_3660 isolated from feces of a patient with CD exhibited higher fecal level of 18:0/18:1 LysoPS than those in untreated germ-free mice. These findings indicate that colonization by the dysbiotic microbiota found in patients with CD links to the promotion of LysoPS generation in the intestine.



### LysoPS exaggerates colitis by driving immunopathological Th1 responses through P2Y10 receptor-dependent metabolic reprograming

To determine the role of LysoPS in the progression of CD, we analyzed effect of 18:1 LysoPS on two colitis models, such as 2,4,6-trinitrobenzenesulfonic acid solution (TNBS)-induced colitis and adoptive T cell transfer-induced colitis. In both colitis models, intraperitoneally injection of LysoPS aggravated large intestinal pathology with increased number of IFN-γ-producing $CD4^+$ T cells. In Th1-polarized cells, LysoPS augmented glycolytic activity through P2Y10 receptor, which linked to elevated IFN-γ and intracellular ROS production and promoted cell proliferation (Figure 1). To assess the impact of *P2ry10* deficiency in $CD4^+$ T cells on LysoPS-induced intestinal pathology, *Rag2*$^{-/-}$ mice were transferred wild-type or *P2ry10*$^{-/y}$*P2ry10b*$^{-/y}$ naive T cells and some groups were administered LysoPS intraperitoneally. LysoPS drastically enhanced body weight loss and worsened large intestinal pathology in *Rag2*$^{-/-}$ mice that received wild-type naive T cells but not in mice that received *P2ry10*$^{-/y}$*P2ry10b*$^{-/y}$ naive T cells. In this context, LysoPS increased the number of IFN-γ$^+$ $CD4^+$ T cells in the large intestinal lamina propria of *Rag2*$^{-/-}$ mice that received wild-type cells, whereas it did not affect the number of these cells in mice that received *P2ry10*$^{-/y}$*P2ry10b*$^{-/y}$ $CD4^+$ T cells. Thus, P2Y10 receptor activation by LysoPS elicits immunopathological Th1 responses, which leads to the aggravation of intestinal inflammation.

Regulation of lipid metabolites, including leukotrienes and prostaglandins, is an emerging therapeutic approach for inflammatory disorders. Thus, the bioactive lipid LysoPS could be a putative diagnostic biomarker and a therapeutic target for CD.

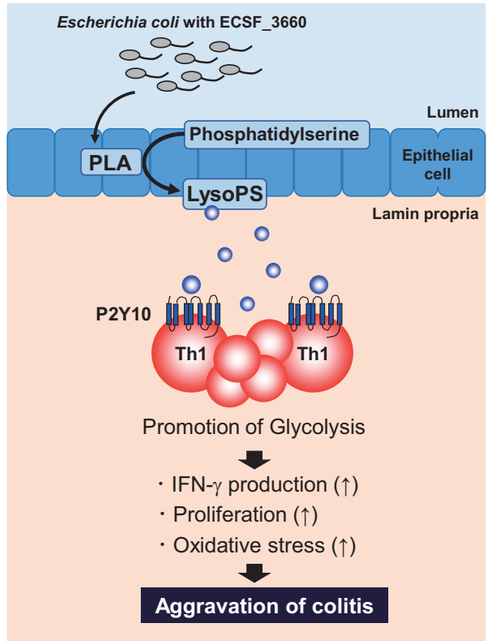


**Figure.**
**LysoPS aggravates Th1-dependent colitis via P2Y10 receptor.**
Elevation of intestinal lysophosphatidylserine (LysoPS) concentration due to microbial phospholipase A (PLA)-mediated hydrolysis of phosphatidylserine in the host cell membrane causes progression of intestinal inflammation by eliciting immunopathological Th1 responses through promotion of glycolysis via P2Y10 receptor.

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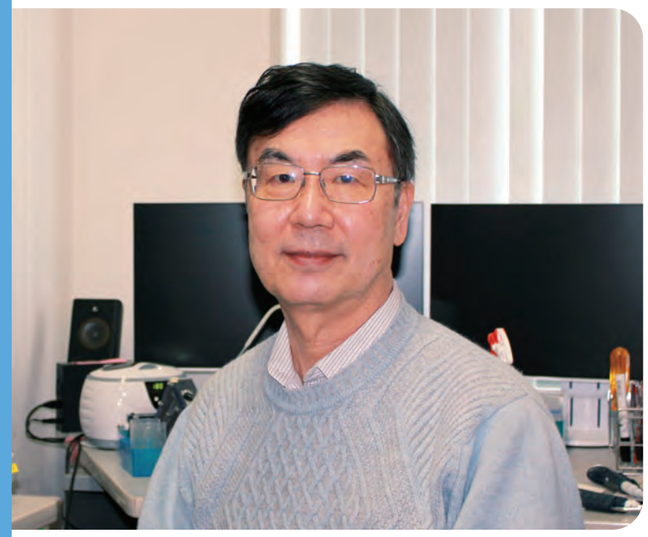
# Experimental Immunology



## Shimon Sakaguchi, MD/PhD

| Professor | Shimon Sakaguchi |
|---|---|
| Associate Professor | Kenji Ichiyama |
| Assistant Professor | Yusuke Takeshima |
| Postdoctoral Fellow | 4 |
| Research Assistant | 3 |
| Visiting Scientist | 14 |
| Support Staff | 6 |

This laboratory studies: (i) the cellular and molecular basis of immunologic self-tolerance, in particular the roles of regulatory T cells (Treg); (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.

Treg cells, which specifically express the transcription factor Foxp3, are actively engaged in the maintenance of immunological self-tolerance and homeostasis. Treg development and function is coordinated by the establishment of the Treg-specific epigenetic landscape and the transcription factor network involving FoxP3. While FoxP3 functionally acts as a strong repressor of *Il2*, *Ifng*, and other genes upon Treg activation, the Treg epigenome encompassing Treg-specific CpG hypomethylation, chromatin accessibility and histone modifications, reinforces activation of enhancers, especially super-enhancers, associated with Treg signature genes such as *Foxp3*, *Cd25* and *Ctla4*, and stabilizes their expression. Notably, the establishment of such Treg-specific epigenome is FoxP3-independent and a large portion of Treg-specific super-enhancers are concurrently activated at the precursor stage, prior to FoxP3 expression (Kitagawa et al., 2017). These findings suggest that genetic and epigenetic alterations at Treg function-associated genes may be causative of autoimmune and other inflammatory diseases by affecting Treg development and function (reviewed in Sakaguchi 2020).

In 2021, we addressed how Treg signature gene loci, in particular the *Foxp3* locus, become activated along Treg cell differentiation in the thymus. At the *Foxp3* gene locus, the mammalian conserved non-coding sequences (CNSs) have been highlighted as the key functional enhancer elements for induction and stabilization of Foxp3 expression. To determine how they developmentally contribute to Treg lineage specification, we showed that among *Foxp3* CNSs, the promoter-upstream CNS0 and the intergenic CNS3, which bound distinct transcription factors, were activated at early stages of thymocyte differentiation prior to *Foxp3* promoter activation, with sequential genomic looping bridging these regions and the promoter. While deletion of either CNS0 or CNS3 partially compromised thymic Treg cell generation, deletion of both completely abrogated the generation and impaired the stability of Foxp3 expression in residual natural Treg cells. As a result, CNS0/3-double-deleted mice succumbed to lethal systemic autoimmunity/inflammation. Thus, coordinated activation of *Foxp3* CNS0 and CNS3 initiates and stabilizes Foxp3 gene expression, thereby crucially controlling Treg cell development, maintenance, and consequently immunological self-tolerance (Kawakami, Kitagawa et al., Immunity, 2021).

Foxp3-expressing $CD4^+CD25^+$ Tregs constitutively and highly express the immune checkpoint receptor CTLA-4, whose Treg-specific deficiency causes severe systemic autoimmunity (Wing et al., Science 2008). As a key mechanism of Treg-mediated suppression, Treg-expressed CTLA-4 downregulates the expression of CD80/CD86 costimulatory molecules on antigen-presenting cells (APCs). We have shown that Treg-expressed CTLA-4 facilitates Treg-APC conjugation and immune synapse formation. The immune synapses thus formed provide a stable platform whereby Tregs are able to deplete CD80/CD86 molecules on APCs by extracting them via CTLA-4-dependent trogocytosis. The depletion occurs even with Tregs solely expressing a mutant CTLA-4 form lacking the cytoplasmic portion required for its endocytosis. Furthermore, CD80 downregulation or blockade by Treg-expressed membrane CTLA-4 or soluble CTLA-4 Ig, respectively, disrupts cis-CD80/PD-L1 heterodimers and increases free PD-L1 on dendritic cells (DCs), expanding a phenotypically distinct population of $CD80^{lo}$ free-$PD\text{-}L1^{hi}$ DCs. Taken together, Tregs are able to inhibit the T-cell stimulatory activity of APCs by reducing their CD80/CD86 expression via CTLA-4-dependent trogocytosis. This CD80/CD86 reduction on APCs is able to exert dual suppressive effects on T-cell immune responses by limiting CD80/CD86 costimulation to naïve T cells and by increasing free-PD-L1 available for the inhibition of PD-1 expressing effector T cells. Blockade of CTLA-4 and PD-1/PD-L1 in combination may therefore synergistically hinder Treg-mediated immune suppression, thereby effectively enhancing anti-tumor immune responses, with occasional development of autoimmunity (Tekguc et al., PNAS, 2021).

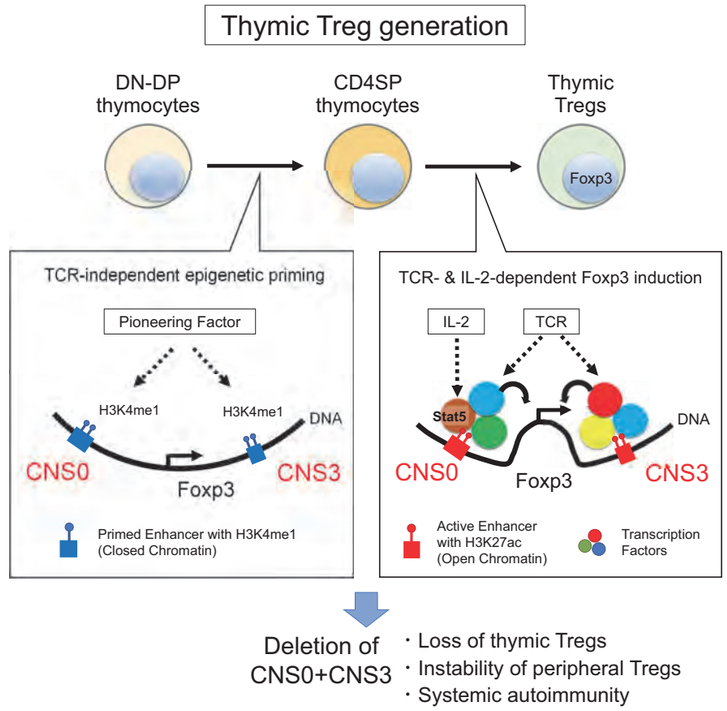



**Figure.**
At the *Foxp3* gene locus, the promoter-upstream CNS0 and then the intergenic CNS3 are activated at early stages of thymocyte differentiation prior to *Foxp3* promoter activation, with sequential genomic looping among these regions. While deletion of either CNS0 or CNS3 partially impairs thymic Treg cell generation, deletion of both completely abrogates the Treg generation, evoking systemic autoimmunity/inflammation.

## Recent Publications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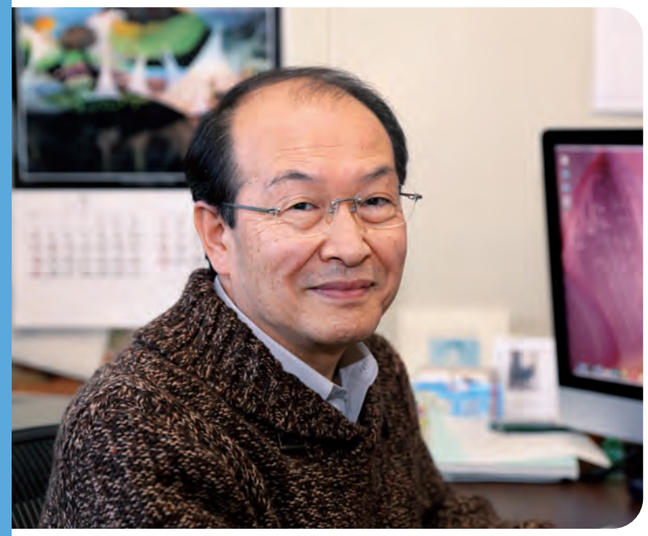
# Cell Signaling




## 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 activation through inhibitory checkpoint receptors

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 of CTLA4 and PD-1, we have analyzed the dynamic regulation of other inhibitory co-stimulation receptor, LAG3. This inhibitory receptor was also colocalized with the TCR-MC upon TCR stimulation to mediate inhibition of T cell activation. Since the association 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 (Figure).

### 2. Inhibitory mechanism of T cell activation through phosphatases

We have analyzed negative regulation of T cell activation, particularly 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.

### 3. Regulation of T cell function by innate signaling

We have also analyzed the modulation of T cell function by innate-like signals. During such analyses, we found that Receptor-interacting serine/threonine-protein kinase 1 (RIPK1) plays a critical role in T cell function and differentiation, particularly on metabolic regulation and senescence. We are analyzing T cell-specific RIPK1-deficient mice to analyze its function of T cells on metabolism and aging.

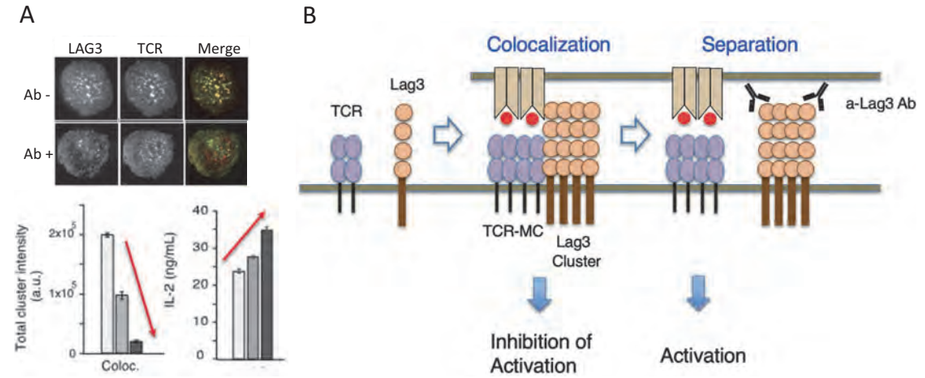



**Figure.**
**Negative regulation of T cell activation through LAG3 colocalized with TCR microcluster.**
A. LAG3 generates clusters colocalized with TCR-microcluster (MC) upon T cell activation. anti-LAG3 Ab inhibited the colocalization between TCR-MC and LAG3 clusters while enhanced IL-2 production. B. Schematic illustration indicating that colocalization of LAG3 clusters with TCR-MC is critical for LAG3 to induce suppressive function of T cell activation which is inhibited by anti-LAG3 Ab.

## Recent Publications


1. Takemori T, Sugimoto-Ishige A, et al. Establishment of a monoclonal antibody against human NTCP that blocks HBV infection. J Virol. 96:e0168621 (2022).
2. Kumagai A, Nara T, Uematsu M, Kakinuma Y, Saito T, and Masuda K. Development and characterization of a unique anti-IgE monoclonal antibody cross-reactive between human and canine IgE. Immun Inflamm. Dis. 9:1740-1748 (2021).
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4. Imanishi T and Saito T. T Cell co-stimulation and functional modulation by innate signals. Trends Immunol. 41:200-212 (2020).
5. Sasaki T, Yajima T, Shimaoka T, Ogawa S, Saito T, Yamaoka K, Takeuchi T and Kubo M. Synergistic effect of IgG4 antibody and CTLs causes tissue inflammation in IgG4-related disease. Int Immunol. 32:163-174 (2020).

# Lymphocyte Differentiation

## Tomohiro Kurosaki, MD/PhD

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|---|---|
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| Research Assistant | 2 |
| Support Staff | 4 |

The coronavirus disease 2019 (COVID-19) pandemic, caused by the β-coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a global health crisis. In this year, in order to make broadly protective vaccines against SARS-related coronaviruses, we designed new types of vaccines, and tested whether they work in the mouse model system.

SARS-related coronaviruses may cause future outbreaks, therefore broadly protective vaccines are urgently needed. When looking carefully at the structure of the receptor binding region (RBD) of the spike protein of the SARS-CoV-2 virus, it is composed of the head and the core sub-region, the former and the latter of which can directly block the entry of SARS-CoV-2, and contribute to the overall structural stability of the RBD region, respectively.

Given that the core sub-domain is structurally well conserved among SARS-related viruses, we intended to make new type of vaccines to elicit neutralizing antibodies against the core sub-domain. For this purpose, we introduced N-linked glycans onto the SARS-CoV-2 RBD surfaces and used them as immunogens in a mouse model. These types of vaccines indeed elicited significant neutralizing activity for not only SARS-CoV-2 but SARS-related viruses such as bat WIV1-CoV.

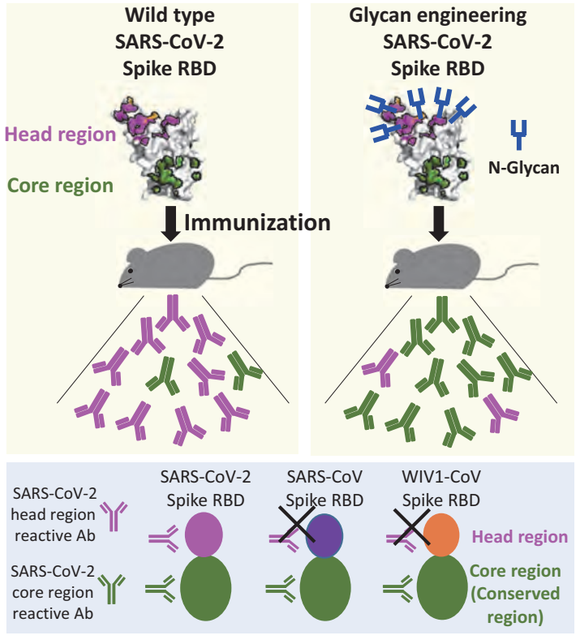


**Figure.**
**How to make protective vaccines against not only SARS-CoV-2, but SARS-CoV and WIV1-CoV viruses.**
In order to induce the antibodies predominantly against the regions that are structurally conserved in RBD among SARS-related viruses, glycan engineering was performed to mask the dominant epitope on the RBD head region which is a structurally non-conserved region. When mice are immunized with this modified RBD vaccine, as expected, antibodies that recognize the structurally conserved core-RBD region of not only SARS-CoV-2 but also other related SARS-related viruses such as SARS-CoV, WIV1-CoV are predominantly induced and also showed a high protective effect against these SARS-related viruses.

## Recent Publications


1. Inoue T, Shinnakasu R, Kawai C, Ise W, Kawakami E, Sax N, Oki T, Kitamura T, Yamashita K, Fukuyama H, Kurosaki T. Exit from germinal center to become quiescent memory B cells depends on metabolic reprograming and provision of a survival signal. J Exp Med. 218(1):e20200866 (2021).
2. Shinnakasu R, Sakakibara S, Yamamoto H, Wang PH, Moriyama S, Sax N, Ono C, Yamanaka A, Adachi Y, Onodera T, Sato T, Shinkai M, Suzuki R, Matsuura Y, Hashii N, Takahashi Y, Inoue T, Yamashita K, Kurosaki T. Glycan engineering of the SARS-CoV-2 receptor-binding domain elicits cross-neutralizing antibodies for SARS-related viruses. J Exp Med. 218(12):e20211003 (2021).
3. Tanaka S, Ise W, Baba Y, Kurosaki T. Silencing and activating anergic B cells. Immunol Rev. doi: 10.1111/imr.13053 (2021).
4. Yeh CH, Finney J, Okada T, Kurosaki T, Kelsoe G. Primary germinal center-resident T follicular helper cells are a physiologically distinct subset of CXCR5hiPD-1hi T follicular helper cells. Immunity 55(2):272-289 (2022).
5. Tsao HW, Kaminski J, Kurachi M, Barnitz RA, Dilorio MA, LaFleur MW, Ise W, Kurosaki T, Wherry EJ, Haining WN, Yosef N. Batf-mediated epigenetic control of effector CD8+ T cell differentiation. Sci Immunol. 7(68):eabi4919 (2022).

# Malaria Immunology


## Cevayir Coban, MD

Professor | Cevayir Coban


Malaria infection often leads to severe complications such as cerebral malaria. Even after the recovery from severe illness, individuals are repeatedly re-infected. The immune pathology causing severe complications and the reasons of the lack of sterile immunity against malaria have been an intense research topic in our laboratory.

To investigate the molecular pathogenesis underlying cerebral malaria, we investigated the spatial distribution of the 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 demonstrated that *Plasmodium berghei* ANKA (*Pb*ANKA) parasites significantly accumulated in the olfactory bulb (OB) of mice, compared with the other parts of the brain, including the cerebral cortex, cerebellum and brainstem (*Matsuo-Dapaah et al., Int Immunology, 2021*). We expect that CUBIC clearing of whole ECM brain might greatly advance our understanding of human cerebral malaria (Figure 1).

The germinal center (GC) in secondary lymphoid organs is a very important structure where somatic hypermutation and clonal selection are coupled for antibody affinity maturation against infections including malaria. However, how GCs are formed and regulated is incompletely understood. We recently identified an unexpected role of Tank-binding kinase-1 (TBK1) as a crucial B cell-intrinsic factor for GC formation. In the absence of TBK1 in B cells, B cells failed to form GC despite normal Tfh cell differentiation. The TBK1 phosphorylation elevates in B cells during GC differentiation and regulates the balance of IRF4/BCL6 expression by limiting CD40 and BCR activation through noncanonical NF-κB and AKTT308 signaling. In the absence of TBK1, CD40 and BCR signaling synergistically enhanced IRF4 expression in Pre-GC, leading to BCL6 suppression, and therefore failed to form GCs. As a result, memory B cells generated from TBK1-deficient B cells fail to confer sterile immunity upon reinfection, suggesting that TBK1 determines B cell fate to promote long-lasting humoral immunity (*Lee et al., J Exp. Medicine, 2022*).

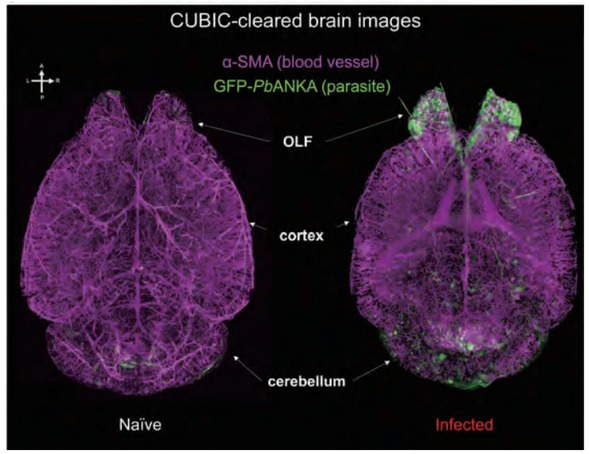


Figure 1.
Using a new three-dimensional CUBIC tissue-clearing method to examine the brain during experimental cerebral malaria (*Matsuo-Dapaah et al., Int. Immunology, 2021*). The 3-dimensional (3D) images of single microvessels deep in the whole brain after CUBIC cleared in Naïve and PbANKA (GFP tagged)-infected mice brains. The GFP-tagged parasites are preferentially accumulated in the capillaries and tissue of the olfactory bulb.

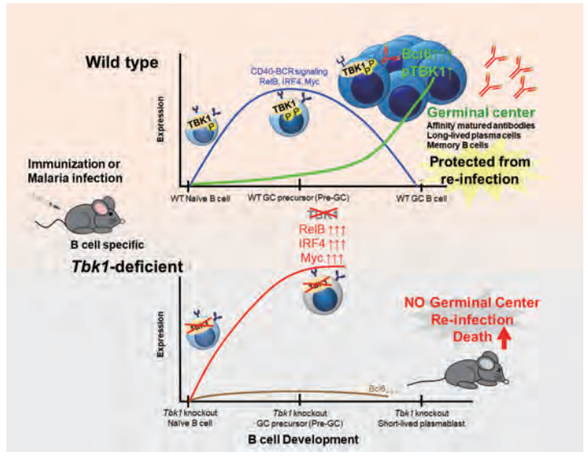


Figure 2.
**B cell-intrinsic TBK1 is essential for germinal center formation during infection and vaccination in mice (*Lee et al., J Exp. Medicine, 2022*).**
A schematic diagram illustrating the mechanism of B cell fate decision controlled by TBK1 through fine tuning of CD40/BCR signaling to negatively regulate IRF4 and c Myc in Pre GC. As a result, in the absence of TBK1 in B cells GC is not formed and high quality antibodies which protects against re-infection could not be produced.

## Recent Publications


1. 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).
2. 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).
3. Coban C. The host targeting effect of chloroquine in malaria. Curr Opin Immunol. 66:98-107 (2020).
4. Lee MSJ, Natsume-Kitatani Y, Temizoz B, Fujita Y, Konishi A, Matsuda K, Igari Y, Tsukui T, Kobiyama K, Kuroda E, Onishi M, Marichal T, Ise W, Inoue T, Kurosaki T, Mizuguchi K, Akira S, Ishii KJ, Coban C. B cell-intrinsic MyD88 signaling controls IFNγ-mediated early IgG2c class switching in response to a particulate adjuvant. Euro J Immunol. doi:10.1002/eji.201848084 (2019).
5. Coban C, Lee MSJ, Ishii KJ. Tissue-specific immunopathology during malaria infection. Nat Rev Immunol. doi:10.1038/nri.2017.138 (2018).

# Vaccine Science

## Ken J. Ishi, MD/PhD

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| Support Staff | 2 |

**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.

Below are some of our recent works conducted in FY2021

### *1. Primary Cilia in the Skin: Functions in Immunity and Therapeutic Potential*

The skin is the biggest organ and provides a physical and immunological barrier against pathogen infection. The distribution of primary cilia in the skin of mice has been reported, but which cells in human skin have them has not, and we still know very little about how they change in response to immune reactions or disease. This review introduces several studies that describe mechanisms of cilia regulation by immune reaction and the physiological relevance of cilia regulating proliferation and differentiation of stroma cells, including skin-resident Langerhans cells. Based on such background and potential of research on primary cilia, we published the review article as shown in the subheading and research article entitled; Increase in primary cilia in the epidermis of patients with atopic dermatitis and psoriasis, where we elucidate the incidence of primary cilia in skin inflammation and the potential mechanism underlying the dysregulation of keratinocytes. Significant increases in ciliated cells were observed in the patients with atopic dermatitis and psoriasis skin samples compared with normal skin samples. An increase of ciliated cells in the epidermis may impair keratinocyte differentiation under stress conditions caused by inflammation in both AD and psoriasis patients.

### *2. A CpG oligonucleotide annealed to a complementary strand with an amphiphilic chain unit, acts as a potent cancer vaccine adjuvant by targeting draining lymph nodes*

Robust induction of cancer-antigen-specific CD8+ T cells is essential for the success of cancer peptide vaccines, which are composed of a peptide derived from a cancer-specific antigen and an immune-potentiating adjuvant, such as a Toll-like receptor (TLR) agonist. Efficient delivery of a vaccine antigen and an adjuvant to antigen-presenting cells in the draining lymph nodes (LNs) holds key to maximize vaccine efficacy. We developed S-540956, a novel TLR9-agonistic adjuvant consisting of B-type CpG ODN2006 (also known as CpG7909), annealed to its complementary sequence oligodeoxynucleotide (ODN) conjugated to a lipid; it could target both a cancer peptide antigen and a CpG-adjuvant in the draining LNs. S-540956 accumulation in the draining LNs and activation of plasmacytoid dendritic cells (pDCs) were significantly higher than that of ODN2006. Mechanistic analysis revealed that S-540956 enhanced the induction of MHC class I peptide-specific CD8+ T cell responses via TLR9 in a CD4+ T cell-independent manner. In mice, the therapeutic effect of S-540956-adjuvanted with a human papillomavirus (HPV)-E7 peptide vaccine against HPV-E7-expressing TC-1 tumors was significantly better than that of an ODN2006-adjuvanted vaccine. Our findings demonstrate a novel adjuvant discovery with the complementary strand conjugated to a lipid, which enabled draining LN targeting and increased ODN2006 accumulation in draining LNs, thereby enhancing the adjuvant effect. Our findings imply that S-540956 is a promising adjuvant for cancer peptide vaccines and has a high potential for applications in various vaccines, including recombinant protein vaccines.

### *3. Mechanisms of immunogenicity of, and reactogenicity to, LNP-mRNA vaccines*

Messenger (m) RNA vaccines such as those used to prevent COVID-19 owe part of their success to methylation that masks immunostimulatory properties of the mRNA, but the immunological mechanisms of adjuvanticity are unclear. Two new studies reveal distinct mechanisms for innate sensing of this hidden adjuvant. With two recent papers, we proposed a new mechanism by which LNP and RNA exert the adjuvanticity in vivo (Figure).

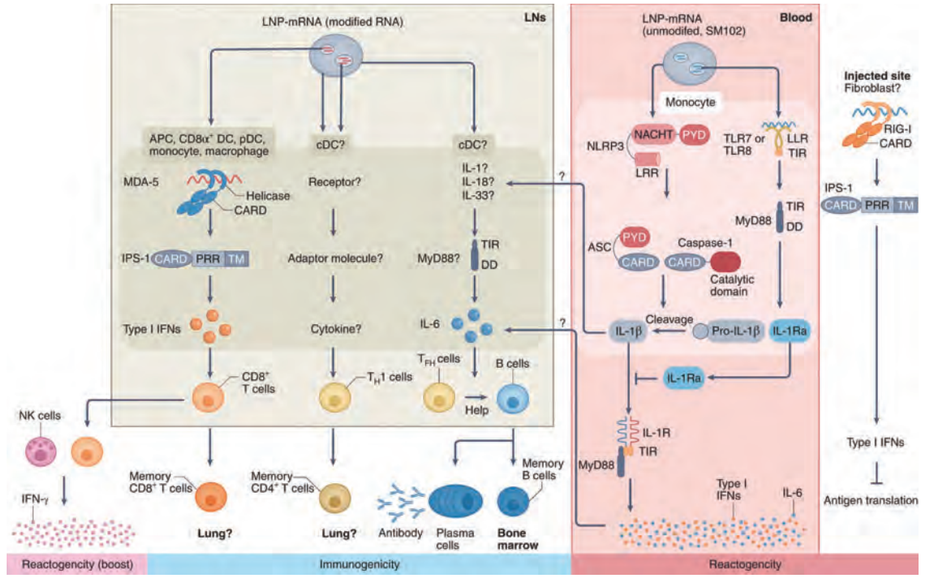
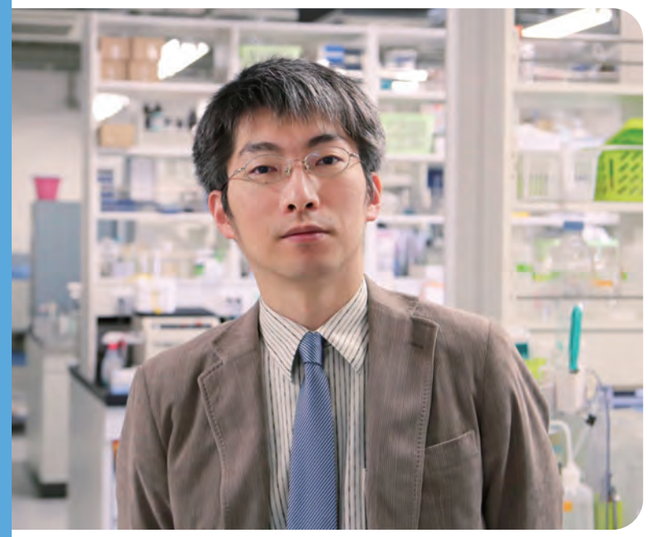



**Figure.**
In the lymph nodes (LNs), modified RNA sensed by MDA-5 results in the production of type I interferons (IFNs). Type I interferons induce antigen-specific $CD8^+$ T cell responses. LNPs can activate innate immune responses in lymph nodes and elicit production of IL-6 that is essential for the induction of $T_{FH}$ cells and GC B cells. These pathways are important for the immunogenicity of LNP-mRNA vaccines. When the mRNA is unmodified and a specific ionizable lipid (such as SM-102) is contained in the LNP, the mRNA is recognized by TLR7 and/or TLR8 and the ionizable lipid is recognized by the NLRP3 inflammasome in monocytes. Inflammasome-induced members of the IL-1 family trigger further inflammatory cytokine production. IL-1Ra is also produced as a negative feedback loop to inhibit IL-1 signaling and reduce inflammation. Unmodified mRNA is also detected by RIG-I, which results in the production of type I interferons and thus interferes with antigen translation. After booster vaccination, modified RNA–induced type I interferons also activate natural killer (NK) cells to produce IFN-γ. These signaling pathways might affect the reactogenicity of LNP-mRNA vaccines. APC, antigen-presenting cell; DC, dendritic cell; pDC, plasmacytoid dendritic cell; cDC, conventional dendritic cell; CARD, caspase-recruitment domain; PRR, proline-rich region; TM, transmembrane domain; TIR, Toll–IL-1R domain; DD, death domain; $T_H1$ cells, type 1 helper T cells; NACHT, nucleotide-binding domain; PYD, pyrin domain; LRR, leucine-rich repeat; ASC, apoptosis-associated speck-like protein containing a caspase-recruitment domain.

## Recent Publications


1. Hioki K, Hayashi T, et al. Machine learning-assisted screening of Herbal MedicineExtracts as vaccine adjuvants Frontiers in Immunology 2022 in press.
2. Kobiyama K, Ishii KJ. Making innate sense of mRNA vaccine adjuvanticity. Nat Immunol. 23(4):474-476 (2022).
3. Temizoz B, Hioki K, Kobari S, Jounai N, Kusakabe T, Lee MSJ, Coban C, Kuroda E, Ishii KJ. Anti-tumor immunity by transcriptional synergy between TLR9 and STING activation. Int Immunol. 2022 in press.
4. Lee MSJ, Inoue T, Ise W, et al. B cell-intrinsic TBK1 is essential for germinal center formation during infection and vaccination in mice. J Exp Med. 219(2):e20211336 (2022).
5. Nakagawa T, Tanino T, Onishi M, et al. S-540956, a CpG Oligonucleotide annealed to a complementary strand with an amphiphilic chain unit, acts as a potent cancer vaccine adjuvant by targeting draining lymph nodes. Front Immunol. 12:803090 (2021).

# Immunoparasitology




## Masahiro Yamamoto, PhD

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| **Professor** | **Masahiro Yamamoto** |
| Associate Professor | **Miwa Sasai** |
| Postdoctoral Fellow | **1** |
| Support Staff | **2** |


T cells play a critical role in adaptive immunity for pathogen elimination and tumor surveillance (Gaud et al., 2018). T cells express a TCR-CD3 complex containing TCRαβ and CD3ζγεδεζ subunits on their surfaces (Fu et al., 2014). Under physiological conditions, T-cell activation is initiated by engagement of TCRs on T cells and peptides in complex with major histocompatibility complex (pMHC) molecules on antigen-presenting cells (APCs) (Samelson and Klausner, 1988). After binding of the TCR to the pMHC, tyrosine residues of immunoreceptor tyrosine-based activation motifs in the cytosolic region of the CD3ζ chain are phosphorylated by lymphocyte protein tyrosine kinase (Lck), recruiting zeta-chain-associated protein kinase of 70 kDa (ZAP70) (Courtney et al., 2018). Subsequently, ZAP70 phosphorylates the transmembrane adaptor molecule linker for the activation of T cells (LAT), which serves as a signaling hub and recruits other signaling molecules to the plasma membrane, including SH2-domain-containing leukocyte protein of 76 kDa (SLP76) and phospholipase C γ1 (PLCγ1) (Dustin and Choudhuri, 2016). The LAT/SLP76 complex together with interleukin-2-inducible T-cell tyrosine kinase (ITK) activates PLCγ1, catalyzing hydrolysis of phosphatidylinositol 4,5-bisphosphate ($PIP_2$) to produce inositol 1,4,5-trisphosphate ($IP_3$) and diacylglycerol (DAG) (Rhee, 2001). $IP_3$ and DAG are important for incrementing cytosolic $Ca^{2+}$ levels to activate NFAT and phosphorylate MAP kinases and protein kinase C θ (PKCθ), which activates NF-κB, resulting in production of various cytokines, T-cell proliferation, and T-cell effector function (Rao and Hogan, 2009; Samelson, 2011).

Although these core TCR signaling molecules are shared between $CD4^+$ and $CD8^+$ T cells, it is widely assumed that CD4 and CD8 are not accessory molecules but co-receptors that potentiate activation signals (Eichmann et al., 1989; Janeway, 1992; Parnes, 1989). Both CD4 and CD8α co-receptors augment CD3-TCR signaling, since the cytoplasmic regions of both CD4 and CD8α interact with Lck (Rudd et al., 1988; Veillette et al., 1988). Since Lck preferentially binds CD4 rather than CD8α (Veillette et al., 1989), TCR signaling pathways in $CD4^+$ and $CD8^+$ T cells are quantitatively different. However, given the shared signaling molecules, it is widely accepted that TCR signaling pathways in $CD4^+$ and $CD8^+$ T cells are qualitatively identical (Artyomov et al., 2010; Fu et al., 2014; Gaud et al., 2018). The cytoplasmic tail of CD8α contains a CXCP motif important for Lck binding (Shaw et al., 1990; Turner et al., 1990). CD8α-deficient mice restored with a mutant CD8α in which the Lck-binding motif is replaced with alanine display milder phenotypes in terms of $CD8^+$ T-cell activation and T-cell development in the thymus than do CD8-deficient mice restored with tailless CD8α (Chan et al., 1993; Fung-Leung et al., 1993), suggesting that there are other signaling molecules that bind to the cytoplasmic regions of CD8α (Zamoyska, 1994). However, such signaling molecules that specifically bind CD8α and differentiate the $CD8^+$ T-cell receptor signaling pathway are unknown.

Phospholipase C (PLC) regulates cellular concentrations of $PIP_2$ as substrate or $IP_3$ and DAG as products to control complex cellular homeostasis. $IP_3$ and DAG act as second messengers (Kadamur and Ross, 2013). $IP_3$ binds to the $IP_3$ receptor on the endoplasmic reticulum and increases cytosolic $Ca^{2+}$ levels. $Ca^{2+}$ and DAG activate protein kinase C (Rhee, 2001). The mammalian PLC family consists of thirteen members classified into six subtypes (β, γ, δ, ε, ζ, and η) based on amino acid sequence and domain structure (Kadamur and Ross, 2013). All PLCs possess a highly conserved catalytic core containing an N-terminal pleckstrin homology (PH) domain, an EF-hands motif, a split X+Y catalytic domain, and a C2 domain (Kadamur and Ross, 2013). In TCR signaling, PLCγ1 plays a critical role in activation of both $CD4^+$ and $CD8^+$ T cells under physiological conditions (Samelson, 2002). In contrast, bacterial superantigens activate TCR signaling pathways independently of PLCγ1 (Bueno et al., 2006). Moreover, such superantigen-induced activation does not require Lck, ZAP70, or CD4. Furthermore, PLCβ inhibition and PLCβ1 silencing each partially block superantigen-induced T-cell activation (Bueno et al., 2006), suggesting that PLCβ1 is involved in activation under pathological conditions. PLCβ4 is the last member of the PLCβ family and is highly expressed in the retina and cerebellar Purkinje cells (Peng et al., 1997; Watanabe et al., 1998). PLCβ4 deficiency leads to defects in the visual process and ataxia, suggesting its neurological significance (Jiang et al., 1996; Kano et al., 1998; Kim et al., 1997). Despite these established neurological roles, the immune functions of PLCβ4, especially in T-cell signaling pathways, remain unexplored (Kawakami and Xiao, 2013).

In this study, we examine the roles of PLCβ4 in TCR signaling and immune response and find that PLCβ4 is selectively required for activation of TCR signaling in $CD8^+$ T cells and physiologically important for anti-parasitic and anti-tumor adaptive immune responses.

## PLCβ4 plays an important role in $CD8^+$ T cell-mediated host defense against *T. gondii* and anti-tumor immunity

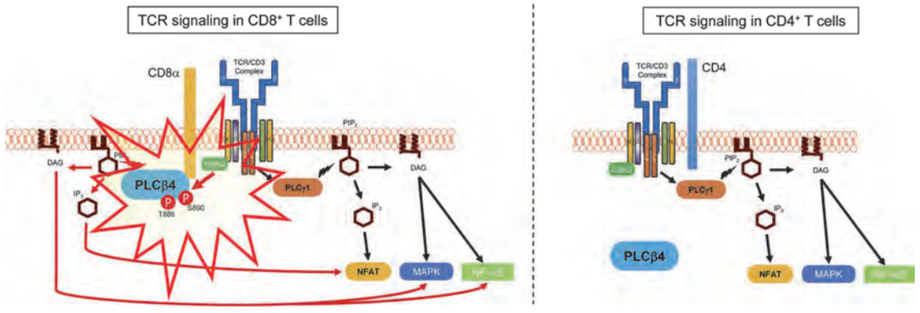
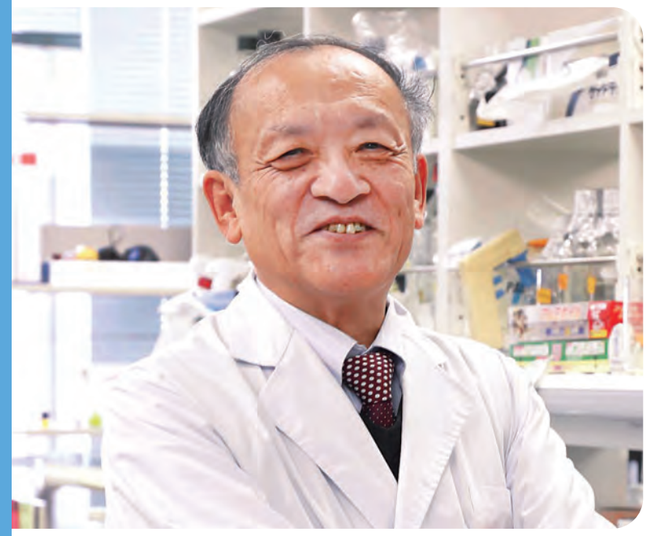



**Figure.**
Uncovering a novel role of PLCβ4 in selectively mediating TCR signaling in $CD8^+$ but not $CD4^+$ T cells.

## Recent Publications


1. 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).
2. Pradipta A, Bando H, Ma JS, Tanaka S, Sasai M, Yamamoto M. Plasmodium UIS3 avoids host cell-autonomous exclusion that requires GABARAPs but not LC3 and autophagy. Parasitol Int. 83:102335 (2021).
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).
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).
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).

# Biochemistry & Immunology



## Shigekazu Nagata, PhD

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| Professor | Shigekazu Nagata |
| Assistant Professor | Takaharu Sakuragi |
| Postdoctoral Fellow | 1 |
| Research Assistant | 3 |
| Visiting Scientist | 2 |
| Support Staff | 1 |

Phospholipids are asymmetrically distributed between 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 phospholipid 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. The PtdSer, thus exposed to the cell surface, works as an "eat me" signal for apoptotic cells to macrophages. The PtdSer-exposure is also observed in activated platelets, lymphocytes and mast cells, capacitated sperm, aged erythrocytes, exosomes, and enveloped viruses.

We previously identified two P4-type ATPases (ATP11A and 11C) and their subunit CDC50A as flippases at plasma membranes (Segawa et al. Science 2014). ATP11A and 11C flip PtdSer, but not phosphatidylcholine (PtdCho), maintaining the asymmetric distribution of PtdSer. A group in Tohoku University Medical School detected a de novo heterozygous point mutation of ATP11A in a patient with developmental delays and neurological deterioration. Mice carrying the corresponding mutation died perinatally of neurological disorders. This mutation caused an amino acid substitution (Q84E) in the first transmembrane segment of ATP11A, and the mutant ATP11A flipped PtdCho (Figure 1). Aberrant PtdCho flipping markedly decreased the concentration of PtdCho in the outer leaflet of plasma membranes, whereas sphingomyelin (SM) concentrations in the outer leaflet increased. This change in the distribution of phospholipids altered cell characteristics, including cell growth, cholesterol homeostasis, and sensitivity to sphingomyelinase. These results provided insights into the physiological importance of the substrate specificity of plasma membrane flippases for the proper distribution of PtdCho and SM (Segawa et al. J. Clin. Invest. 2021).

Two families of membrane proteins (TMEM16 and XKR) support the scrambling of phospholipids at plasma membranes. Among ten members of the TMEM16 family, TMEM16F ubiquitously expressed and functions as $Ca^{2+}$-dependent scramblases. The TMEM16F gene is mutated in human Scott syndrome patients, a congenital bleeding disorder, indicating that TMEM16F is responsible for exposing PtdSer to activate blood clotting factors (Suzuki et al. Nature 2010). The XKR family comprises nine members. XKR8 is ubiquitously expressed and forms a binary complex with Basigin or Neuroplastin, an Ig-super family protein (Suzuki et al. Science 2013; PNAS 2016). XKR8 carries a caspase-recognition site at the C-terminal tail region, and its cleavage during apoptosis is necessary to function as a scramblase. The PtdSer, thus on the dead cell's surface, is recognized by macrophages for engulfment. A loss-of-function mutation of Xkr8 causes inefficient engulfment of apoptotic cells leading to the activation of autoimmunity and male infertility (Kohno et al. PNAS 2019; Yamashita et al. MCB 2020).

Combining cryo-EM and X-ray crystallography, we determined the tertiary structure of the human XKR8-Basigin complex at a resolution of 3.8 Å (Sakuragi et al. Nat. Struct. Mol. Biol. 2021). Its membrane-spanning region adopts a cuboid-like structure (Figure 2). A single PtdCho molecule was present in a hydrophobic cleft on the surface exposed to the outer leaflet of the plasma membrane. Nine charged residues are placed from top to bottom as a staircase inside the molecule. Two were essential for stabilizing the complex, while six were for scrambling phospholipids in inward and outward directions, providing a pathway for the translocation of phospholipids. A tryptophan residue (W45) was present between the head group of PtdCho and the extracellular end of the staircase. Its mutation to alanine made the Xkr8-Basigin complex constitutively active, indicating that the W45 may play a gatekeeper in regulating its scramblase activity. The structure of Xkr8-Basigin would provide insights into the molecular mechanisms underlying phospholipid scrambling.

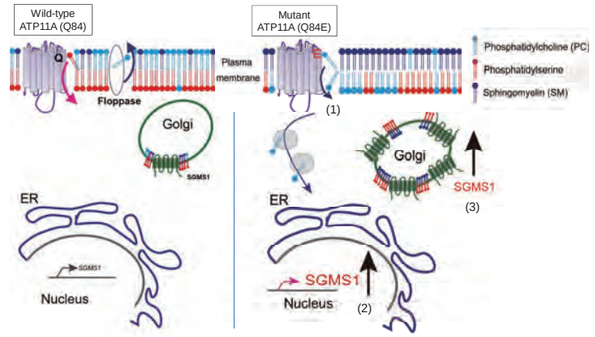


**Figure 1.**
A sublethal point mutation of human ATP11A that changes the substrate specificity. The ATP11A flippase translocates phosphatidylserine (PtdSer), but not phosphatidylcholine (PtdCho), from the outer to the inner leaflet of plasma membranes, maintaining the asymmetric distribution of PtdSer. A point Q84E mutation of ATP11A found in a patient with developmental delays and neurological deterioration allows PtdCho binding at the substrate entry site for flipping (1). The PtdCho flipped into the inner leaflet of the plasma membranes up-regulates the expression of the sphingomyelin (SM) synthase (SGMS1) gene (2). The SGMS1 then converts PtdCho to SM at the Golgi apparatus (3). SM then moves to the outer leaflet of the plasma membranes. The reduced PtdCho and increased SM concentrations at the outer leaflet of plasma membranes have a deleterious effect on cells, including cell growth, cholesterol homeostasis, and sensitivity to sphingomyelinase.

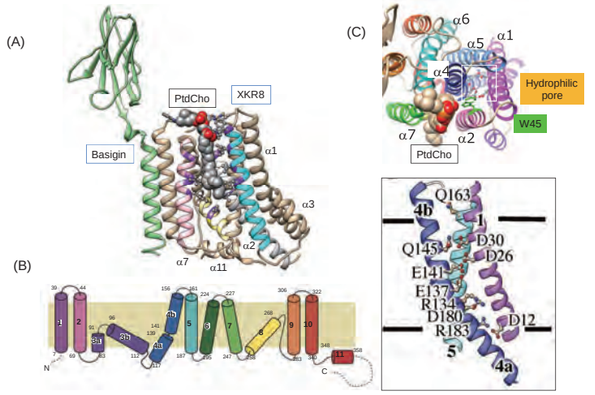


**Figure 2.**
The tertiary structure of the human XKR8-Basigin complex that scrambles phospholipids. (A) The tertiary structure of the human XKR8-Basigin complex is represented in a ribbon diagram. There is a cleft surrounded by hydrophobic residues in the upper middle region of the molecule, and phosphatidylcholine (PtdCho) is inserted in the cleft. (B) α-helices of hXkr8 are numbered and schematically shown. (C) Close-up side (lower panel) and top views of the hydrophilic pore (boxed area) carrying 9 charged amino acids (Glu, Asp, and Arg) in the α1, α 4, and α 5 helices. The side chains of charged amino acids are represented in a colored ball and stick model. PtdCho is present in the cleft on the surface. W45 (green) at the top of α2 seems to serve as a gatekeeper for scrambling phospholipids through the pore.

## Recent Publications


1. Ochiai Y, Suzuki C, Segawa K, Uchiyama Y, and Nagata S. Inefficient development of syncytiotrophoblasts in the Atp11a-deficient mouse placenta. Proc Natl Acad Sci. USA. 119 in press.
2. 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).
3. Segawa K, Kikuchi A, et al. A sublethal ATP11A mutation associated with neurological deterioration causes aberrant phosphatidylcholine flipping in plasma membranes. J Clin Invest. 131:e148005 (2021).
4. Sakuragi T, Kanai R, et al. The tertiary structure of the human Xkr8-Basigin complex that scrambles phospholipids at plasma membranes. Nat Struct Mol Biol. 28:825-834 (2021).
5. Caronni N, Piperno GM, et al. TIM4 expression by dendritic cells mediates uptake of tumor-associated antigens and anti-tumor responses. Nat Commun. 12:2237 (2021).

# Molecular Neuroscience

## Toshihide Yamashita, MD/PhD

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| Assistant Professor | Hiroshi Nakagawa |
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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.

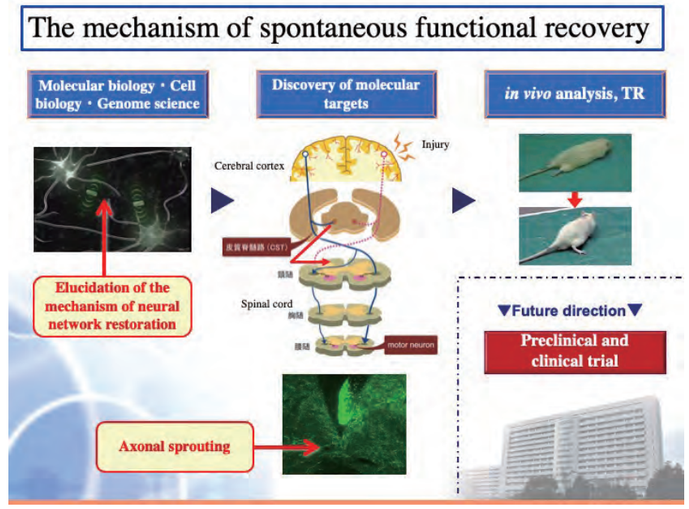


Figure 1.
The mechanism of spontaneous functional recovery.

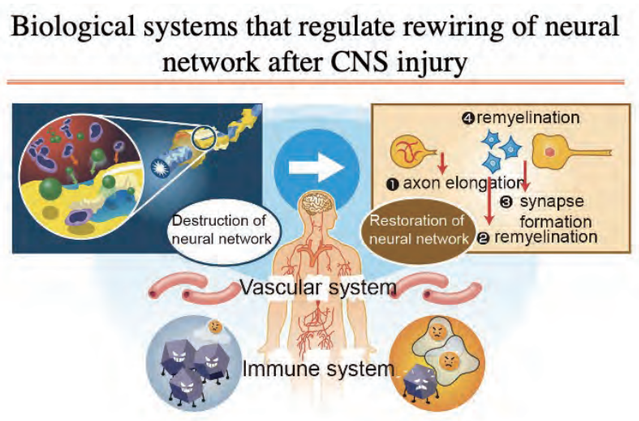


Figure 2.
Biological systems that regulate rewiring of neural network after CNS injury.

## 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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| | **Eri Ishikawa** |
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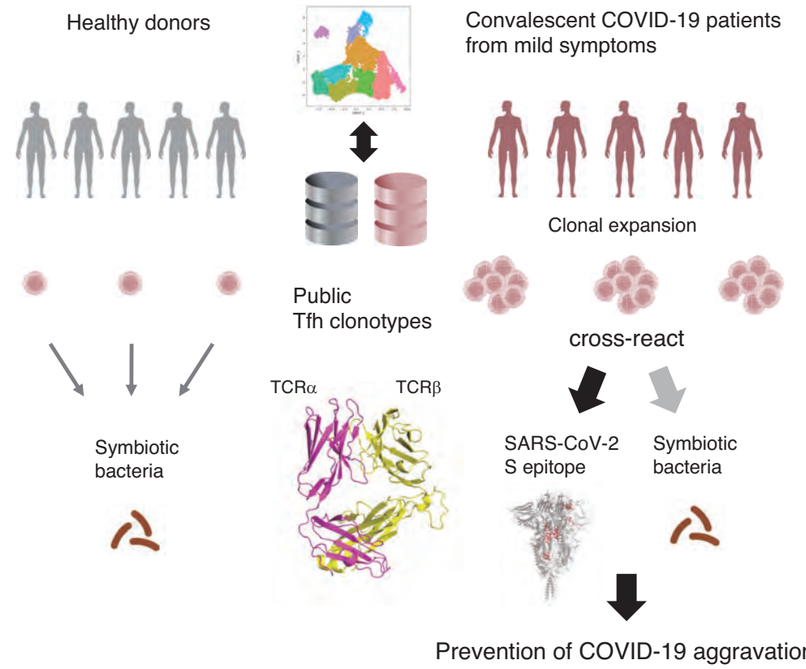
| Research Assistant | **8** |
| Visiting Scientist | **5** |
| Support Staff | **5** |

### Identification of conserved SARS-CoV-2 spike epitopes that expand public cTfh clonotypes in mild COVID-19 patients

Adaptive immunity is a fundamental component in controlling COVID-19. In this process, follicular helper T (Tfh) cells are a subset of $CD4^+$ T cells that mediate the production of protective antibodies; however, the SARS-CoV-2 epitopes activating Tfh cells are not well characterized. Here, we identified and crystallized TCRs of public circulating Tfh (cTfh) clonotypes that are expanded in patients who have recovered from mild symptoms. These public clonotypes recognized the SARS-CoV-2 spike (S) epitopes conserved across emerging variants. The epitope of the most prevalent cTfh clonotype, $S_{864\text{-}882}$, was presented by multiple HLAs and activated T cells in most of healthy donors, suggesting that this S region is a universal T cell epitope useful for booster antigen. SARS-CoV-2-specific public cTfh clonotypes also cross-reacted with specific commensal bacteria. In this study, we identified conserved SARS-CoV-2 S epitopes that activate public cTfh clonotypes associated with mild symptoms.

### Symbiotic bacteria-dependent expansion of MR1-reactive T cells causes autoimmune pancreatitis

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) developed chronic inflammation. *Bcl11b*$^{\Delta iThy}$ mice lacked conventional T cells and MAIT cells, whereas $CD4^+$IL-$18R^+$ αβ T cells expressing a skewed *Traj33* $(Jα33)^+$ T cell receptors (TCR) accumulate in the periphery, which are necessary and sufficient for the pathogenesis. The disorders observed in *Bcl11b*$^{\Delta iThy}$ mice were ameliorated by MR1-deficiency, transfer of conventional T cells, or germ-free conditions. We further determined the crystal structure and identified the antigen of the TCR expressed by pathogenic T cells. Here, we show that MR1-reactive T cells become pathogenic in a symbiotic bacteria-dependent manner.




### Recent Publications


1. Lu X, Hosono Y, Nagae M, Ishizuka S, 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).
2. Nagata M, Toyonaga K, Ishikawa E, et al. Helicobacter pylori metabolites exacerbate gastritis through C-type lectin receptors. *J Exp Med*. 218:e20200815 (2021).
3. Imai T, Matsumura T, Mayer-Lambertz S, et al. Lipoteichoic acid anchor triggers Mincle to drive protective immunity against invasive group A Streptococcus infection. *Proc Natl Acad Sci U S A*. 115: E10662-E10671 (2018).
4. Nagata M, Izumi Y, Ishikawa E, et al. Intracellular metabolite β-glucosylceramide is an endogenous Mincle ligand possessing immunostimulatory activity. *Proc Natl Acad Sci U S A*.114: E3285-E3294 (2017).
5. Toyonaga K, Torigoe S, Motomura Y, et al. C-Type Lectin Receptor DCAR Recognizes Mycobacterial Phosphatidyl-Inositol Mannosides to Promote a Th1 Response during Infection. *Immunity* 45:1245-57 (2016).

# Stem Cell Biology and Developmental Immunology

## Takashi Nagasawa, MD/PhD

| | |
|---|---|
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| Associate Professor | Yoshiki Omatsu |
| 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 these findings, 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 (Omatsu at al. *Immunity* 2010; Shimoto et al. *Blood* 2017).

In addition, we determined the nature of CAR cells, showing that CAR cells are mesenchymal stem cells, which give rise to adipocytes and osteoblasts (Seike et al. *Genes Dev*. 2018) 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 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 the role of Runx transcription factors 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, is predominantly expressed in CAR cells. CAR cells and HSC niches are normally formed and maintained in mice lacking Runx1 or Runx2 in CAR cells. However, 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 and hematopoietic progenitor cells in 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 is induced by Foxc1 and enforced expression of Runx1 decreases 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. Clinically, expression of Runx1 and Runx2 was reduced in CAR cells in a mouse model of marrow fibrosis, which is a significant complication of myeloproliferative neoplasns (MPN) that affects up to 20% of patients and is associated with a poor prognosis. These results strengthen the claim that CAR cells are the bone marrow specific fibroblastic reticular cells, which express specific and critical transcription factors, including Foxc1, Ebf 1/3, and Runx1/2, providing HSC niches and bone.

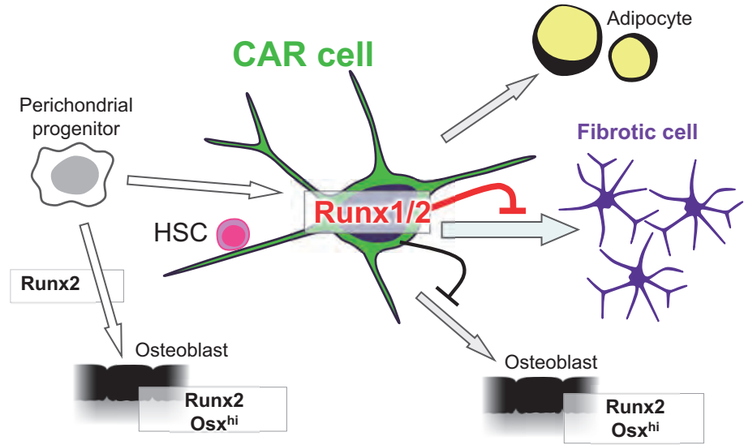


**Figure 1.**
**The functions of Runx1/2 in CAR cell maintenance.**
CAR cells require Runx1 or Runx2 to prevent their fibrotic conversion and maintain HSCs and hematopoiesis in adult bone marrow.

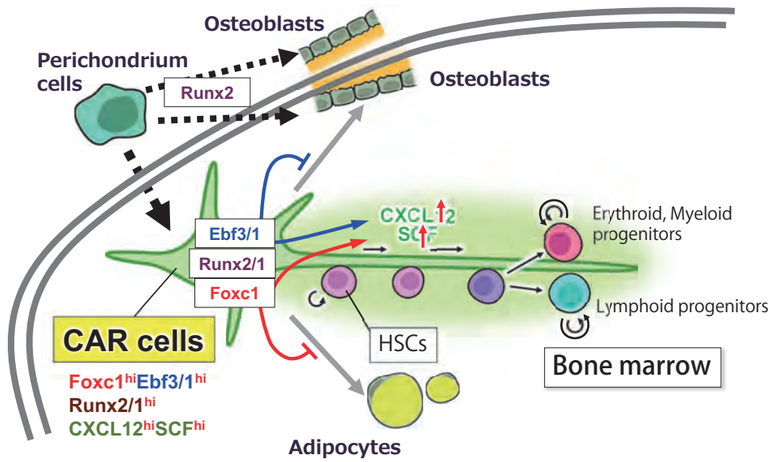


**Figure 2.**
**The development and functions of CAR cells.**
CAR cells are the major cellular component of non-hematopoietic cells in bone marrow characterized by several salient features in both mouse and human. The transcription factors, Foxc1, Ebf1/Ebf3, and Runx1/2 and cytokines, CXCL12 and SCF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

## Recent Publications


1. Omatsu Y, Aiba S, Maeta T, Higaki K, Aoki K, Watanabe H, et al. Runx1 and Runx2 inhibit fibrotic conversion of cellular niches for hematopoietic stem cells. *Nat Commun*. in press (2022).
2. Omatsu Y, Higaki K, Nagasawa T. Cellular Niches for Hematopoietic Stem Cells and Lympho-Hematopoiesis in Bone Marrow During Homeostasis and Blood Cancers. *Curr Top Microbiol Immunol*. 434:33-54 (2021).
3. Nakagawa T, Jörg DJ, Watanabe H, Mizuno S, Han S, Ikeda T, Omatsu Y, Nishimura K, Fujita M, Takahashi S, Kondoh G, Simons BD, Yoshida S, Nagasawa T. A multistate stem cell dynamics maintains homeostasis in mouse spermatogenesis. *Cell Rep*. 37(3):109875 (2021).
4. Omatsu Y, Nagasawa T. Identification of microenvironmental niches for hematopoietic stem cells and lymphoid progenitors-bone marrow fibroblastic reticular cells with salient features. *Int Immunol*. 33(12):821-826 (2021).
5. Aoki K, Kurashige M, Ichii M, Higaki K, Sugiyama T, Kaito T, Ando W, Sugano N, Sakai T, Shibayama H; HANDAI Clinical Blood Club, Takaori-Kondo A, Morii E, Kanakura Y, Nagasawa T. Identification of CXCL12-abundant reticular cells in human adult bone marrow. *Br J Haematol*. 193(3):659-668 (2021).

# Aging Biology

## Eiji Hara, PhD

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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. Therefore, the accumulation of senescent cells, which is often seen with ageing 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, particularly with ageing, are poorly understood. This year we discovered that both gut bacteria and respiratory viruses induce cellular ageing in vivo through the following two studies.

(1) Reports of "post-acute COVID-19 syndrome," in which the inflammatory response persists even after SARS-CoV-2 has disappeared, are increasing but the underlying mechanisms of post-acute COVID-19 syndrome remain unknown. Here we show that SARS-CoV-2 infected cells trigger senescence-like cell-cycle arrest in neighboring uninfected cells in a paracrine manner via virus-induced cytokine production. In cultured human cells or bronchial organoids, these SASR-CoV-2 infection-induced senescent cells express high levels of a series of inflammatory factors known as senescence-associated secretory phenotypes (SASPs), in a sustained manner, even after SARS-CoV-2 is no longer detectable. We also show that the expression of the senescence marker *CDKN2A* and various SASP factor genes is increased in the pulmonary cells of patients with severe post-acute COVID-19 syndrome. Furthermore, we find that mice exposed to 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 undetectable, which could be substantially reduced by the administration of senolytic drugs. The sustained infection-induced paracrine senescence described here may be involved in the long-term inflammation caused by SARS-CoV-2 infection.

(2) Emerging evidence is revealing that alterations in gut microbiota are associated with colorectal cancer (CRC). However, very little is currently known about whether and how gut microbiota alterations are causally associated with CRC development. Here we show that 12 faecal bacterial taxa are enriched in CRC patients in two independent cohort studies. Among them, 2 *Porphyromonas* species are capable of inducing cellular senescence, an oncogenic stress response, through the secretion of the bacterial metabolite, butyrate. Notably, the invasion of these bacteria is observed in the CRC tissues, coinciding with the elevation of butyrate levels and signs of senescence-associated inflammatory phenotypes. Moreover, although the administration of these bacteria into $Apc^{\Delta 14/+}$ mice accelerate the onset of colorectal tumours, this is not the case when bacterial butyrate-synthesis genes are disrupted. These results suggest a causal relationship between *Porphyromonas* species overgrowth and colorectal tumourigenesis, and butyrate-induced senescence may be one of the mechanisms.

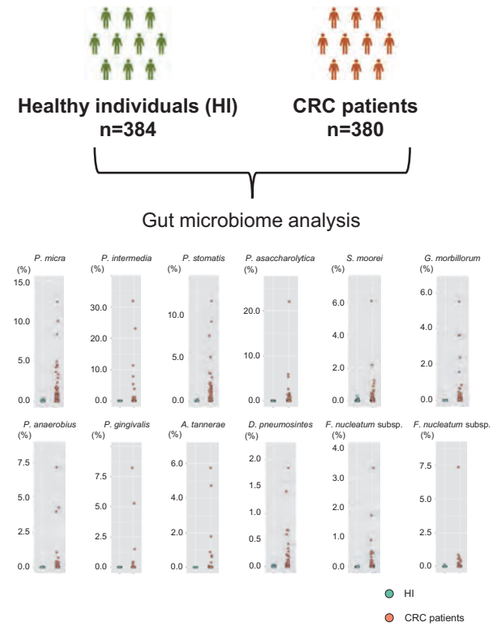


**Figure 1.**
Twelve bacterial taxa that were abundant in CRC patients and rarely present in healthy individuals were identified.

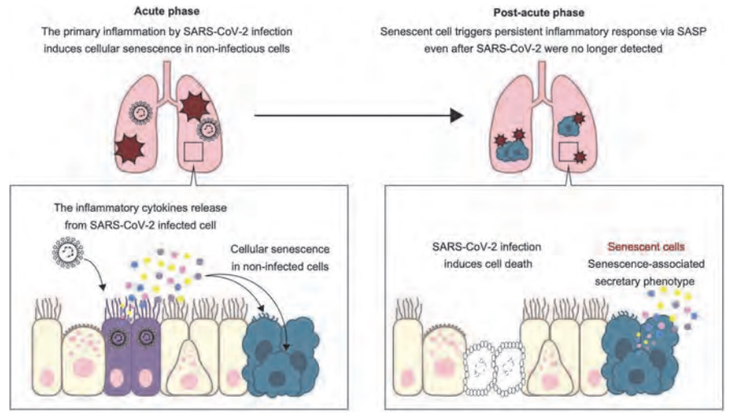


**Figure 2.**
Induction of cellular senescence and persistence of inflammatory response by SARS-CoV-2

## Recent Publications


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

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).

3. Wakita M, Takahashi A, Sano O, Loo TM, Imai Y, Narukawa M, Iwata H, Matsudaira T, Kawamoto S, Ohtani N, Yoshimori T, Hara E. A BET family protein degrader provokes senolysis by targeting NHEJ and autophagy in senescent cells. *Nat. Commun*. 11:1935 (2020).

4. Takahashi A, Loo TM, Okada R, Kamachi F, Watanabe Y, Wakita M, Watanabe S, Kawamoto S, Miyata K, Barber G N, Ohtani N, Hara E. Downregulation of cytoplasmic DNases is implicated in cytoplasmic DNA accumulation and SASP in senescent cells. Nat. Commun. 9:1249 (2018).

# 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 induces *SRC* gene expression via the Smad pathway in coupled with additional transcription factors, and determined the promoter and enhancer regions located upstream of the *SRC* gene. The upregulation of Src contributes to the progression of TGF-β–induced epithelial-mesenchymal transition. In addition, we identified CDCP1 as a Src-activating membrane glycoprotein in lipid rafts. Upregulation of CDCP1 induces prominent activation of Src and the STAT3 pathway, which promotes the invasive activity of epithelial cells. We also found 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. These findings suggest that CDCP1 is a co-receptor of MET (Fig. 1). Furthermore, ablation of CDCP1 suppresses the compensatory renal hypertrophy, indicating that CDCP1 is required for the HGF-MET signaling even in vivo. CDCP1 and MET are crucial for promoting cancer cell invasion; therefore, we expect this study to identify a potential therapeutic target in some types of cancer.

### 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. Subsequent analysis revealed that p18 functions by forming a hetero-heptamer complex (Ragulator), consisting of p18, p14, MP1, HBXIP, p10, RagA/C, and it is required for activation of mTORC1 on lysosomes. Conditional KO of p18 in the epidermis showed that p18-mTORC1 is crucial not only for anabolism of bio-materials, but also for catabolism via autophagy, indicating that p18 is tightly associated with the regulation of mTORC1 nutrient signaling in vivo. Recent studies in the intestinal tissues revealed that the p18-mediated mTORC1 signaling promotes the anabolic metabolism required for robust production of mucin in goblet cells. These findings underscore the critical role of p18 in the regulation of metabolic homeostasis in various tissues and cells. To further analyze the regulation of the p18 complex at the molecular level, we previously determined the crystal structure of Ragulator. This revealed that p18 wraps around the other components of Ragulator and provided significant insights into the role of p18-mediated regulation of mTORC1 on lysosomes. Recent analysis using p18 KO cells that lack regulatory components of Rag GTPase, such as GATOR1 and FLCN, showed that p18-Ragulator complex provides a regulatory platform that is indispensable for amino acid-dependent regulation of mTORC1 (Fig. 2). These findings identified the interacting molecular surface as a potential therapeutic target in lifestyle diseases, such as diabetes mellitus and cancer, both of which are linked to dysfunction of the mTORC1 pathway.

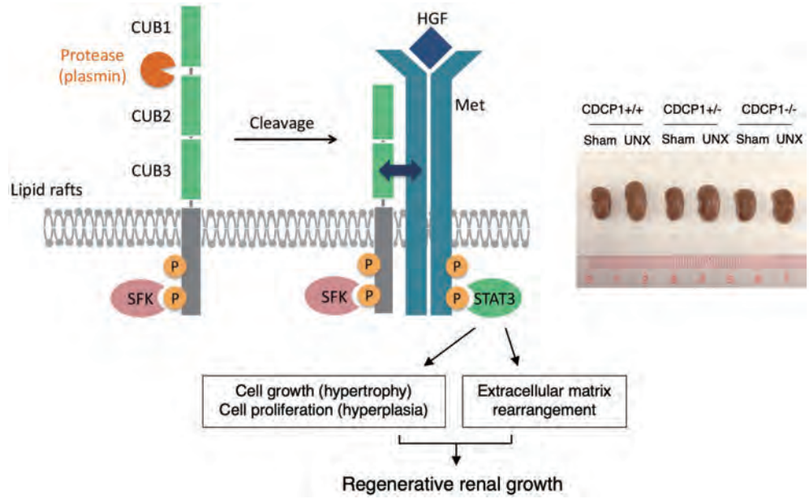


**Figure 1.**
Role of the CDCP1-Met-Src-STAT3 axis in regenerative renal growth.

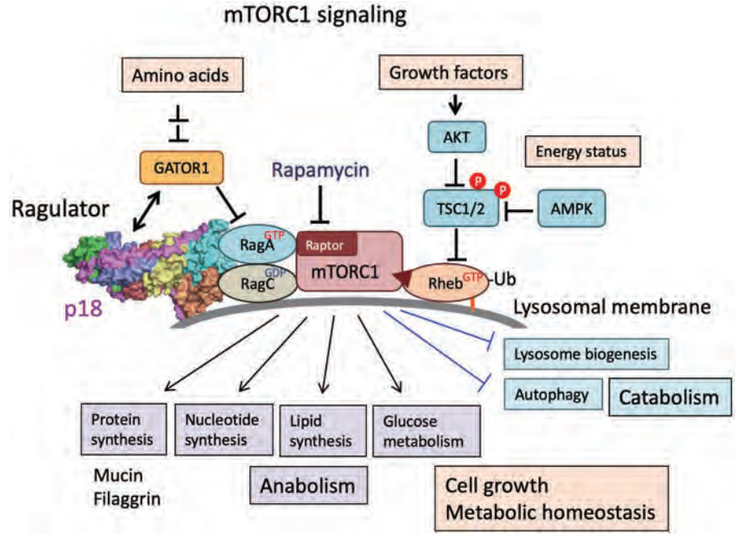


**Figure 2.**
Function and regulation of the mTORC1 signaling on lysosomal membrane.

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1. 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).
2. Kawase N, Sugihara A, Kajiwara K, Hiroshima M, Akamatsu K, Nada S, Matsumoto K, Ueda M, Okada M. SRC kinase activator CDCP1 promotes hepatocyte growth factor-induced cell migration/invasion of a subset of breast cancer cells. J Biol Chem. 298(3):101630 (2022).
3. Liu Y, Soh WT, Kishikawa JI, et al. An infectivity-enhancing site on the SARS-CoV-2 spike protein targeted by antibodies. Cell. 184(13):3452-3466.e18 (2021).
4. Nakatani T, Tsujimoto K, Park J, Jo T, Kimura T, Hayama Y, Konaka H, Takayoshi 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. Nature Commun. 12(1):3333 (2021).
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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|---|---|
| Professor | Nobuyuki Takakura |
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| Postdoctoral Fellow | 2 |
| Research Assistant | 6 |
| Support Staff | 3 |

Angiogenesis, a process of new blood vessels from preexisting vasculature, is involved in the progression of many diseases, such as cancer, retinopathy, inflammatory diseases, and so on and is one of drug target to control these diseases. Many types of cells such as pericytes, fibroblasts, leukocytes, platelets, and organ specific cells including cancer cells interact with endothelial cells to generate tissue and disease specific blood vessels. Since angiogenesis is a complex process, it is difficult to recapitulate it *in vitro*. Therefore, in vivo models are required for the study of vascular change and drug development.

Several in vivo models analyzing angiogenesis have been developed so far, i.e., tumor model, ischemia hind limb model, corneal transplantation model and so on in mice; however, it is difficult to translate experimental results from mouse to human blood vessels. In order to overcome this issue, we tried to establish a new model visualizing human blood vessels by using (cancer) patient derived xenograft (PDX) in immunodeficient mice. The reason why we used tumor tissue is because tumor blood vessels have highly angiogenic potential. We chose the cranial window model to monitor human blood vessels because this uses skull bone for fixing the cover glass to enable a stable rigid fixation allowing long-term observation for up to a year.

Using this method, we were able to observe human blood vessels in tumor tissues over the long term, for at least 49 days. As depicted in Figure, human blood vessels continuously grew and connected with mouse blood vessels. Human specific blocking antibody against vascular endothelial growth factor receptor 2 (VEGFR2) inhibited angiogenesis in a human endothelial cell specific manner. Therefore, this model provides images of the kinetics of changes to the vasculature within the tissue of the same individual mouse, suggesting that we can analyze the molecular mechanism of human angiogenesis precisely and search the methods to accelerate or suppress angiogenesis by using this model.

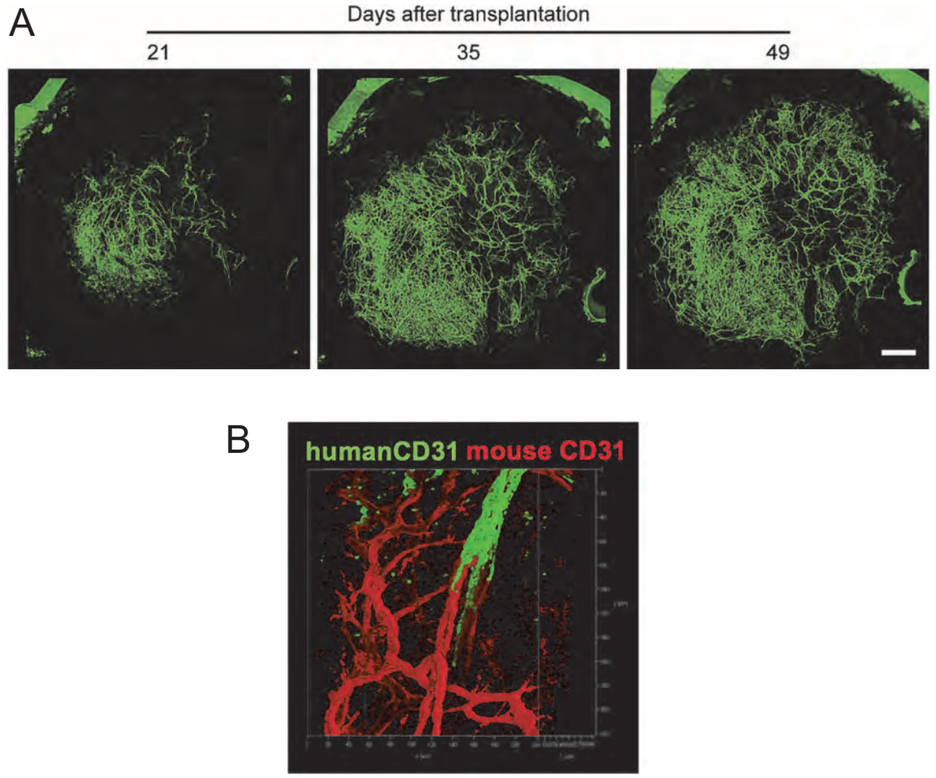


**Figure.**
**Visualization of human blood vessels formed in PDX mouse model.**
(A) Tumors freshly isolated from patient were cut into 1-2 mm fragments and were transplanted into a cranial window in NOD-Scid mice. In several time points, mice were intravenously injected with anti-human CD31 monoclonal antibody to visualize the human blood vessels (green) specifically in vivo. Blood vessels were observed by confocal microscopy under isoflurane anesthesia. (B) Mice as described above were intravenously injected with human specific anti-CD31 antibody (green) and mouse specific anti-CD31 antibody (red) and connection between human and mouse blood vessels was observed in PDX mouse model.

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2. Naito H, Wakabayashi T, Ishida M, Gil CH, Iba T, Rahmawati FN, Shimizu S, Yoder MC, Takakura N. Isolation of tissue-resident vascular endothelial stem cells from mouse liver. Nat Protoc. 15:1066-1081 (2020).
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 |
| Associate Professor | Yumi Matsuoka-Nakamura |
| Research Assistant | 4 |
| Visiting Scientist | 1 |
| Support Staff | 1 |

**Cell surface-expressed Ro52/IgG/HLA-DR complex is targeted by autoantibodies in patients with inflammatory myopathies**

Cellular misfolded proteins are transported to the cell surface by HLA class II molecules and recognized by autoantibodies. We found that the specific autoantibodies against the Ro52/IgG/HLA-DR complex were positive in 90% and 93% of patients with dermatomyositis who were positive for anti-MDA5 and anti-ARS antibodies, respectively. Furthermore, changes in the serum antibody titers correlated with laboratory values reflecting symptoms. These results suggest that antibodies to Ro52/IgG/HLA-DR may be involved in the pathogenesis of a subgroup of inflammatory myopathies.

**Clinical and laboratory parameters predicting cancer in dermatomyositis patients with anti-TIF1γ antibodies**

Dermatomyositis is an autoimmune disorder strongly associated with cancer development. We reported that a close pathophysiological relationship among myositis, cancer and skin involvements in dermatomyositis patients with anti-TIF1γ antibodies. Some inflammatory cytokines, particularly TNF and TNF receptor families may support cancer prediction in dermatomyositis patients with anti-TIF1γ antibodies.

**Regulatory B cells in immune-mediated cutaneous disorders (On-going)**

Regulatory B cells (Bregs) are regarded as a group of B cells which converge accelerated immune reactions by secreting IL-10. Dysfunction of Bregs has been reported in autoimmune disorders including systemic lupus erythematosus both in patients and murine models. However, since B cells are rarely found in the skin lesions, the role of B cells in cutaneous disorders has not been established. We found that Breg population is skewed in immune-mediated cutaneous disorders such as drug eruption and alopecia areata and B cells are presumed to exert causative role in the augmented T1 response in these disorders.

**Keratinocyte IL-36 receptor/MyD88 signaling mediates *Malassezia*-induced skin inflammation**

Among skin commensal fungi, *Malassezia* species exist on nearly all human skin surfaces. The pathophysiology of *Malassezia*-associated skin diseases remains poorly understood due in part to the lack of appropriate animal models. We newly developed a *Malassezia* skin infection model and found that *Malassezia*-induced IL-17- dependent skin inflammation and control of fungal infection are mediated via keratinocyte IL-36 receptor/MyD88 signaling.

**Loricrin and NRF2 coordinate epidermal cornification**

The skin epidermis undergoes a specialized mode of the cell-death program cornification, producing the stratum corneum (SC) compared to the “protein alloy.” The keratin-associated protein loricrin (LOR) promotes the maturation of the SC by generating extensive disulfide cross-linkages and appears indispensable for cornification. Following previous research suggesting LOR’s antioxidative property, we have found that LOR confers protection against carcinogenic electrophiles produced by epidermal resident Langerhans cells capturing polyaromatic hydrocarbons.

**GD3 May Suppress the Functional Activities of Benign Skin T Cells in Cutaneous T-Cell Lymphoma**

In cutaneous T-cell lymphoma (CTCL), which arises from skin-tropic memory T cells, malignant T cells and benign T cells are confined in the same skin lesions. Disialoganglioside with three glycosyl groups (GD3) is increasingly expressed on the surface of solid malignant tumor cells and takes part in tumor progression and suppression of tumor immunity. However, the role of GD3 in CTCL is not well-understood. Therefore, we analyzed the role of GD3 in cutaneous lymphoma. We found that GD3 from the malignant T cells was involved in suppressing the Th17 activity of the benign T cells independent of the regulation of resident memory T cell differentiation in CTCL.

**Distribution of hypomelanotic macules in tuberous sclerosis complex**

We treat a hereditary disorder called TSC with tumors, neurological symptoms and vitiligo due to the activation of mTORC1. However, the pathophysiology of the neurological symptoms and vitiligo in TSC are still unknown. In order to investigate the pathogenesis of vitiligo in TSC, which shows a unique shape and distribution, we reported that the activation of mTORC1 during development might cause the characteristic distribution of vitiligo via abnormal melanocyte migration.

**Collagen homeostasis paradoxically maintains in vitiligo lesion (On-going)**

In a daily clinic experience, 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 vitiligo 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.

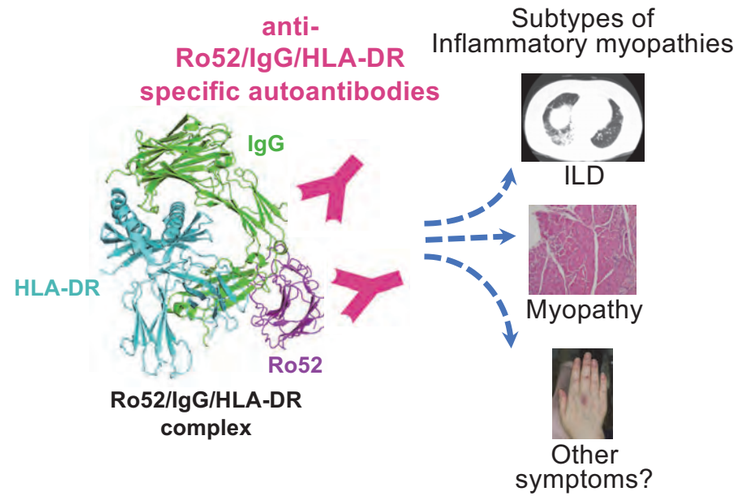



**Figure.**
Ro52, an intracellular Fc receptor, formed Ro52/IgG/HLA-DR complex and was transported to the cell surface. Sequential changes in serum anti-Ro52/IgG/HLA-DR specific autoantibody titers were correlated with changes in the disease status in patients with dermatomyositis.

## Recent Publications


1. Arase N, Tsuji H, Takamatsu H, Jin H, Konaka H, Hamaguchi Y, Tonomura K, Kotobuki Y, Ueda-Hayakawa I, Matsuoka S, Hirano T, Yorifuji H, Murota H, Ohmura K, Nakashima R, Sato T, Kumanogoh A, Katayama I, Arase H, Fujimoto M. Cell surface-expressed Ro52/IgG/HLA-DR complex is targeted by autoantibodies in patients with inflammatory myopathies. J Autoimmun. 126:102774 (2022).

2. Miyachi H, Wakabayashi S, Sugihira T, Aoyama R, Saijo S, Koguchi-Yoshioka H, Fujimoto M, Núñez G, Matsue H, Nakamura Y. Keratinocyte IL-36 Receptor/MyD88 Signaling Mediates Malassezia-Induced IL-17-Dependent Skin Inflammation. J Infect Dis. 223(10):1753-1765 (2021).

3. Kume M, Kiyohara E, Matsumura Y, Koguchi-Yoshioka H, Tanemura A, Hanaoka Y, Taminato M, Tashima H, Tomita K, Kubo T, Watanabe R, Fujimoto M. Ganglioside GD3 May Suppress the Functional Activities of Benign Skin T Cells in Cutaneous T-Cell Lymphoma. Front Immunol. 12:651048 (2021).

4. Takahashi A, Hattori S, Sakai E, Yang L, Katayama I, Fujimoto M, Wataya-Kaneda M. Distribution of hypomelanotic macules in tuberous sclerosis complex: A retrospective cohort study. J Am Acad Dermatol. S0190-9622(21)02282-9 (2021).

5. Okiyama N, Ichimura Y, Shobo M, Tanaka R, Kubota N, Saito A, Ishitsuka Y, Watanabe R, Fujisawa Y, Nakamura Y, Murakami A, Kayama H, Takeda K, Fujimoto M. Immune response to dermatomyositis-specific autoantigen, transcriptional intermediary factor 1γ can result in experimental myositis. Ann Rheum Dis. 80(9):1201-1208 (2021).

# Innate Immune Systems

## Kazuyo Moro, PhD

| Professor | Kazuyo Moro |
|---|---|
| Postdoctoral Fellow | 1 |
| Research Assistant | 2 |
| Visiting Scientist | 3 |
| Support Staff | 2 |

We have investigated the remarkable properties of innate immunity through studying group 2 innate lymphoid cells (ILC2), an innate lymphocyte lineage that we identified in 2010. ILC2 contribute to immune responses by secreting effector cytokines such as IL-5 and IL-13 and regulate the functions of both immune and non-immune cells. ILC2 play a pathogenic role in allergic diseases in barrier tissues including lungs, intestines, and skin. Aiming at advancing therapeutic strategies, we dissect how ILC2 form communication networks with other cells and how these networks malfunction in disease.

### 1. Posttranscriptional regulation of ILC2 homeostatic function via TTP

ILC2s are unique in their ability to produce low levels of type 2 cytokines at steady-state. However, it was unknown how this constitutive cytokine production is regulated. In this study, we demonstrate post-transcriptional regulation through tristetraprolin (TTP) is crucial for the regulation of constitutive type 2 cytokine production by ILC2s at an appropriate level for homeostasis. TTP binds to AU-rich elements (AREs) in the 3' UTRs of the mRNAs and promotes their degradation, often acting as a component of a negative feedback loop for cytokine production by destabilizing mRNA. However, we found that ILC2s highly express TTP in the steady state, while losing this expression following IL-33 stimulation, suggesting that continuous mRNA degradation by TTP prevents cytokine production in the steady state, while loss of TTP by IL-33 stimulation allows ILC2s to produce large amounts of type 2 cytokines. As a result, retroviral overexpression of TTP in ILC2s markedly suppresses IL-5 and IL-13 production under IL-33 stimulation through their mRNA degradation. Luciferase assay further demonstrates that TTP directly regulates Il5 expression through AREs in the 3' UTR of Il5 mRNA, suggesting that IL-5 is a novel target of TTP. Moreover, Il13 expression was found to be indirectly regulated by TTP, however, we did not focus on elucidating the associated detailed mechanism in this study. Finally, we confirm that ILC2s from TTP-deficient mice overproduce IL-5 and IL-13 even in steady state, leading to the dysregulation of eosinophil homeostasis. Collectively, TTP maintains the homeostatic function of ILC2s by suppressing IL-5 and IL-13 production via mRNA degradation.

### 2. ILC2 in bone marrow regulate osteoclastogenesis in a reciprocal manner via RANKL

ILC2s are tissue-resident cells that play different roles in different organs by sensing environmental factors in their surroundings. Initially, it was thought that ILC2s in bone marrow (BM) are progenitors for systemic ILC2s, which migrate to other organs and acquire effector functions. However, accumulating evidence that ILC2s differentiate in peripheral tissues suggests that BM ILC2s may play a specific role in the BM as a unique effector per se. We demonstrated that BM ILC2s highly express RANKL, a robust cytokine for osteoclast differentiation and activation, and that RANKL expression on ILC2s is up-regulated by IL-2 and IL-7. BM ILC2s co-cultured with BMMs in the presence of IL-7 induce the differentiation of TRAP-positive osteoclasts in a RANKL-dependent manner. In contrast, BM ILC2s stimulated with IL-33 down-regulate RANKL expression and convert BMM differentiation into M2 macrophage-like cells rather than osteoclasts by GM-CSF and IL-13 production. These results suggest that ILC2s regulate osteoclast activation and contribute to bone homeostasis in both steady-state and IL-33-induced inflammation.

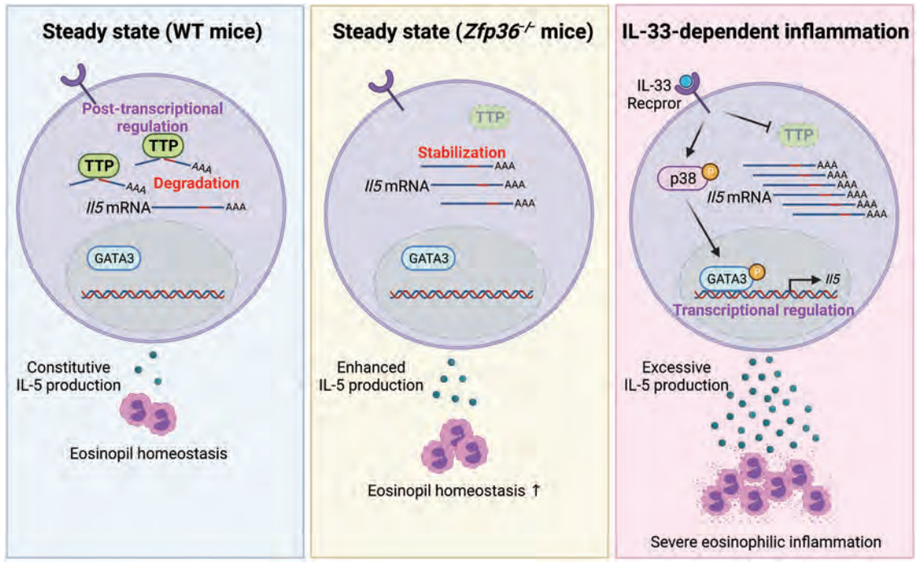



**Figure.**
IL-33 induces a large amount of IL-5 from activated-ILC2s by transcriptional regulation. On the other hand, in steady-state ILC2s, excessive cytokine production is suppressed by TTP-mediated posttranscriptional regulation.

## Recent Publications


1. Momiuchi Y, Motomura Y, Suga E, Mizuno H, Kikuta J, Morimoto A, Mochizuki M, Otaki N, Ishii M & Moro K. Group 2 innate lymphoid cells in bone marrow regulate osteoclastogenesis in a reciprocal manner via RANKL, GM-CSF and IL-13. Int Immunol. 33:573-585 (2021).
2. Matsuyama T, Machida K, Motomura Y, Takagi K, Doutake Y, Tanoue-Hamu A, Kondo K, Mizuno K, Moro K & Inoue H. Long-acting muscarinic antagonist regulates group 2 innate lymphoid cell-dependent airway eosinophilic inflammation. Allergy. 76:2785-2796 (2021).
3. Kobayashi T, Motomura Y & Moro K. The discovery of group 2 innate lymphoid cells has changed the concept of type 2 immune diseases. Int Immunol. 33:705-709 (2021).
4. Kiniwa T & Moro K. Localization and site-specific cell-cell interactions of group 2 innate lymphoid cells. Int Immunol. 33:251-259 (2021).
5. Hikichi Y, Motomura Y, Takeuchi O & Moro K. Posttranscriptional regulation of ILC2 homeostatic function via tristetraprolin. J Exp Med. 218 (2021).

# Human Single Cell Immunology

## James Badger Wing, PhD

| Associate Professor | James Badger Wing |
|---|---|
| Assistant Professor | Jonas N. Søndergaard |
| Postdoctoral Fellow | 2 |
| Research Assistant | 1 |
| Support Staff | 1 |

The Human Single Cell Immunology Lab focuses on single cell biology and its applications to human immunology. Recent advances in single cell technologies, such as mass cytometry (also known as CyTOF) have revealed high levels of heterogeneity among immune cells. This makes it uniquely well placed to obtain a wide-ranging picture of the human immune system. Primarily we use mass cytometry to explore two areas, autoimmunity and infectious diseases.

The devastating outbreak of SARS-CoV2 and the associated COVID-19 disease has had a severe impact on both the global community and Japan. While recent advances in vaccination have given some hope of respite the proven ability of the virus to mutate and potentially generate vaccine resistant strains indicates that there can be no relaxation of our urgent efforts to better understand the effects of this disease (Grubaugh et al, Cell, 2021). A critical part of this effort is to understand the changes to the immune system of infected patients since both viral clearance and most symptoms of the disease are mediated by the immune system (Mathew et al. Science, 2020).

In order to advance our ability to understand these complex systems, we have designed and optimized mass cytometry protocols for the detailed assessment of immune cell phenotypes in humans. Using our expertise in mass cytometry to respond to the challenge of the COVID-19 pandemic we have assessed changes to the immunophenotype of COVID-19 patients in collaboration with the Okada, Kumanogoh, Sakaguchi and Okuzaki groups. Importantly we are simultaneously able to assess many other types of cells such as cytotoxic CD8 T-cells and antibody producing B-cells all of which may be critical to the outcome of COVID-19. **Figure 1**. As part of this we found that Tregs appear to be central hubs of related to the severity sex and age of patients. In particular reference to the role of T-follicular regulatory cells (Tfr) we found that there is a sex biased imbalance of Tfr and antibody producing plasma cells in patients with Severe COVID-19. **Figure 2**. Since Tfr are known to regulate the frequency of plasma cells this may give some indication of the reason for the sex imbalanced production of autoantibodies that has been seen in COVID-19 patients.

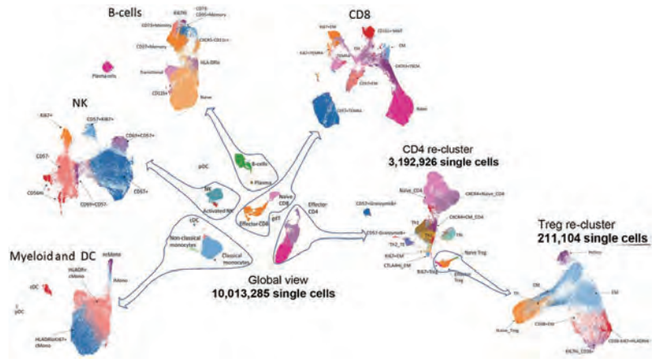



Figure 1.
*Multi-level assessment of immunophenotype of COVID-19 patient peripheral blood mononuclear cells.*

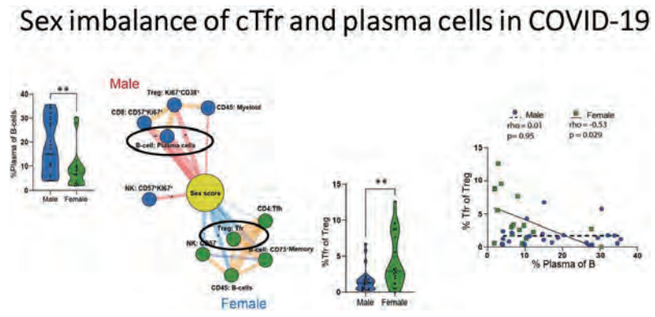



Figure 2.
***Sex correlations with cellular phenotypes in COVID-19.***
*Top 5 cellular correlations with male and female sex in severe COVID-19 patients. Edge width is proportional to correlation strength. Edges connecting to other cellular subsets are positive correlations between indicated subsets. Layout by ForceAtlas2 using edge weights as input. Violin plots of sex specific differences of indicated cellular populations in severe COVID-19 patients. Data from* JN. Søndergaard et al. Preprint on MedRXiv 2022.

## Recent Publications


1. Søndergaard JN, et al. Regulatory T-cells are central hubs for age-, sex- and severity-associated cellular networks during COVID-19. Preprint on MedRXiv (2022). Currently under review.
2. Wing JB & Sakaguchi S. Using mass cytometry to address Tfh and Tfr heterogeneity. Methods in Molecular Biology. "T follicular helper cells". 47-57 (2022).
3. Tekguc M, Wing JB, Osaki M, Long J, and Sakaguchi S. Treg-expressed CTLA-4 depletes CD80/CD86 by trogocytosis, releasing free PD-L1 on antigen-presenting cells. PNAS. 118 (30) e2023739118 (2021).
4. Wing JB, et al. Scalable, multimodal profiling of chromatin accessibility, gene expression and protein levels in single cells. Nature Biotechnology 39(10):1246-1258 (2021).
5. Tulyeu J, Søndergaard JN, Sakaguchi S and Wing JB. Isolation and Characterization of Both Human and Mouse Tfh/Tfr Cells. Current Protocols 1(11):e283 (2021).

# Human Immunology (Single Cell Genomics)

## Daisuke Okuzaki, PhD

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

The Laboratory of Human Immunology was established in November 2019 for the purpose of accelerating the application of single cell sequencing technology to improve the connection between fundamental science research and clinical applications. One of the major research projects we are currently focused on is a genomics project in collaboration with the Trauma and Acute Critical Care Center in the Osaka University Graduate School of Medicine. Many of the patients sent to the Trauma and Acute Critical Care Center have acute inflammation and suffer from systemic inflammatory response syndrome (SIRS). The severity of SIRS usually has to be clinically classified within several hours upon patient arrival. Classification is based on clinical criteria such as fever, hypothermia, tachycardia, tachypnea or leucopoenia. Although treatment of the clinical syndrome is a top priority in the Trauma and Acute Critical Care Center, progress in the research of its pathological classification and associated molecular mechanism has not advanced in Japan as it has in Europe and America.

We hereby kick started a collaboration research project involving the measurement and collection of biomolecular data such as DNA, RNA and protein from patients biomaterials, while integrated with cutting edge single cell sequencing technology. In the past year prospective observational study, we collected ELISA, RNA sequencing, Olink and single cell sequencing data from patients three days and seven days post arrival. The resulting multiomics data, combined with clinical pathology and bioinformatic multi-dimensional analysis, were utilized to classify SIRS patients based on molecular properties into pathological classifications such as sepsis, ARDS, PCAS, trauma, heat stroke or burn. We are currently working on improving the classifications, which can be used as a guideline for proper treatment selection upon arrival.

Our proposal to search for appropriate biomarkers using proteome and transcriptome profiling techniques and to apply them clinically was selected as a project for the AMED " Research Program on Emerging and Re-emerging Infectious Diseases. This project is being conducted in cooperation with the Trauma and Acute Critical Care Center at Osaka University and the Osaka Prefectural Nakakawachi Emergency and Critical Care Center.

Furthermore, with COVID-19 raging worldwide, it is also classified as a SIRS and the course of treatment for patients brought to Osaka University Hospital should be preferably decided prior to arrival. Currently there are no conventionally established biomarkers or drug treatments for COVID 19. In order to search for the appropriate biomarkers to reflect the pathological classification for COVID-19, cohorts were divided into the three groups of research, development and verification. Applying the same strategy as the aforementioned SIRS studies, we collected experimental data using RNA sequencing, mass spectrometry, Olink proteomics, and single cell RNA sequencing. Through the integration with panomics data, a set of “pathological molecular scores” and “pathological correlated clusters” were defined. The novel “pathological classification” is determined by the defined clusters and scores, and enables selective therapeutic intervention depending on the COVID-19 symptoms.

On the other hand, the importance of evaluating vaccination efficacy is gradually increasing given the current nationwide vaccination efforts. In order to elucidate the pathological mechanism of the novel coronavirus as well as develop potential treatments, single cell RNA sequencing was performed for peripheral blood samples that were taken from healthy individuals prior and post vaccination. In connection with the Joint Research Coronavirus Task Force and serving as a member of the All Handai research team, we are currently joining forces with the young researchers of IFReC as well as the Graduate School of Medicine and the Faculty of Medicine, and analyzing the peripheral blood samples taken from the patients sent to Osaka University Hospital during all four waves of COVID-19 in Japan. Currently, we are analyzing multiple aspects of the molecular properties of immune cells from dozens of patients.

We have co-authored two papers in which we applied single cell RNA sequencing. They include a study of PTH-induced bone formation conducted by Morimoto et al., published in Nat Commun. 2021 and a successful observation of hematopoietic recovery supported by innate lymphoid cells under stress conditions performed by Sudo T, et al., published on J Exp Med. 2021.

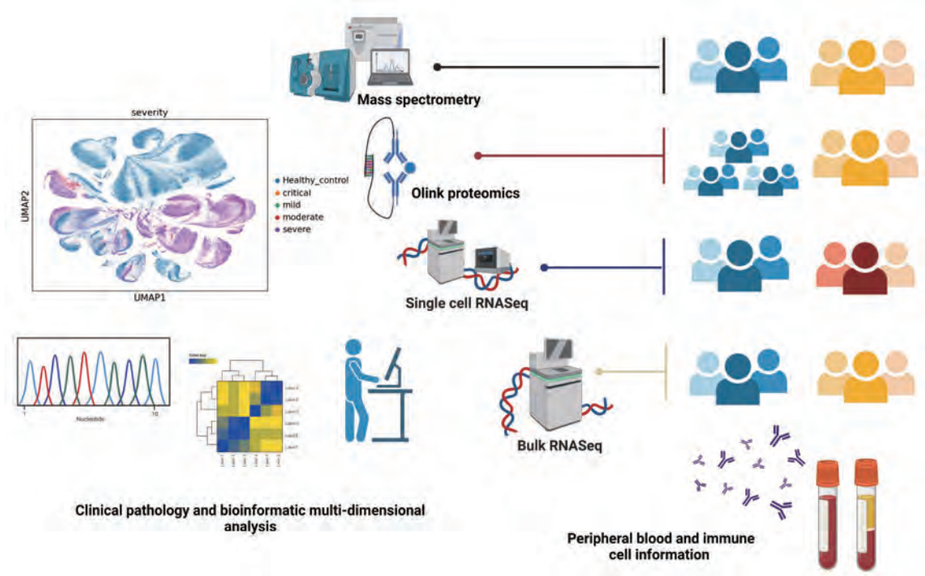



**Figure.**
Overview of the research process.

## Recent Publications


1. Uenaka M, Yamashita E, Kikuta J, Morimoto A, Ao T, Mizuno H, Furuya M, et al. Osteoblast-derived vesicles induce a switch from bone-formation to bone-resorption in vivo. Nat Commun. 13(1):1066 (2022).
2. Kidani Y, Nogami W, Yasumizu Y, Kawashima A, Tanaka A, Sonoda Y, Tona 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).
3. Ebihara T, Matsumoto H, Matsubara T, Togami Y, Nakao S, Matsuura H, Kojima T, et al. Cytokine Elevation in Severe COVID-19 From Longitudinal Proteomics Analysis: Comparison With Sepsis. Front Immunol. 12:798338 (2022).
4. Al Kadi M, Ishii E, Truong DT, Motooka D, Matsuda S, Iida T, Kodama T, Okuzaki D. Direct RNA Sequencing Unfolds the Complex Transcriptome of Vibrio parahaemolyticus. mSystems. 6(6):e0099621 (2021).
5. Morimoto A, Kikuta J, Nishikawa K, Sudo T, Uenaka M, Furuya M, Hasegawa T, et al. SLPI is a critical mediator that controls PTH-induced bone formation. Nat Commun. 12(1):2136 (2021).

# Immune Homeostasis


## Yasutaka Okabe, PhD

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


### Tissue Macrophage Biology

Tissue-resident macrophages are present in virtually every mammalian tissue and they are essential components for the maintenance of tissue homeostasis. They perform tissue-specific functions that are critical for normal tissue physiology. The abnormalities of tissue-resident macrophage functions are often linked to various pathologies including osteopetrosis, type 2 diabetes, immune deficiency, and neurodevelopmental diseases. The focus of the laboratory is to understand molecular mechanisms that generate a diversity of tissue-resident macrophage phenotypes as well as the roles of macrophages in tissue homeostasis. The heterogeneity of tissue-resident macrophage phenotypes is considered to be functional specialization as a consequence of their adaptation to local tissue environments (Figure). Tissue-resident macrophages, in response to tissue environmental cues, activate the corresponding functional polarization programs which accompany the induction of specific gene-expression programs. We have characterized tissue-specific transcriptional programs of resident macrophages and identified hundreds of genes that are selectively expressed in tissue macrophages (Okabe and Medzhitov, Cell, 2014). Among these genes, we found transcription factor GATA6 is uniquely expressed in macrophages that reside in the peritoneal cavity. We reported that GATA6 acts as a master transcriptional regulator for functional specialization of peritoneal macrophages, as the deletion of GATA6 gene in peritoneal macrophages resulted in the defect of peritoneal specific gene expression program. Additionally, we and other groups found GATA6-mediating program control macrophage positioning within the peritoneal cavity, local proliferation, and peritoneal-specific immune responses. We identified retinoic acid, a lipophilic molecule derived from vitamin A, plays an essential role in the induction of GATA6 gene in peritoneal macrophages. Together, these results provide insight into the mechanism of generation of tissue specialization of resident macrophages.

### Regulation of Body Cavity Immunity

Body cavities (peritoneal, pleural, and pericardial cavities) are fluid-filled spaces lined with a layer of mesothelial cells, and accommodate visceral organs and other structures in the animal body. The body cavity represents a unique place for immune cell distribution; some of the immune cells such as B-1 lymphocytes are predominantly present in the body cavity but they are rarely found in the other lymphoid organs. These lymphocytes constantly migrate from the body cavity into peripheral tissues and contribute to whole-body immune regulation such as natural antibodies and gut IgA production. Therefore, it is increasingly appreciated that the body cavity plays unreplaceable functions in the immune system. However, the mechanisms regulating the development, localization, and activation of immune cells in the body cavity remain largely unknown. We study the regulating mechanisms of the body cavity immune system and its roles in the infection and injury.

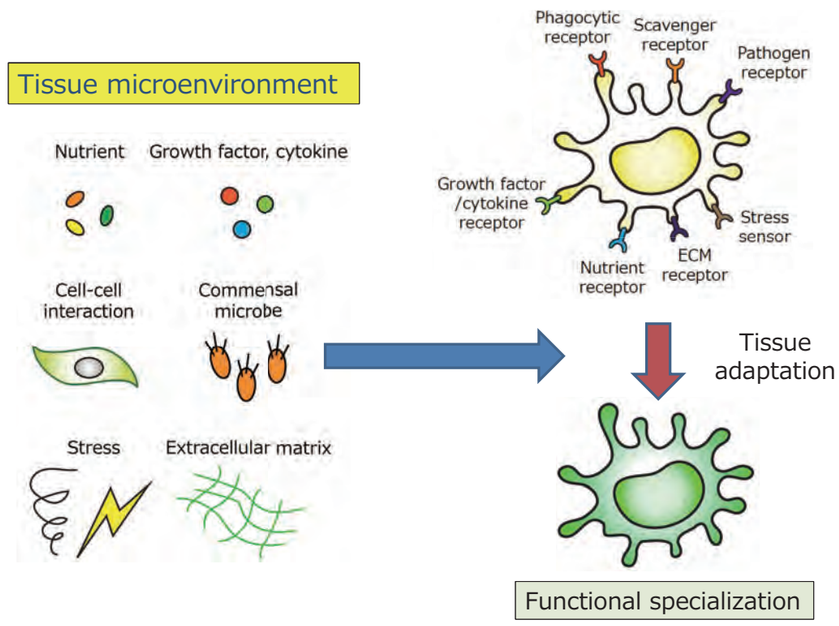



**Figure.**
Local tissue environments induce functional specialization of tissue-resident macrophages.


### Recent Publications

1. Okabe Y. Immune Niche Within the Peritoneal Cavity. Current Topics of Microbiology and Immunology. 434:123-134 (2021).
2. Okabe Y. Molecular Control of the Identity of Tissue-Resident Macrophages. Int Immunol. 30:485-491 (2018).
3. Okabe Y & Medzhitov R. Tissue Biology Perspective on Macrophages. Nat Immunol. 17:9-17 (2016).
4. 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

| | |
|---|---|
| Professor | Naoki Hosen |
| Associate Professor | Hisashi Kato |
| Assistant Professor | Kana Hasegawa |
| | Kentaro Fukushima |
| | Jiro Fujita |
| | Michiko Ichii |
| | Yasutaka Ueda |
| | TomoakiUeda |
| 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 MMG49 CAR T-cell for MM is now on-going.

### Identification of a novel target antigen for multiple myeloma

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 recently found that MM can be specifically targeted by a new monoclonal antibody that recognizes a ubiquitously expressed protein CD98 heavy chain (hc)/SLC3A2. 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.

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

The above findings suggest that cancer immunotherapeutic targets may yet be identified in many cell-surface proteins that undergo post-translational changes, even if the expression of the proteins themselves is not cancer-specific. Thus, 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 are now selecting ones recognizing antigen structures highly specific for AML cells. In the case of solid tumors, while inefficient trafficking of CAR-T cells to tumor sites and immune-suppressive tumor microenvironment hamper development of effective CAR-T cell therapy, the most critical problem is lack of appropriate cancer-specific target antigens. We start trying to find cell surface antigen structures for various types of cancers in collaboration with several departments treating cancers in Osaka University Hospital.

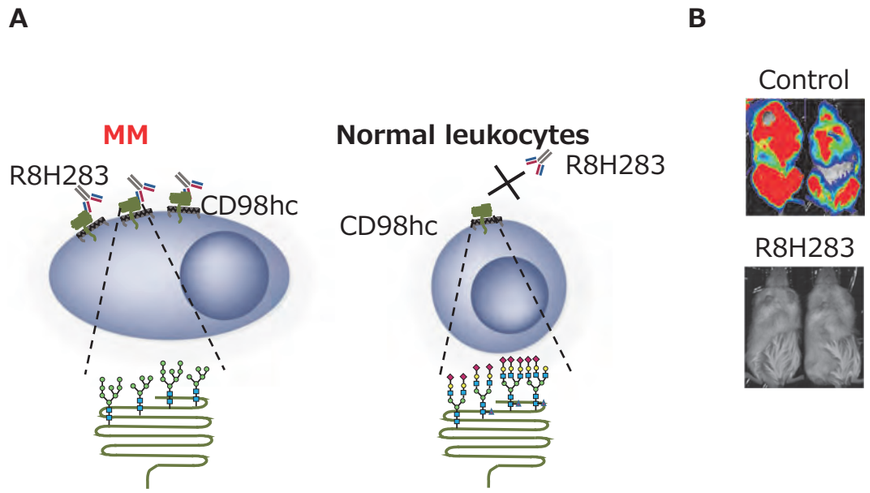



**Figure.**
**R8H283, a novel antibody against CD98hc, a widely expressed protein, can specifically targets myeloma cells.**
(A) The glycoforms of CD98hc present on normal leukocytes were distinct from those present on MM cells, which may explain the lack of R8H283 reactivity to normal leukocytes. Indicated glycan structure is just a conceptual scheme. Glycans actually attached to CD98hc has huge varieties. (B) R8H283 exerts anti-MM effectsn in a mouse xenograft model.

## Recent Publications


1. 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).
2. Ozawa T, Fujii K, Sudo T, et al. Special AT-Rich Sequence-Binding Protein 1 Supports Survival and Maturation of Naive B Cells Stimulated by B Cell Receptors. J Immunol 208:1937-1946 (2022).
3. Shingai Y, Yokota T, Okuzaki D, et al. Autonomous TGFbeta signaling induces phenotypic variation in human acute myeloid leukemia. Stem Cells 39:723-736 (2021).

# Microbiology and Immunology

**Nobuhiko Kamada, PhD**

Professor | Nobuhiko Kamada

Our team has been studying the role of the gut microbiota in the pathogenesis of the gastrointestinal disease, such as inflammatory bowel disease (IBD). We developed a humanized gnotobiotic mouse model that enables one to examine the functional impact of gut dysbiosis associated with IBD. Using this model, we identify potential pathobionts enriched in IBD patients and are responsible for disease induction and progression. We have identified potential pathobionts that elicit inflammasome activation in host immune cells (e.g., macrophages) and the intestinal epithelium. Some of the identified pathobionts appear to have adherent–invasive *E. coli* (AIEC)–like phenotypes. In addition to intestinal inflammation, we observed that persistent gut colonization by AIEC bacteria leads to the development of intestinal fibrosis. Moreover, we have identified unique metabolic pathways used by pathobionts to maximize their fitness in the inflamed gut. We discovered that AIEC bacteria reprogram their metabolism in the inflamed gut and upregulate genes related to L-serine catabolism. AIEC, but not commensal *E. coli* strains, use L-serine catabolism; a unique metabolic function that confers a fitness advantage on AIEC over commensal competitors.

Also, we focus on the microbial and immunological cross-talk between mucosal tissues in the pathogenesis of gastrointestinal diseases. In this regard, we study the oral–gut axis in IBD pathogenesis. Recent studies have demonstrated that the enrichment of oral bacteria in the intestinal mucosa of IBD patients may contribute to proinflammatory processes in the gut. We aim to identify the mechanisms by which oral disease exacerbates intestinal inflammation. We have discovered that periodontitis results in the expansion of pathobionts in the oral cavity. Amassed oral pathobionts are ingested and colonize the colonic mucosa. Ectopic colonization of oral pathobionts elicits the activation of the inflammasome in lamina propria macrophages, which may compound their colitogenic capacities. In parallel, oral bacteria–reactive Th17-skewed T cells arise de novo in the oral cavity during periodontitis. Oral bacteria–reactive T cells are imprinted with gut tropism and migrate to the inflamed gut. Once in the gut, the oral bacteria–reactive T cells are activated by ectopically colonized oral pathobionts. Thus, together, oral pathobionts and pathogenic T cells, which are generated as a result of oral inflammation, exacerbate gut inflammation.

Note: The PI is cross-appointed at the IFReC and the University of Michigan (USA) from September 2021. These research projects were conducted at the University of Michigan. We are currently establishing new projects on host-microbe interactions in gastrointestinal disease at the IFReC.

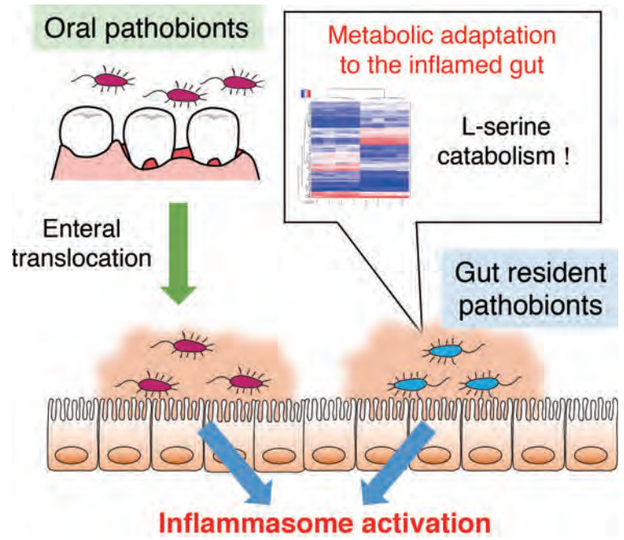



**Figure.**
(Left) Ectopic gut colonization by oral pathobionts elicits inflammasome activation in the colonic mucosa. (Right) Gut resident pathobionts, such as adherent-invasive *Escherichia coli*, reprogram their metabolism in the inflamed gut. L-serine catabolism up-regulated during inflammation confer pathobionts a competitive fitness advantage over competing commensals. Gut pathobionts promote intestinal inflammation via the activation of the inflammasome.

## Recent Publications

1. 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*. 6(23):e148543 (2021).

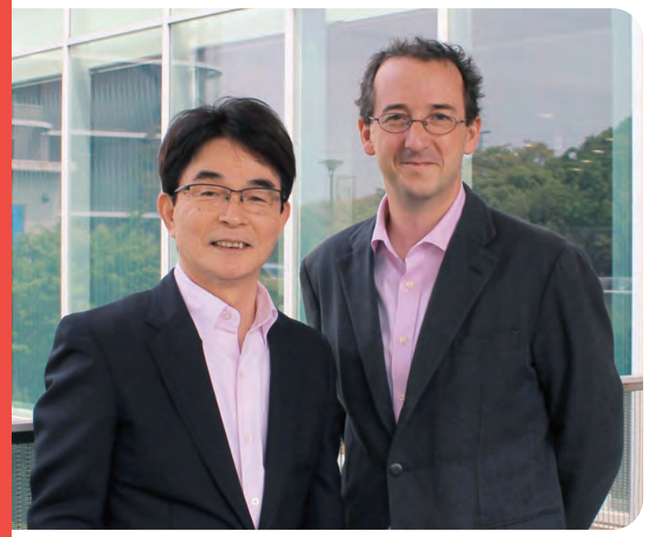
2. Hayashi A, Nagao-Kitamoto H, Kitamoto S, Kim CH, Kamada N. The butyrate-producing bacterium *Clostridium butyricum* suppresses *Clostridioides difficile* infection via neutrophil- and antimicrobial cytokine-dependent but GPR43/109a-independent mechanisms. *J Immunol*. 206(7):1576-1585 (2021).

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

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

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

# Single Molecule Imaging




## Toshio Yanagida, PhD
## Ben Seymour, MD/PhD

| | |
|---|---|
| Professor | Toshio Yanagida<br>Ben Seymour |
| Associate Professor | Mitsuhiro Iwaki |
| Support Staff | 2 |


One of the major puzzles in medicine is why people are so susceptible to developing chronic pain after injury or inflammation. Indeed around 20-30% of the population develop chronic pain at some point in their life, making it the leading cause of disability and one of the greatest unmet needs across the entire medical spectrum. Our lab focuses on understanding the core mechanisms of pain in the brain, and testing the broad hypothesis that injury and inflammation set-off hyper-protective behaviours that evolved to protect us during recuperation, but that are extremely prone to cause continuation of pain after the insult has resolved.

We have received a generous award from the UK Medical Research Council / Versus Arthritis, to test this idea in more detail. Recently, we have been exploring brain-mediated hyper-protective behaviours in patients with rheumatoid arthritis, and found both behavioural and brain evidence that characterizes precisely how this is implemented, and how it is likely to generate a suite of symptoms that includes pain, fatigue, and mood changes. To probe this idea more robustly – especially to test whether these changes plays a causative role in chronification of pain after injury or inflammation, we have developed a new digital experimental platform for studying behaviour in patients. This platform is an open digital laboratory that can be used by researchers and applied to multiple cross-sectional and longitudinal clinical cohorts. This exists on a free software platform that also includes sophisticated computational analysis tools, as well as a data sharing database that allows normative and comparative data analysis.

An inherent prediction of our results is that brain mechanisms that cause pain and fatigue can be targeted by technology-based approaches. We have received a generous award from the UK Engineering and Physical Sciences Research Council to establish an infrastructure for developing this, exploiting UK-Japan collaboration. In particular, we are focusing on non-invasive technologies that include brain stimulation, virtual reality, digital therapy and neurofeedback tools, which are integrated and applied in a mechanism-targeted strategy. Our ultimate goal is to design and deliver low-cost, accessible technologies that can have a transformative impact on chronic pain across different societies and populations.

In our other work, we are also studying fundamental pathways for adaptive learning in the brain. Diseases such as inflammatory arthritis causes a complex fluctuating pattern of pain. We have been studying how the brain can learn this temporal pattern, and have found that the brain generates an internal model of the pain-causing state using Bayesian statistical learning. This is formed in the front-parietal cortex (Mancini et al, 2021).

We are also developing single molecule imaging techniques for mechanobiology of immune cell. To visualize the mechanical force in cells, we previously developed DNA-based force sensor, Nanospring (NS). This is a protein-sized spring molecule and can be fully stretched by pN force. The force-extension relation was quantified using acoustic force spectroscopy and we confirmed the stiffness and the robustness against solution condition. We attached RGDfK peptide (ligand of integrin receptor) to the end of the NS and adsorbed to the glass bottom dish, then monitored the single molecule traction force of human skin fibroblast via integrin receptors (Figure 2). We could successfully observe the extension of the fluorescently labeled NS underneath focal adhesions using total internal reflection microscopy. The NS length was dynamically changed at second time scale and the force varied at the range of 0.1 to 25 pN. This is the first report to observe dynamical change of the traction force at the single molecule level. The observation reports us fruitful information regarding the molecular mechanism of cellular mechanosensing. Recently, it is known that T cell receptor (TCR) senses specific magnitude and direction of force via membrane receptor when T cell interact with antigen presenting cell (APC). Our new methodology reported here should be powerful to clarify the mechanical-induced immune response.

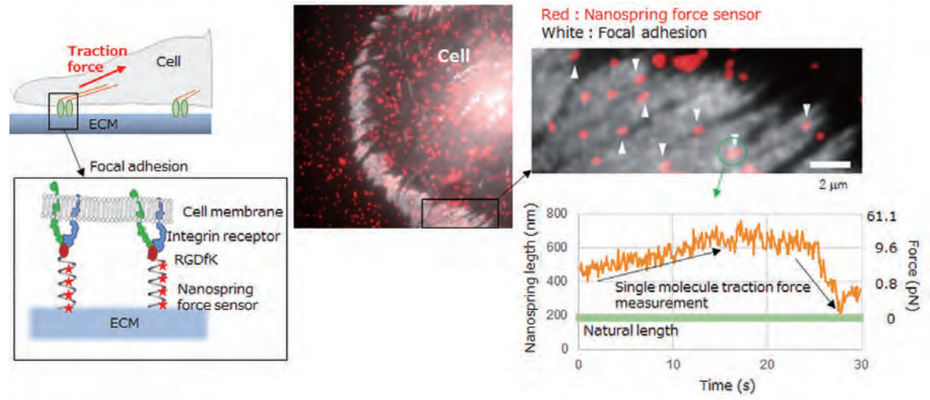



**Figure.**
Single molecule traction force microscopy measured by DNA-based force sensor, Nanospring to clarify the mechanical-induced immune response.

## Recent Publications


1. Marcucci L, Fukunaga H, Yanagida T, Iwaki M. The synergic role of actomyosin architecture and biased detachment in muscle energetics: insights in cross bridge mechanism beyond the lever-arm swing. Int J Mol Sci. 22:7037 (2021).

2. Tanaka SC, Yamashita A, Yahata N, Itahashi T, Lisi G, Yamada T, et al. A multi-site, multi-disorder resting-state magnetic resonance image database. Scientific data 8 (1):1-15 (2021).

3. Mancini F, Zhang S, Seymour B. Learning the statistics of pain: computational and neural mechanisms. bioRxiv 2021.

# Immunology and Cell Biology

## Masaru Ishii, MD/PhD

| Professor | Masaru Ishii |
|---|---|
| Associate Professor | Junichi Kikuta |
| Assistant Professor | Yutaka Uchida |
| | Akito Morimoto |
| | Kentaro Fujii (SA) |
| Postdoctoral Fellow | 3 |
| Research Assistant | 5 |
| Visiting Scientist | 1 |
| Support Staff | 2 |

### 1. Intravital bone imaging revealing osteoclast and osteoblast dynamics *in vivo*

We developed a novel imaging system to visualize inside bones using intravital multiphoton microscopy. Using this methodology, we succeeded in visualizing the *in vivo* behaviors of osteoclast and osteoblast as well as the activity of bone-resorbing by osteoclasts. In addition to development of methodology, we are also updating various materials for bone imaging such as mouse models to visualize osteoclast and osteoblast and probes to visualize the bone resorption activity of osteoclasts. Our major contributions to the field of bone biology research are as follows:

- Identifying S1P (Sphingosine-1-phosphate) as a key factor in controlling the migratory behavior of osteoclast precursors *(Nature 2009, J. Exp. Med. 2010)*.
- Showing the substantial contribution of S1P-mediated migration of bone cells by generating S1P transporter deficient mice *(J. Clin. Invest. 2012)*.
- Identifying Vitamin D as significantly suppressing bone destruction by modulating S1P-mediated migration of osteoclast precursor *(Proc. Natl. Acad. Sci. USA 2013)*.
- Proposing two distinct mature osteoclast functional states: bone-resorbing mature osteoclasts and non-resorbing mature osteoclasts *(J. Clin. Invest. 2013)*.
- Showing the importance of epigenetic processes of osteoclast in the regulation of cellular metabolism and differentiation *(Nat. Med. 2015)*.
- Developing a new fluorescence probe for detecting bone surface pH to visualize bone resorption by mature osteoclasts *(Nat. Chem. Biol. 2016)*.
- Applying intravital bone imaging to drug efficacy evaluation and elucidation of mechanism of action *(Ann. Rheum. Dis, 2018; JBMR plus, 2018)*.
- Showing the importance of cell to cell communication between mature osteoblasts and mature osteoclasts for bone homeostasis *(Nat. Commun. 2018)*.
- Identifying SLPI (secretory leukocyte protease inhibitor) as a key mediator for PTH-induced bone formation *(Nat. Commun. 2021)*.

Bone metabolism is regulated by the cooperative activity between bone-forming osteoblasts and bone-resorbing osteoclasts. However, the mechanisms mediating the switch between the osteoblastic and osteoclastic phases have not been fully elucidated. We identified a specific subset of mature osteoblast-derived extracellular vesicles that inhibit bone formation and enhance osteoclastogenesis. Intravital imaging reveals that mature osteoblasts secrete and capture extracellular vesicles, referred to as small osteoblast vesicles (SOVs). Co-culture experiments demonstrate that SOVs suppress osteoblast differentiation and enhance the expression of receptor activator of NF-κB ligand, thereby inducing osteoclast differentiation. We also elucidate that the SOV-enriched microRNA miR-143 inhibits Runt-related transcription factor 2, a master regulator of osteoblastogenesis, by targeting the mRNA expression of its dimerization partner, core-binding factor β. In summary, we identified SOVs as a mode of cell-to-cell communication, controlling the dynamic transition from bone-forming to bone-resorbing phases *in vivo (Nat. Commun. 2022)*.

### 2. Identification of disease associated macrophages

Intravital imaging provides information of dynamic parameters, is useful for identifying a novel subset of macrophages. Inside live bones, osteoclasts and osteoblasts are constantly generated. Under well-controlled bone destruction and formation processes, bone constantly undergoes destruction and formation to maintain homeostasis. In our previous study, we developed a unique technology to collect and analyze cells from arthritic joints, successfully identifying a new type of bone-destroying osteoclast that contributes to RA (rheumatoid arthritis), called Arthritis-associated osteoclastogenic Macrophages, or "AtoMs" (Nat. Immunol. 2019). We found that the arthritis-inducing abnormal osteoclasts have distinct properties and origins from normal osteoclasts involved in bone metabolism. By targeting the differentiation and functions of arthritis-inducing abnormal osteoclasts, we will be able to establish innovative therapeutic strategies and next-generation of anti-RA drugs that do not affect normal osteoclasts and normal bone homeostasis.

### 3. Application of multiphoton microscopy for human cancer diagnosis

Intravital imaging with multiphoton microscopy is an undoubtedly powerful tool for dissecting live cellular dynamics in intact tissues and organs and thus useful for studying immune system dynamics *in vivo*. However, the application is currently limited in animal models and may not be for analyzing human samples. By collaborating with companies (supported by AMED) we are developing a new microscopy system for applying human tissues and organs *in vivo*. Currently, we have succeeded in visualizing non-labelled human normal and cancer tissues, which can be used for differential diagnosis *(Sci. Rep.*, 2017*)*. In the follow-up study *(Cancer Res.2021)*, we applied near-infrared excitation and nonlinear optics to visualize unstained human epithelial tissues of the cervix uteri by constructing images with third-harmonic generation (THG) and second-harmonic generation (SHG). THG images enabled evaluation of nuclear morphology in a quantitative manner with six parameters after image analysis using deep learning. It was also possible to quantitatively assess intraepithelial fibrotic changes based on SHG images and another deep learning analysis. Our method enables real-time noninvasive diagnosis of cervical lesions, thus constituting a potential tool to dramatically change early detection. This study proposes a novel method for diagnosing cancer, which enables visualization of histologic features of living tissues without the need for any biopsy or staining dye.

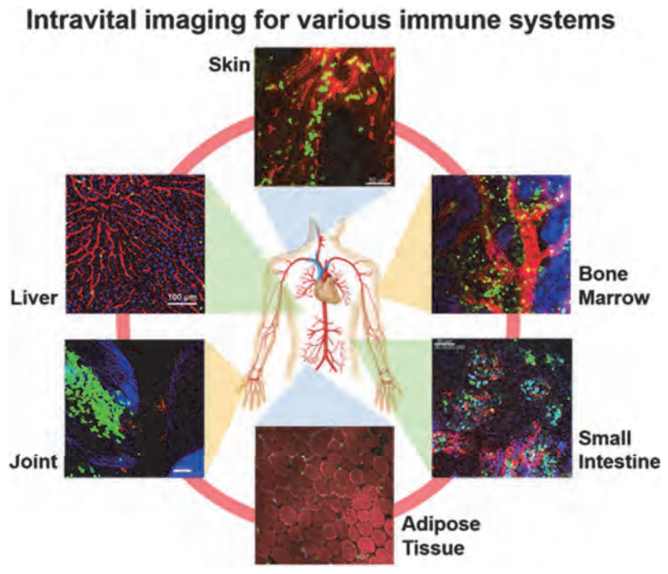



**Figure.**
*In vivo* cellular dynamics in various immune systems. Immune cells are highly dynamic and interconnect various tissues and organs, by forming a 'soft-wired' network. We are elucidating the basic principle controlling the dynamic nature of immune cells by visualizing *in vivo* behaviors using advanced imaging techniques.

## Recent Publications


1. Uenaka M, et al. Osteoblast-derived vesicles induce a switch from bone-formation to bone-resorption in vivo. Nat Commun. 13(1):1066 (2022).
2. Morimoto A, et al. SLPI is a critical mediator that controls PTH-induced bone formation. Na. Commun. 12:2136 (2021).
3. Sudo T, et al. Group 2 innate lymphoid cells support hematopoietic recovery under stress conditions. J Exp Med. 218:e20200817 (2021).
4. Matsui T, et al. Nonlinear optics with near-infrared excitation enable real-time quantitative diagnosis of human cervical cancers. Cancer Res. 80:3745-3754 (2020).
5. Hasegawa T, et al. Identification of a novel arthritis-associated osteoclast precursor macrophage regulated by FoxM1. Nat Immunol. 20:1631-43 (2019).

# Nuclear Medicine

## Jun Hatazawa, MD/PhD

| Professor | Jun Hatazawa |
|---|---|
| Associate Professor | Hiroki Kato |
| Assistant Professor | Tadashi Watabe |
| Postdoctoral Fellow | 2 |
| Research Assistant | 2 |
| Support Staff | 1 |

### Phase I Clinical Trial of $Na^{211}At$ for intractable Thyroid Cancers

Since the first use of $^{131}I$ for thyroid diseases (1951) and $^{60}Co$ for external irradiation source (1953) in Japan, medical radioisotopes were mainly imported from abroad. Due to senile nuclear reactors in Canada, Europe, and South Africa, reduced workers during COVID-19 pandemic, and limited transport capacity by approved airlines, a supply of medical RI became limited and unstable. After the policy change by Japanese government from import to domestic production (July, 2021), a rebuild of supply chain of medical RI is promoted under the Japan Atomic Energy Commission and regulatory authorities of the government. It consisted of target production for $^{99}Mo$ and $^{225}Ac$, neutron/proton/deuteron/electron irradiation, purification and quality control of target RI, labeling and distribution of radiopharmaceuticals, and waste disposal management. The action plan will be finalized by the end of May 2022.

In this situation, Phase I clinical trial of Astatine-211 ($^{211}At$) NaAt started in December 2021 at Osaka University Hospital for intractable thyroid cancers under the support of AMED (Principal Investigator: Dr. Tadashi Watabe). This is the first clinical trial in the world. The first patient study was completed without any adverse event. Another project to use of $^{211}At$ is to treat brain tumors by means of $^{211}At$ labelled gold nanoparticle (Kato H, et al., 2021). $^{211}At$ is an alpha particle emitting radioisotope in the halogen family with 20 times higher biological effect than gamma-ray. Chemical and biological nature of Astatine-211 is similar to Iodine-131, a beta-emitting radioisotope used for the treatment of well-differentiated thyroid cancers. Astatine-211 production and purification procedures were developed in Research Center for Nuclear Physics, Osaka University (Ikeda H, et al.). Astatine-211 labeled cancer cell specific compounds are now under development in Osaka University.


- Watabe T, Kaneda-Nakashima K, Liu Y, et al. Enhancement of $^{211}At$ Uptake via the Sodium Iodide Symporter by the Addition of Ascorbic Acid in Targeted α-Therapy of Thyroid Cancer. J Nucl Med. 2019 Sep;60(9):1301-1307
- Hiroki Kato, Xuhao Huang, Yuichiro Kadonaga, et al. Intratumoral administration of astatine-211-labeled gold nanoparticle for alpha therapy, J Nanobiotechnology. 2021 Jul 28;19(1):223.
- Ikeda H, Hayashi Y, Takahashi N, et al. Appl Radiat Isot. 2018 Sep;139:251-255.


## Phase I Clinical Trial of $Na^{211}At$

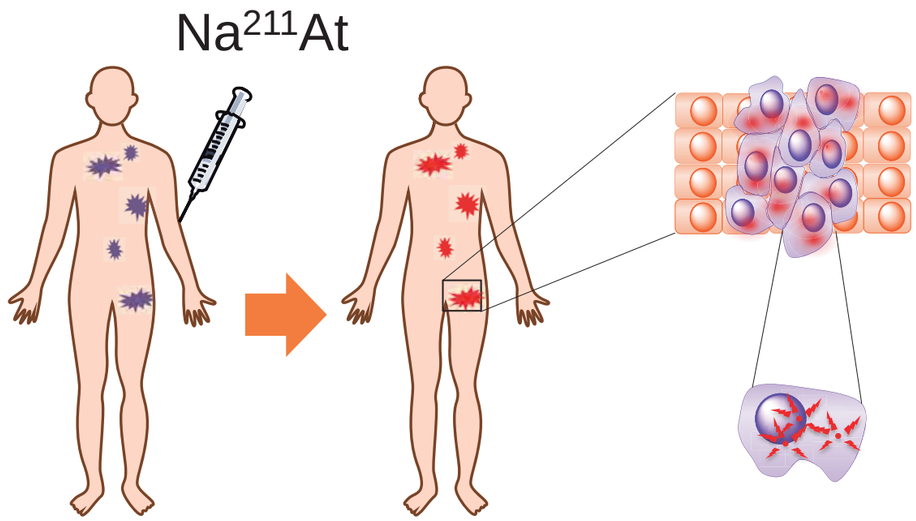
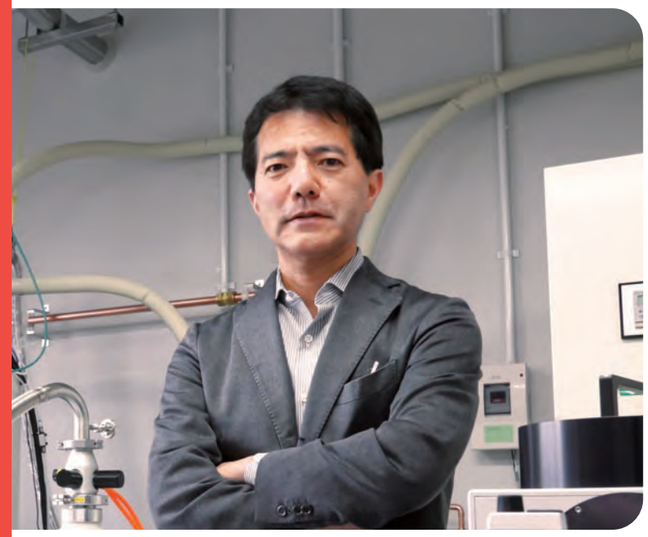



**Figure.**
$Na^{211}At$ solution is intravenously administered to a patient with metastatic thyroid cancer. $Na^{211}At$ is specifically transported into metastatic thyroid cancer cells similar to $Na^{131}I$. Since $^{211}At$ has short pass length of alpha-particle within several cells and higher energy, it has higher cell killing effect associated with less normal cell damage.

## Recent Publications


1. Hiroki Kato, Xuhao Huang, Yuichiro Kadonaga, Daisuke Katayama, Kazuhiro Ooe, Atsushi Shimoyama, Kazuya Kabayama, Atsushi Toyoshima, Atsushi Shinohara, Jun Hatazawa, Koichi Fukase. Intratumoral administration of astatine-211-labeled gold nanoparticle for alpha therapy, J Nanobiotechnology. 19(1):223 (2021).
2. Shirakami Y, Watabe T, Obata H, Kaneda K, Ooe K, Liu Y, Teramoto T, Toyoshima A, Shinohara A, Shimosegawa E, Hatazawa J, Fukase K. Synthesis of [$^{211}At$]4-astato-L-phenylalanine by dihydroxyboryl-astatine substitution reaction in aqueous solution. Sci Rep. 11(1):12982 (2021).
3. Hatazawa J. The Clinical Value of Breast Specific Gamma Imaging and Positron Imaging: An Update. Seminars in Nuclear Medicine. https://doi.org/10.1053/j.semnuclmed.2022.02.005 (Review article)

# Chemical Imaging Techniques




## Kazuya Kikuchi, PhD

| Professor | Kazuya Kikuchi |
|---|---|
| Associate Professor | Yuichiro Hori |
| Assistant Professor | Masafumi Minoshima |
| Postdoctoral Fellow | 2 |
| Research Assistant | 1 |
| Visiting Scientist | 1 |
| Support Staff | 1 |


Protein degradation plays an essential role in maintaining cellular homeostasis, with protein misfolding and uncontrolled protein aggregation responsible for a range of neurodegenerative diseases, including Huntington's disease, Alzheimer's disease and amyotrophic lateral sclerosis (ALS). Damaged or misfolded proteins in cells are normally removed by the ubiquitin-proteasome and/or lysosomal proteolytic pathways, which hydrolyse them into small peptides and amino acids. The ability to use a probe to fluorescently detect the production/degradation of short-lived proteins (SLPs) in real time would be particularly useful, because many of the key roles they play in cell regulatory processes are not fully understood.

We here developed a new trifunctional 'contact quenching' "OFF–ON–OFF" fluorescence probe (F3-DNB) for the rapid labeling of PYP-tag proteins (Fig. 1). This probe contains a PYP-tag binding 7- hydroxycoumarin ligand connected to a fluorescein fluorophore through a PEG linker. The central coumarin ligand unit is attached to a pendant dinitrobenzene (DNB) quenching unit through a labile thiophenol ester bond that can undergo a trans-thioesterification reaction with the thiol group of the Cys69 residue of a PYP-tag protein, which cleaves the quenching unit to produce a fluorescent PYP-tag-bound probe. A DNB quencher moiety was included in the probe design to avoid steric interactions between the fluorophore and the coumarin ligand that were known to result in slow PYP-tag protein labeling kinetics. A structurally related F3-DNB2 probe was also prepared containing a more activated thiophenol leaving group that we reasoned would label PYP-tag proteins more effectively. Finally, a third F5-DNB2 probe containing a longer PEG5 linker was synthesized, because previous studies had shown that close interaction between the probe's fluorophore and ligand units could produce steric congestion that decreased the rate of labeling of PYP-tag.

In vitro treatment of PYP-tag with F5-DNB2, the most reactive probe, 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 (Fig. 2A). SDS-PAGE analyses of the protease cleavage products revealed no gel bands present for intact probe-labeled PYP proteins, thus confirming that both had undergone proteolytic digestion (Fig 2B). 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 SLPs in cellular systems using an OFF–ON–OFF fluorescence switch was performed. Mouse ornithine decarboxylase (MODC) was chosen as a potential SLP 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. Efficient proteasomal degradation of the expressed fusion construct between PYP-tag and MODC$^{422\text{-}461}$ was confirmed with western blot analyses of cellular extracts from the transfected HEK293T cells. 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. 2C). The rapid protein labeling kinetics, high fluorescent intensities and modular nature of this new probe system means it should find widespread use for real-time fluorescence visualization of the expression/degradation of a wide range of short-lived PYP-tag proteins, thus enabling it to be used as a diagnostic tool to inform numerous biological studies and drug discovery programs.

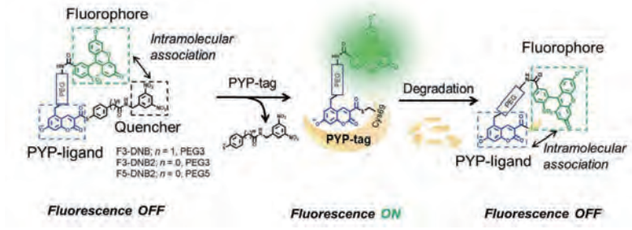



**Figure 1.**
Structures and mechanism of action of the modular PYP-tag ligands (F3-DNB, F3-DNB2 and F5-DNB2) used as "OFF–ON–OFF" probes for the fluorescent detection of protein degradation.

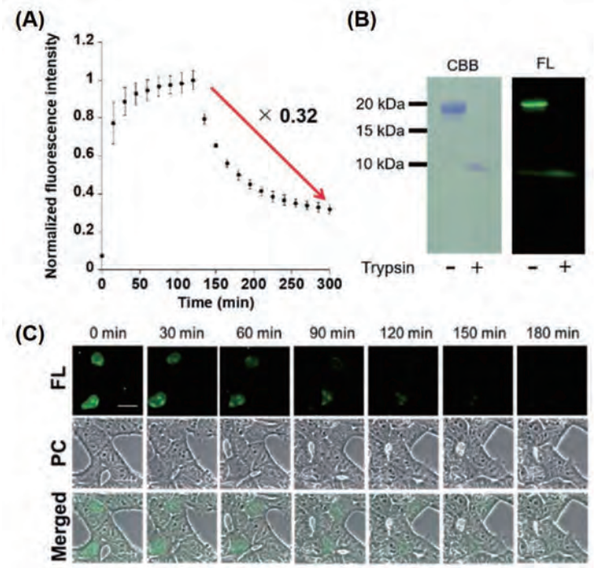



**Figure 2.**
(A) Protein degradation detected from the "OFF–ON–OFF" fluorescence response of F5-DNB2. PYP$^{NQN}$ incubated with F5-DNB2 for 120 min and then reacted with trypsin in 20 mM HEPES buffer (pH 7.4), 150 mM NaCl, and 1.0% DMSO at 37 °C. Fluorescence intensities recorded using excitation and emission wavelengths of 500 and 521 nm. (B) SDS-PAGE analyses of protein degradation products. CBB = Coomassie Brilliant Blue; FL = Fluorescence. (C) Time-lapse fluorescence imaging studies of the degradation of a short-lived PYP-tag protein in cells. HEK293T cells expressing HA-PYP$^{NQN}$-NLS-MODC$^{422\text{-}461}$ in presence of dicetylated F5-DNB2 (1.0 μM). Excitation at 473 nm. Scale bar = 20 μm. PC = Phase Contrast; FL = Fluorescence.

## Recent Publications


1. Reja SI, Hori Y, Kamikawa T, Yamasaki K, Nishiura M, Bull SD, & Kikuchi K. 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).
2. Tsukazaki H, Kikuta J, Ao T, Morimoto A, Fukuda C, Tsuda E, Minoshima M, Kikuchi K, Kaito T, & Ishii M. Anti-Siglec-15 antibody suppresses bone resorption by inhibiting osteoclast multinucleation without attenuating bone formation. *Bone* **152**:116095 (2021).
3. Konishi Y, Okunishi A, Sugihara F, Nakamura T, Akazawa K, Minoshima M, & Kikuchi K. Development of off-on switching $^{19}$F MRI probes for cathepsin K activity detection. *Bull Chem Soc. Jpn*. 94:1690-1694 (2021).
4. Kowada T, Arai K, Yoshimura A, Matsui T, Kikuchi K, & Mizukami S. Optical manipulation of subcellular protein translocation using a photoactivatable covalent labeling system. *Angew Chem Int. Ed.* **60**:11378-11383 (2021).
5. Hori Y, Nishiura M, Tao T, Baba R, Bull SD, & Kikuchi K. Fluorogenic probes for detecting deacylase and demethylase activity towards post-translationally-modified lysine residues. *Chem Sci.* **12**:2498-2503 (2021).

# Immune Response Dynamics


## Kazuhiro Suzuki, MD/PhD

| | |
|---|---|
| **Professor** | **Kazuhiro Suzuki** |
| Assistant Professor | Akiko Nakai |
| Research Assistant | 2 |
| Support Staff | 2 |


Our laboratory has been studying the interactions between the nervous and immune systems with a special focus on the roles of adrenergic nerves, which constitute the efferent arc of the sympathetic nervous system, in the control of adaptive immune responses. Our study revealed a mechanism by which adrenergic nerves control lymphocyte trafficking through lymph nodes. Inputs from adrenergic nerves to the $\beta_2$-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 nerve activity (Suzuki et al, J. Exp. Med. 2016). In search of factors that mediate the crosstalk of signaling between the two different types of G protein-coupled receptors (GPCRs), the $\beta_2$-adrenergic receptor and chemokine receptors, we identified a protein complex consisting of copper metabolism MURR1 domain-containing (COMMD) 3 and COMMD8 (COMMD3/8 complex), of which the functions had been totally unclear (Fig. A).

We first identified COMMD8 as a protein that binds to the C-terminal tail of a representative chemokine receptor CXCR4. Additional proteomic screening revealed the interaction of COMMD8 with COMMD3. We found that these proteins constitutively formed a complex in the cytosol, but were translocated to the plasma membrane after stimulation of CXCR4. The COMMD3/8 complex interacted with not only CXCR4 but also other G protein-coupled chemoattractant receptors, including CXCR5, CCR7, and the oxysterol receptor EBI2, after activation of the receptors. Interestingly, COMMD3 and COMMD8 were degraded by the proteasome in the absence of the other, and deficiency of either protein produced the same functional consequences, indicating that both COMMD3 and COMMD8 are required for the stability and functions of their complex. Deficiency of COMMD3 or COMMD8 in B cells reduced their chemotactic responses mediated by the receptors to which the COMMD3/8 complex was recruited. These findings reveal that the COMMD3/8 complex is a positive regulator of chemoattractant receptor signaling (Nakai et al., J. Exp. Med. 2019).

Agonist binding to GPCRs activates trimeric G proteins to induce generation of second messengers that modulate downstream signaling. Agonist-occupied GPCRs are phosphorylated by GPCR kinases (GRKs) and subsequently recruit β-arrestins that serve as scaffolds to activate signaling molecules, including mitogen-activated protein kinases (MAPKs). The GRK family consists of seven mammalian members, among which GRK2, GRK3, GRK5, and GRK6 are expressed ubiquitously. Different GRKs phosphorylate distinct sites on the C-terminal tail of the receptor, establishing a barcode that dictates the outcomes of β-arrestin engagement. Thus, specific targeting of GRKs to activated GPCRs is crucial for signal transduction. Our analysis on the mechanism of action of the COMMD3/8 complex demonstrated that this protein complex functions as an adaptor that selectively recruits GRK6 to chemoattractant receptors, which promotes MAPK activation and consequently lymphocyte chemotaxis (Fig. B). It has been suggested that the specificity of GRK recruitment to GPCRs is determined by the relative expression levels of individual GRKs, which vary among cell type, and distinct receptor conformations induced by ligand binding. Our study identifies a GRK-recruiting adaptor, the COMMD3/8 complex, as an additional determinant of GRK specificity for GPCRs (Nakai et al., J. Exp. Med. 2019).

Consistent with the reduced chemotactic responses of COMMD3- and COMMD8-deficient B cells, the mutant B cells showed multiple defects in their migration in vivo (Fig. C). Additionally, deficiency of COMMD3 or COMMD8 in B cells severely impaired production of antigen-specific antibodies (Fig. D), which was accompanied with reduced formation of germinal centers (Fig. E). Therefore, the COMMD3/8 complex plays essential roles in the control of B cell migration and induction of humoral immune responses (Nakai et al., J. Exp. Med. 2019). To test the involvement of the COMMD3/8 complex in autoimmune diseases, we established an experimental system that allowed inducible deletion of the COMMD3/8 complex in moue models of autoimmunity. Notably, deletion of the COMMD3/8 complex in the course of the diseases blocked their progression with suppressed production of autoantibodies. These findings suggest that the COMMD3/8 complex is a potential therapeutic target for autoimmune diseases.

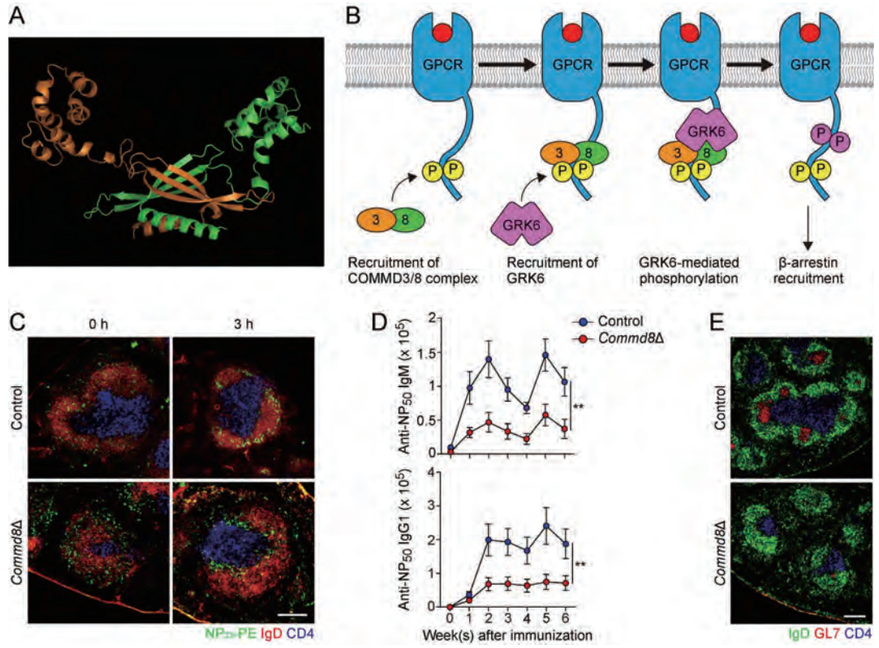



**Figure.**
**Role of the COMMD3/8 complex in GPCR signaling and immune responses.**
(A) *In silico* modeling for the structure of the COMMD3/8 complex. COMMD3, orange; COMMD8, green.
(B) Role of the COMMD3/8 complex in GRK6 recruitment to GPCRs.
(C) Defective intrafollicular migration of COMMD8-deficient (*Commd8Δ*) B cells at an early time point (3 h) after immunization.
(D and E) Impaired antibody production (D) and germinal center formation (E) in B cell-specific COMMD8-deficient mice. Scale bars, 200 μm.

## Recent Publications


1. Nakai A, Leach S and Suzuki K. Control of immune cell trafficking through inter-organ communication. Int Immunol. 33:327-335 (2021).
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 and Nakai A. Immune modulation by neuronal electric shock waves. J Allergy Clin Immunol. 141:2022-2023 (2018).
4. 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).
5. Nakai A, Hayano Y, Furuta F, Noda M and Suzuki K. Control of lymphocyte egress from lymph nodes through $\beta_2$-adrenergic receptors. J Exp Med. 211:2583-2598 (2014).

# Biophotonics

## Nicholas Isaac Smith, PhD

| Associate Professor | Nicholas Isaac Smith |
|---|---|
| Assistant Professor | Nicolas Pavillon |
| | 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 completed work on several projects that advance the sensitivity and interpretability of label-free single cell analysis. We showed that label-free evaluation of immune cells can give us insight at the single-cell level, into a cell phenotype or state. When performing this type of experiment there are some aspects that arise which are not necessarily intuitive. For example, different cell types that are known to be functionally different can turn out to be challenging to discriminate with our methods, while the detection of a change in state (such as activation) can turn out to be easier to detect. The sensitivity of our detection depends on how much difference exists at the molecular level between two targets. The use of optical methods based on spectroscopy means that we do not have single molecule sensitivity, but rather see the ensemble of molecules in the cell that are excited by the laser beam.

We made further improvements towards higher throughput measurement. This was achieved through additional automation of our systems, using machine vision, and automatic targeting of individual cells for Raman analysis. Although fundamentally not comparable to true high-throughput techniques such as FACS, we nevertheless managed to increase the speed of measurement to acquire several thousand cells per hour, without need of constant human intervention. This is beneficial not only for convenience but can also help to improve repeatability.

We completed several works with collaborators including advancements in microscopy techniques with the Fujita group in Applied Physics, in both label-free and probe-based modalities. Dr Lelliot also completed a study with collaborators in the Kumanogoh group, elucidating the role of adhesion in neutrophil extracellular traps (NETs). Neutrophils are known to produce extracellular debris that resembles a physical net, and this process has been implicated in a wide variety of disease conditions, including COVID-19. It is still relatively early in terms of existing knowledge into how NETs form, whether there are different types of NETs, and the pathways involved with them. Here, the work clarified that for NET stimulation specifically related to anti-neutrophil cytoplasmic antibody associated vasculitis, neutrophil adhesion is required. This has implications for the disease pathology and suggests that clinical studies targeting neutrophil adhesion factors may be a promising direction for treatment.

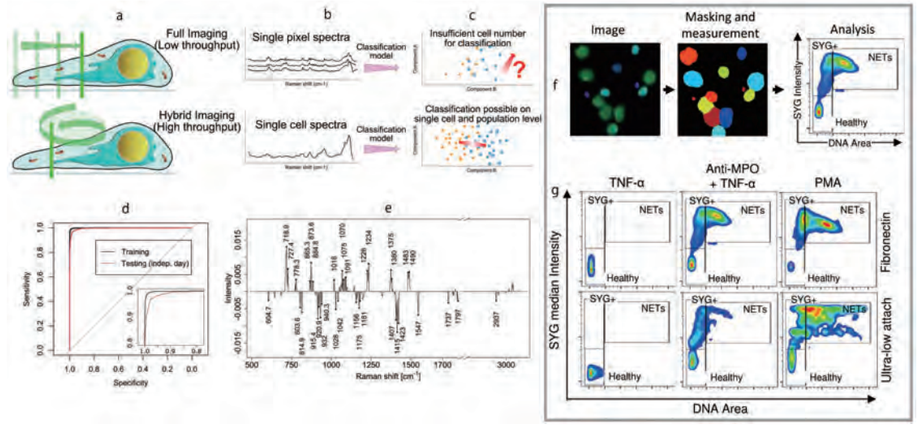



**Figure.**
Classification of cell phenotypes, cell states, and immune responses. Panel (a) shows a schematic of our higher throughput optical spectroscopy mode giving one signature spectra per cell. This data leads to the ability to classify each cell (b). The sensitivity and specificity can be very high, considering that the whole measurement is label-free. Here, we show the accuracy of a model that determines whether a cell had been exposed to LPS or LPS combined with cycloheximide which inhibits the normal protein synthesis response to LPS (d). Even when looking at data from an independent day's experiments, the accuracy remains high. Depending on the method chosen to create the classification model, it is possible to extract information that tells us which molecular bands are most indicative of the inhibition process (e, see Pavillon et al 2021 for details). Panel f shows data from the NET project, here using labels, where the degree of NET formation was quantified by image analysis, so that accurate quantitative characterization of the NET response to different stimuli could be achieved (g).

## Recent Publications


1. Lelliott PM, Nishide M, Pavillon N, Okita Y, Shibahara T, Mizuno Y, Yoshimura H, Obata S, Kumanogoh A, and Smith NI. Cellular Adhesion Is a Controlling Factor in Neutrophil Extracellular Trap Formation Induced by Anti-Neutrophil Cytoplasmic Antibodies. ImmunoHorizons 6 (2):170-183 (2022).
2. Pavillon N, and Smith NI. Deriving accurate molecular indicators of protein synthesis through Raman-based sparse classification. Analyst 146:3633-3641 (2021).
3. 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).
4. Pavillon N and Smith NI. Immune cell type, cell activation, and single cell heterogeneity revealed by label-free optical methods. Sci Rep. 9:17054 (2019).
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 Standley |
|---|---|
| Associate Professor | Kazutaka Kato |
| | Li Songling |
| | Soyoung Park |
| Postdoctoral Fellow | 2 |
| Research Assistant | 3 |

### A. Reversible optical control of T cell activation

Light is an attractive trigger for controlling the reversible assembly and disassembly of biomolecules in a spatiotemporal manner. Our interest is to decipher how the dynamics of T cell receptor (TCR) clustering affects downstream T cell signaling and gene expression. For this purpose, we have devised an optically controllable T cell activation system using TCR-binding aptamers or anti-TCR-antibodies and a light-responsive arylazopyrazole (AAP) module (Park, S. et al. 2021). This technology will provide a structural understanding of T cell activation, which can be applied to single-chain antibody (scFV) receptors in CAR T cells. In this way, we ultimately aim to open the door for a new area of *Optoimmunology* research.

### B. COVID-19 Pandemic

The 2021 year was dominated by efforts to characterize SARS-CoV2 as well as host responses to the virus. In the beginning, we had very little information on B or T cell receptors that targeted SARS-CoV2, so we focused our attention on the main antigen: the spike protein. We first examined the evolution of the spike protein in order to identify residues that appeared to be under selection pressure during evolution (Saputri, D. et al *Front Microbiol*, 2020). We subsequently observed that a number of such "evolutionarily important" residues were among the first to mutate in emerging viruses of concern and proposed that surveying viruses in non-human hosts my provide an opportunity to predict their evolution in humans (Katoh, K. & Standley, D. M. *Commun Biol*, 2021). As the sequences of antibodies that target the spike protein began to trickle in to the public domain, we started to work closely with other groups at IFReC to identify their functions. In particular, Prof. Arase noticed a subset of antibodies that targeted the spike N-terminal domain (NTD) that enhanced, rather than neutralized SARS-CoV2 infection (Liu, Y. et al *Cell* 2021). We hypothesized that the function of such enhancing antibodies was mediated by spike-spike crosslinking, leading to decoupling between the NTD and receptor-binding domain (RBD). We have carried out extensive simulations of antibody-crosslinked spike proteins in the Fugaku supercomputer in order to validate our hypothesis. Encouragingly, spike bound NTDs exhibited significantly larger distances from neighboring RBS than did unbound NTDs, in support of the proposed mechanism **(Figure 2)**. The project is still ongoing, but, if further experimental support for this mechanism is found, it would represent a novel form of antibody-dependent enhancement (Li, S. and Van Eerden, F.J. et al in prep). Finally, in collaboration with Assist. Prof. Atsushi Hoshino at the Kyoto Prefectural University of Medicine, Assoc. Prof. Toru Okamoto at Osaka Univ. RIMD and Prof. Junichi Takagi at Osaka Univ. IPR, we helped to characterize mutations in the Omicron variant in terms of immune escape and ACE2 binding (Ikemura, N.T. et al *Sci. Transl. Med* 2022).

### C. Antibody structural modeling

We have continued to work on antibody structural modeling at various levels. In 2021, we participated in an international collaborative study of CAR T cells that strongly suggested the importance of CAR dimerization in the treatment of B cell acute lymphoblastic leukemia (Singh, N. et al *Nat Med*, 2021). We have also been developing new tool, AbAdapt, for elucidating antibody-antigen interactions using combination of structural modeling, molecular docking and Deep Learning (Davila, A. and Xu, Z. et al. Bioinform. Adv 2022). More recently, we have shown that such predictions can be significantly improved by use of the AlphaFold software (Xu, Z. et al in prep) **(Figure 3)**.

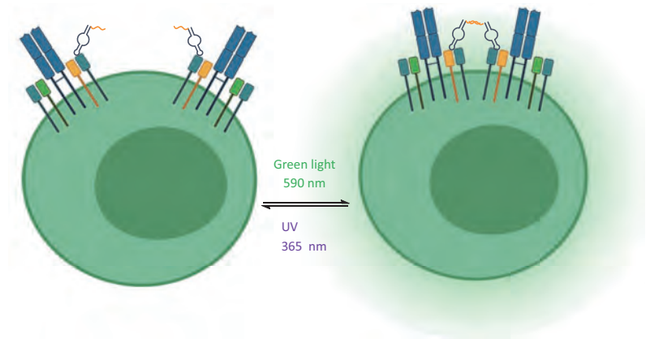



**Figure 1.**
*Conceptual scheme for reversible optical switch for T cell activation using TCR-binding aptamers.*

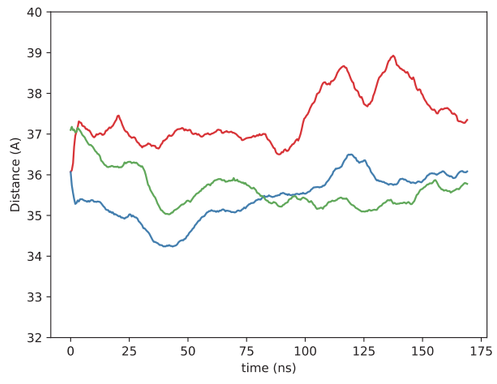



**Figure 2.**
*RBD-NTD distance in the three different spike subunits of the right spike. Spike A (red) is bound by the antibody and has clearly a larger separation between NTD and RBD separation and then the two other spikes.*

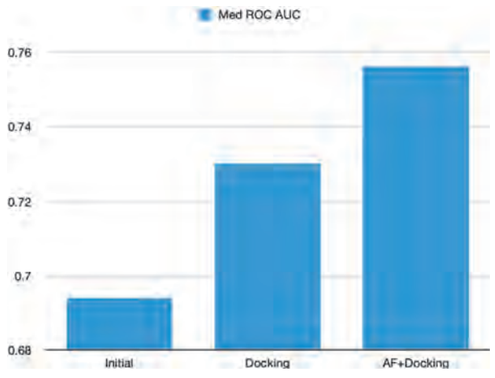



**Figure 3.**
*Antibody-specific epitope prediction. The three bars indicate the performance (Median ROC AUC) for predicting epitope residues on a holdout set of 100 antigens. "Initial" means no antibody information; "Docking" means information from docking antibodies built with Repertoire Builder was used; "AF + Docking" means information from docking antibodies built with AlphaFold was used.*

## Recent Publications


1. Ikemura NT, Inaba T, Arimori T, Motooka D, Katoh K, et al. An engineered ACE2 decoy neutralizes the SARS-CoV-2 Omicron variant and confers protection against infection in vivo. Sci Transl Med. in press (2022).
2. Davila A, Xu Z, Li S, Rozewicki J, et al. AbAdapt: an adaptive approach to predicting antibody–antigen complex structures from sequence. Bioinformatics Advances 2 (2022).
3. Chong YK, Tartey S, Yoshikawa Y, Imami K, Li S, et al. Cyclin J-CDK complexes limit innate immune responses by reducing proinflammatory changes in macrophage metabolism. Sci Signal. 15, eabm5011 (2022).
4. Singh N, Frey NV, Engels B, et al. Antigen-independent activation enhances the efficacy of 4-1BB-costimulated CD22 CAR T cells. Nat Med. 27:842-850 (2021).
5. Liu Y, Soh WT, Kishikawa JI, Hirose M, et al. An infectivity-enhancing site on the SARS-CoV-2 spike protein targeted by antibodies. Cell 184:3452-3466 e3418 (2021).

# Statistical Immunology

## Yukinori Okada, MD/PhD

| Professor | Yukinori Okada |
|---|---|
| Associate Professor | Qingbo S Wang |
| Assistant Professor | Kenichi Yamamoto |
| Research Assistant | 5 |
| Support Staff | 2 |

### Goal of our laboratory

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

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

Microbiomes play substantial roles in biology of a variety of human immune-related diseases. We conducted metagenome-wide association studies (MWAS) of gut microbiome of systemic lupus erythematosus (SLE) utilizing whole-genome shotgun sequencing. We identified increases of *Streptococcus intermedius* and *Streptococcus anginosus* in SLE. Microbiome-metabolome association analysis identified positive dosage correlation of acylcarnitine with Streptococcus intermedius (Tomofuji Y et al. *Ann Rheum Dis* 2021). Further, we constructed an in silico pipeline to quantify gut virome from the existing shotgun sequencing data. We conducted virome-wide association study (VWAS) based on the shotgun sequencing of 476 Japanese which included patients with rheumatoid arthritis (RA), SLE, multiple sclerosis and healthy control subjects. Our VWAS revealed that crAss-like phages, one of the main components of a healthy gut virome, significantly decreased in RA and SLE (**Figure 1**, Tomofuji Y et al. *Ann Rheum Dis* 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. By conducting cross-population and cross-biobank genome-wide association study (GWAS) of >200 human phenotypes for >600,000 individuals of Asian and European ancestries, we identified ~5,000 new associated loci. By applying deconvolution analysis of the GWAS summary statistics, we extracted the variants and genes sharing the related disease etiology, such as RA and SLE (Sakaue S et al. *Nat Genet* 2021). As for our current challenges in the COVID-19 pandemic, we organized the all Japan consortium and massively collected omics resources from the patients with COVID-19. We conducted multi-omics analysis including GWAS, part of which contributed to international collaborations (COVID-19 Host Genetics Initiative. *Nature* 2021).

### Deep sequencing of killer cell immunoglobulin-like receptor genes

The killer cell immunoglobulin-like receptor (KIR) gene region are one of the most complex genomic region in human genome. We conducted deep sequencing of KIR genes in >1,100 individuals of the Japanese population. We implemented a novel *in silico* pipeline to genotype KIR gene variants from the sequencing reads (**Figure 2**). By combining with the whole-genome sequencing data, we constructed an imputation reference panel of the KIR gene region. We conducted a phenome-wide association study of the KIR gene variants using the biobank resources, but found no significant genotype-phenotype correlations (Sakaue S, et al. *Cell Genomics* 2022).

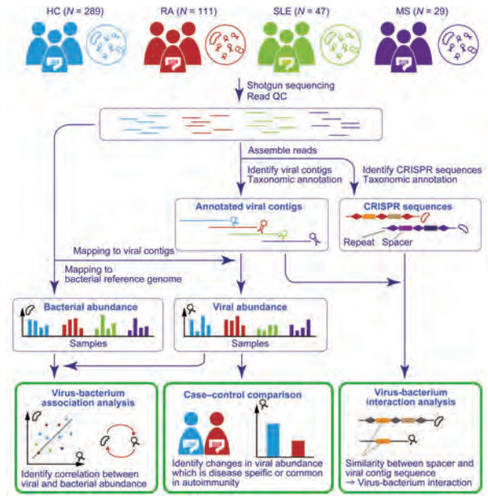



**Figure 1.**
*An in silico* pipeline to quantify gut virome from metagenome shotgun sequencing

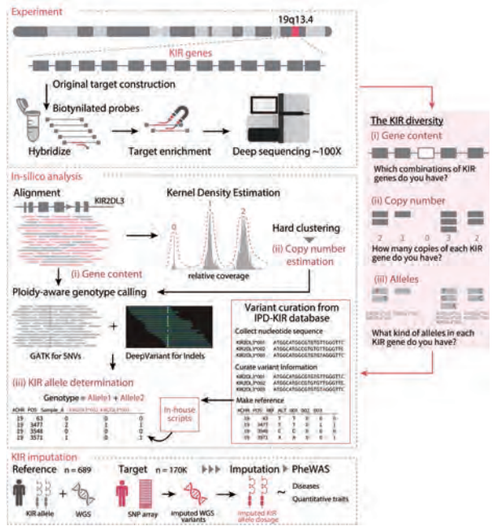



**Figure 2.**
KIRAP (**K**iller **I**mmunoglobulin-like **R**eceptor variant **A**nalytical **P**latform)

## Recent Publications


1. Sakaue S, et al. Decoding the diversity of killer immunoglobulin-like receptors by deep sequencing and a high-resolution imputation method. Decoding the diversity of killer immunoglobulin-like receptors by deep sequencing and a high-resolution imputation method. Cell Genomics 2:100101 (2022).
2. Tomofuji Y, et al. Whole gut virome analysis of 476 Japanese revealed a link between phage and autoimmune disease. Ann Rheum Dis 81:278-288 (2022).
3. Sakaue S, et al. A cross-population atlas of genetic associations for 220 human phenotypes. Nat Genet 53:1415-1424 (2021).
4. Tomofuji Y, et al. Metagenome-wide association study revealed disease-specific landscape of the gut microbiome of systemic lupus erythematosus in Japanese. Ann Rheum Dis 80:1575-1583 (2021).
5. Mari E K Niemi, et al. COVID-19 Host Genetics Initiative. Mapping the human genetic architecture of COVID-19. Nature 600:472-477 (2021).

# 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 linear regression and machine learning to model immune transcriptional regulatory networks. Using the expression level of the regulators as a proxy for their activities we apply these methods to study how transcriptional networks change during immune cell differentiation and disease.

## Applications to immunology

Using single cell transcriptomics, protein expression, immune repertoire and chromatin accessibility we study the differentiation of NKT cells in the thymus and spleen of SKG and WT mice. SKG mice have a mutation in ZAP70 molecule that causes weakened TCR signaling, resulting in a bias towards development of NKT1 compared to NKT2 in the wild type. This approach enables us to understand how changes in regulatory networks during development effect NKT type biases. In a clinical setting, we apply single cell genomics to get insight into IgA nephritis onset and therapies. We analyze the transcriptome and protein expression of immune cells in PBMCs and tonsils from IgAN patients before and after treatment with e.g., tonsillectomy and/or steroid immunosuppression. We combine these datasets with spatial transcriptomics from tonsils to identify factors that lead to successful therapy.

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.

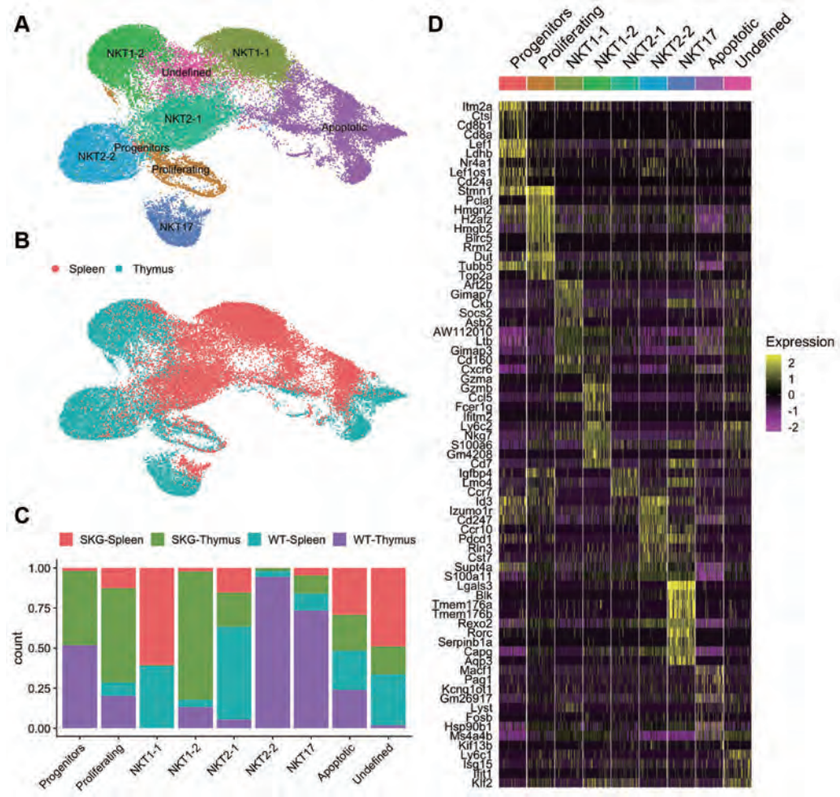



Figure.
Single cell genomics identifies signatures of NKT cells in the thymus and spleen of WT and SKG mice.

## 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 (2020).

4. Teraguchi S, Saputri DS, et al. Methods for sequence and structural analysis of B and T cell receptor repertoires. Comput Struct Biotechnol J. 18:2000-2011 (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 (2014).

# Events & Outreach Activities

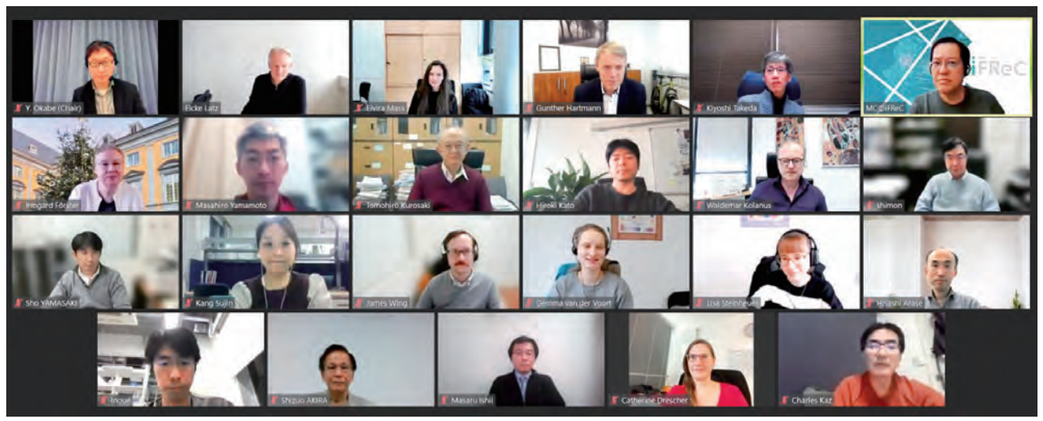
# The 2nd ImmunoSensation$^2$-IFReC Joint Workshop

The 2nd ImmunoSensation2- IFReC Joint Workshop was jointly organized by IFReC and ImmunoSensation$^2$ at the University of Bonn. The two institutes had signed an academic exchange agreement in 2018, and organized this online workshop simultaneously both in Japan and Germany. The workshop provided an opportunity to showcase the latest immunology researches in both countries.

● **Date: December 16-17, 2021**

| Speaker | Title |
|---|---|
| **Kiyoshi Takeda** | Opening Remarks |
| **Joachim Schultze** | Are we ready for swarm immunology? |
| **Masaru Ishii** | Identification of pathogenic tissue-resident macrophages visualized by intravital multiphoton imaging technology. |
| **Christoph Wilhelm** | Fasting and ketogenesis as regulators of protective immunity in severe respiratory viral infections. |
| **Elvira Mass** | Developmental programming of tissue-resident macrophages. |
| **Kazuyo Moro** | Group 2 innate lymphoid cells drive spontaneous pulmonary fibrosis. |
| **Shizuo Akira** | Regnase-1, an endoribonuclease involved in inflammation, immunity and metabolism |
| **Eicke Latz** | Cytosolic exposure of mitochondrial matrix contents activates the NLRP10 and AIM2 inflammasomes. |
| **Sho Yamasaki** | Innate immune sensing of metabolites causes neuroinflammatory diseases. |
| **Eva Bartok** | Characterizing the Innate Immune Response to mRNA Vaccination. |
| **Hiroki Kato** | RNA methyltransferases are critical for Influenza A virus and SARS-CoV-2 replication. |
| **Irmgard Foerster** | Environmental control of immune regulation and metabolism by the AhR/AhRR pathway. |
| **Sujin Kang** | IL-6-targeting therapy for cytokine storms by COVID-19, CAR-T cell therapy and other diseases. |
| **Masahiro Yamamoto** | Role of PLCbeta4 in CD8+ T cell signaling. |
| **Shimon Sakaguchi** | Induction of tumor immunity with long-lasting memory by depleting clonally expanding Tregs in tumor tissues. |
| **Gunther Hartmann & Waldemar Kolanus** | Closing Remarks |


This symposium was supported by "Core-to-Core Program" of Japan Society for the Promotion of Science.



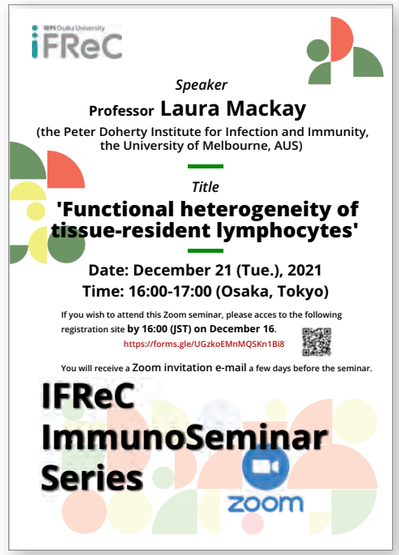
# IFReC Colloquia

To prevent the spread of COVID-19, the IFReC Colloquium, an important event held every two months allowing researchers of IFReC to gather together, has been organized using an online tool in FY 2021.


| No. | Date | Speaker | Title |
|---|---|---|---|
| 50 | May 26, 2021 | **Takahide Itokazu** (Molecular Neuroscience/ Neuro-Medical Science) | Plasticity and recovery from central nervous system damage |
| | | **Yu Miyamoto** (Immunology and Cell Biology) | Resident macrophages give rise to spatial heterogeneity of immune responses in the liver |
| 51 | July 29, 2021 | **Mitsuhiro Iwaki** (Single molecule imaging) | DNA-based force sensor for single-molecule mechanobiology |
| | | **Yicheng Wang** (Immunoglycobiology) | Identification of a lipid scramblase involved in glycosylphosphatidylinositol |
| 52 | Sept. 29, 2021 | **Kentaro Kajiwara** (Oncogene Research) | CDCP1 promotes compensatory renal growth by integrating Src and Met signaling |
| | | **Shintaro Okumura** (Aging Biology) | Identification of gut bacteria abundant in CRC patients that promote tumorigenesis |
| 53 | Nov. 25, 2021 | **Weizhen Jia** (Signal Transduction) | Indispensable role of Galectin-3 in promoting quiescence of hematopoietic stem cells |
| | | **Fumitaka Muramatsu** (Signal Transduction) | Vascular endothelial cells create drug-resistant tumor microenvironment through iron ion regulation by ceruloplasmin |
| | | **Hyota Takamatsu** (Immunopathology) | Caspase-1–mediated secretion of mitochondrial DNA-rich exosomes causes pathological inflammation in Behçet's syndrome |
| 54 | Mar. 17, 2022 | **Floris van Eerden** (Systems Immunology) | Mechanism of SARS-CoV-2 infection-enhancing antibodies |
| | | **Masafumi Minoshima & Shahi Imam Reja** (Chemical Imaging Techniques) | Development of Fluorescent Chemical Probes for Imaging pH in Bone Tissue and Single Molecule Imaging |

# IFReC ImmunoSeminar

IFReC started a series of online seminars called the "ImmunoSeminar Series" in March, 2021. This seminar was well received, and the series have been continued by inviting world-class immunologists to speak at the online seminars through the fiscal year.




| Date | Speaker | Title |
|---|---|---|
| May 19, 2021 | **Shane Crotty** (La Jolla Institute for Immunology, USA) | Adaptive immunity and immune memory to SARS-CoV-2 and COVID-19 |
| June 4, 2021 | **Hao Wu** (Harvard University, USA) | Structural deciphering of immune sensors |
| July 30, 2021 | **Gabriel Nuñez** (University of Michigan, USA) | Host-Microbiota Interactions in Health and Disease |
| Aug. 27, 2021 | **Akiko Iwasaki** (Yale School of Medicine, USA) | Immune responses to SARS-CoV-2 |
| Oct. 15, 2021 | **Zhijian Chen** (University of Texas Southwestern Medical Center, USA) | Igniting an Immune Response with cGAS |
| Nov. 4, 2021 | **Michel Nussenzweig** (Rockefeller University, USA) | Human Immune Responses to SARS-CoV-2 Infection and Vaccination |
| Dec. 20, 2021 | **Laura Mackay** (University of Melbourne, Australia) | Functional heterogeneity of tissue-resident lymphocytes |
| March 11, 2022 | **Feng Shao** (NIBS, Beijing, China) | Pyroptosis in antibacterial and antitumor immunity |

# The 1st International Symposium of CiDER on Microbiology and Immunology

● **Date: January 11, 2022**

Osaka University established the Center for Infectious Disease Education and Research (CiDER) in 2021 as a contribution to conquering infectious diseases. To celebrate its launch, "CiDER Kick-Off Week" was held on january 10-12, 2022. On the second day, "The 1st International Symposium of CiDER on Microbiology and Immunology," an online event mainly organized by IFReC, was held where eminent world scientists gave talks.

| Speaker | Title |
|---|---|
| **Akiko Iwasaki**<br>Yale University, USA | Immune responses to SARS-CoV-2 |
| **Shane Crotty**<br>La Jolla Institute for Immunology, USA | Understanding adaptive immunity and immune memory to SARS-CoV-2 and COVID-19 vaccines |
| **Yohei Yamauchi**<br>University of Bristol, UK | Cell biology of SARS-CoV-2 entry by Neuropilin-1 |
| **Wataru Ise**<br>CiDER/IFReC, Osaka University | Regulation of plasma cell generation and survival for long-term protection from pathogens |
| **James B. Wing**<br>CiDER/IFReC, Osaka University | Age, sex and severity associations of regulatory T-cells in COVID-19 |
| **Yoshihiro Kawaoka**<br>University of Tokyo, Japan | Addressing the threat of emerging viral infections |
| **Hisashi Arase**<br>CiDER/IFReC/RIMD, Osaka University | Neutralizing and infectivity-enhancing antibodies against SARS-CoV-2 |
| **Yoshiharu Matsuura**<br>CiDER/RIMD, Osaka University | Construction of reverse genetics and VLP vaccine for SARS-CoV-2 |

CiDER OSAKA UNIVERSITY


# The 10th WPI Science Symposium for Students “Nano World toward the Future”

● **Date :** December 18, 2021
● **Organizers :** NanoLSI (Kanazawa University), other WPI institutes and JSPS
● **Venue :** Ishikawa Music Hall, Kanazawa

The 10th WPI Science Symposium had an attendance of over 300 high school students, mainly from the Super Science High Schools in Ishikawa Prefecture, and was simultaneously streamed online. At IFReC's booth, we introduced the immunology research activities at Osaka University, and distributed our PR magazine “Imuneco” to attendees.



# Online Outreach Activities

From FY2020, IFReC implemented a new approach to holding its outreach activities by utilizing the web and online tools. The experience gained from holding these virtual events is expected to be highly useful even after COVID-19 restrictions on activities are lifted.

## Research Frontline Lecture for Educators "Let's get to know the latest research at WPI! "

- Date : June 19 & 26, and July 3, 2021
- Organizers : IFReC (Osaka Univ) and other five WPI institutes
- Speakers : Jun Hatazawa (IFReC), Naoto Shirahata (MANA), and others


## Life Science Seminar for High School Students

- Date : July 30, 2021
- Organizers : Senri Life Science Foundation and IFReC (supported by Osaka Prefectural Board of Education)
- Speakers : Kazuyo Moro (IFReC) and two scientists of Osaka University



## Science Agora 2021 "Dialogue for Life"

Neo-Science "Information, AI, Big data for Cutting Edge Researches"

- Date : November 7, 2021
- Organizer : IFReC, ICReDD (Hokkaido University) and IRCN (University of Tokyo)
- Speakers : Naganari Ohkura (IFReC) and two scientists



## SpringX Super School "Protecting our Lives from Infectious Diseases"

- Organizer : © KNOWLEDGE CAPITAL and Center for Infectious Disease Education and Research (CiDER), Osaka University
- Speakers : Wataru Ise, Hisashi Arase, and Yukinori Okada (CiDER/IFReC)



# Japanese Language Class

Japanese language classes are held for overseas researchers / students to alleviate any stress and inconvenience in their research and in their daily lives that may be caused by the language barrier. We offer two classes, one is for beginners, and another is for pre-intermediate level students. In 2021, all classes were organized by the use of an online tool.

**Message from Ms. Kaori Tajima, Japanese language teacher**

Hi. I'm Tajima. I'm really excited to teach Japanese at IFReC, again. The class is focused on speaking. You are expected to talk about various things like your hobbies, your experiences in Japan, your plans for the future and so on, using new grammar and vocabulary that you learn in the class. I hope you enjoy learning Japanese as well as communicating with your class mates.



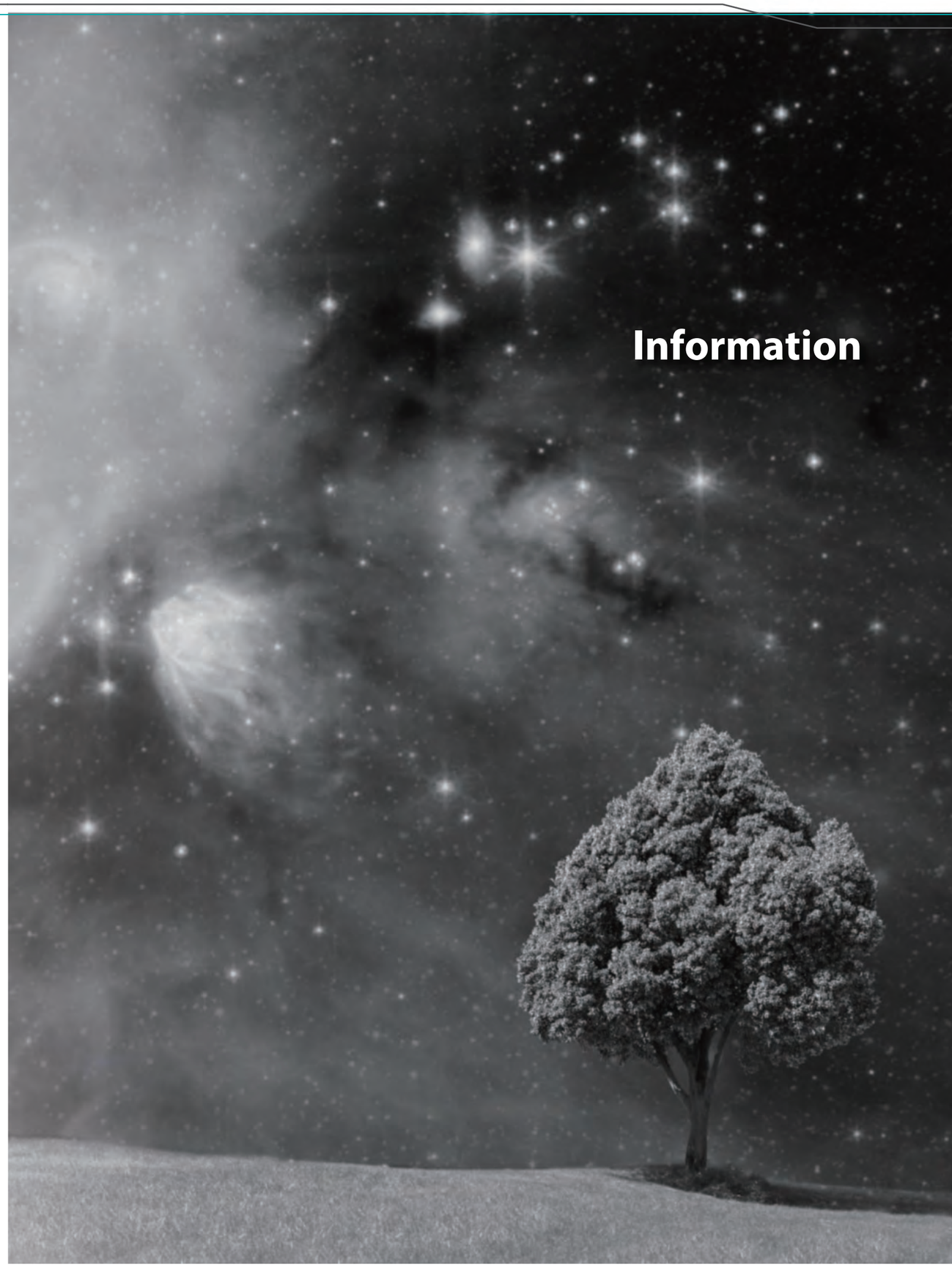
## Focus on Daily Conversion

Learn practical conversations for use in various situations in daily life, such as when shopping or at a restaurant. In addition, learning Japanese for use in your lab and meetings will broaden your communication skills.

## Enjoy Talking with Other Students

Use the Breakout Room to talk in pairs or groups, and you can not only learn Japanese, but also make new friends outside your laboratory or department by sharing fun and useful information. It's easy to stay motivated when you have fellow students to talk with, and they can encourage you to continue learning.





# Major Awards

## Shizuo Akira & Shimon Sakaguchi

**◆The World's Most Influential Scientific Minds 2021**

Highly Cited Researchers are researchers whose theses with large number of citations from all over the world. In 2021, about 6,600 researchers from 21 fields were selected as "the most influential researchers in the world". The three researchers in the two fields were selected from Osaka University, and two (Profs Akira and Sakaguchi) of them were from IFReC.

## Tadamitsu Kishimoto

**◆A Clarivate Citation Laureate 2021**

Since the papers by the laureate have been cited so often in the last two or more decades that these scientists typically rank in the top 0.1% in their research fields. The laureate are recognized to have written multiple high-impact articles over many years.

## Shigekazu Nagata

**◆Pioneer, Australasian Cell Death Society 2021**

In the early 90th, his group in Osaka demonstrated that apoptosis could be induced by a death factor (Fas ligand).

## Hisashi Arase

**The Erwin von Bälz Prize 2021**

The Erwin von Bälz Prize was established in 1964 by Nippon Boehringer Ingelheim Co., Ltd. Arase was awarded First Prize for the theme "Regulation Mechanisms for Viral Infections."

## Taroh Kinoshita

**◆Osamu Hayaishi Memorial Prize 2021**

**◆Toyoichi Ohtawara Award 2021**

Kinoshita has made considerable achievements in the research field of GPI (glycosylphosphatidylinositol)-anchored protein, a series of proteins anchored to the cell membrane (commented by the Hayaishi Prize committees).

## James B. Wing

**◆Osaka University Prize 2021**

The prize is given to the faculty members of Osaka University, who have made remarkable contributions to the education, research, and organizational management. Wing was awarded for "the studies for immune dynamics of regulatory T cells using single cell analysis".

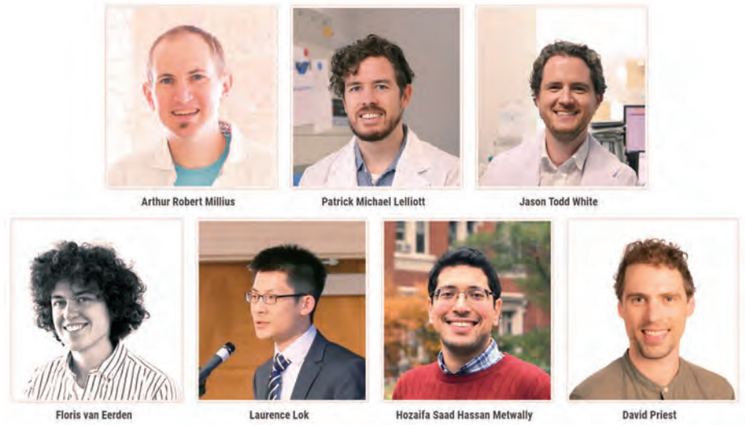
## Ryu Okumura & Yasutaka Motomura

**◆JSI Young Investigator Award 2021**

Okumura (Lab Mucosal Immunology) and Motomura (Lab Innate immune systems) were awarded for "Elucidation of the mechanism of colonic homeostasis by Lypd8 expressed by intestinal epithelial cells" and "Elucidation of the pathogenesis of allergy by IL-4/IL-13", respectively.

## Advanced Postdoc Program at IFReC

IFReC is recruiting post-doctoral researchers for its Advanced Postdoc Program. This program offers three-year employment and funding (3 million yen 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.

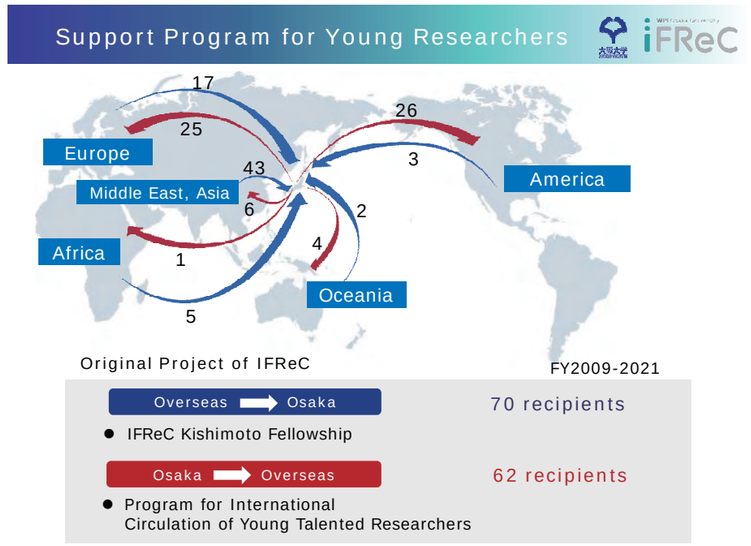

Advanced Postdoc members as of March, 2022.

## 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. IFReC has selected three PIs in FY2018, two in FY2019, and three in FY2020 with the continuous research funding for three fiscal years. The grant is expected to generate excellent research achievements, raise the international recognition of these PIs, and contribute to the acquisition of external research funding. The final debriefing session for the program is held 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. These programs aim to develop the practical skills and abilities of young researchers in international collaborative research and to develop their network with researchers overseas. Since 2009, 132 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. Since 2020, over 40 young scientists have used this system.

# 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.)

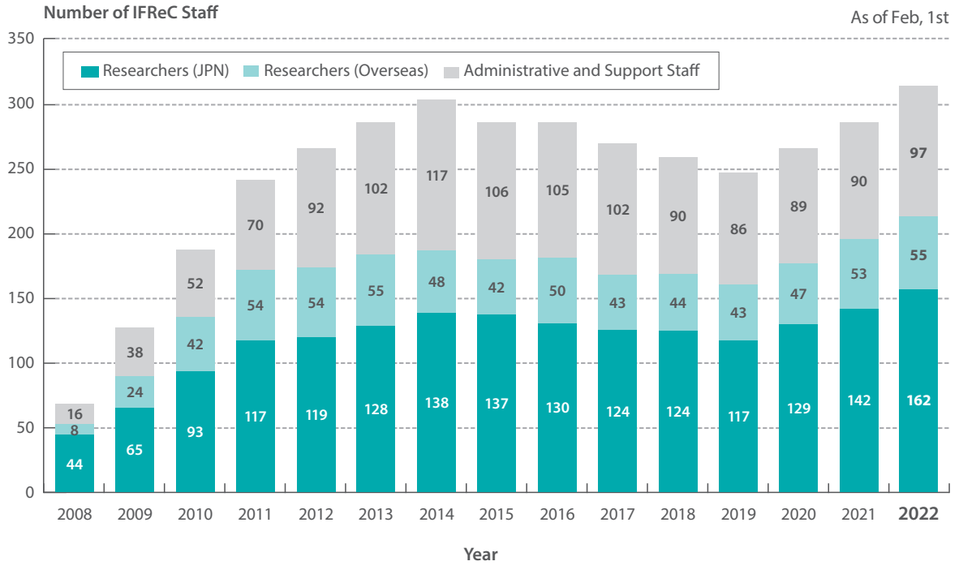
## Core Instrumentation Facility

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

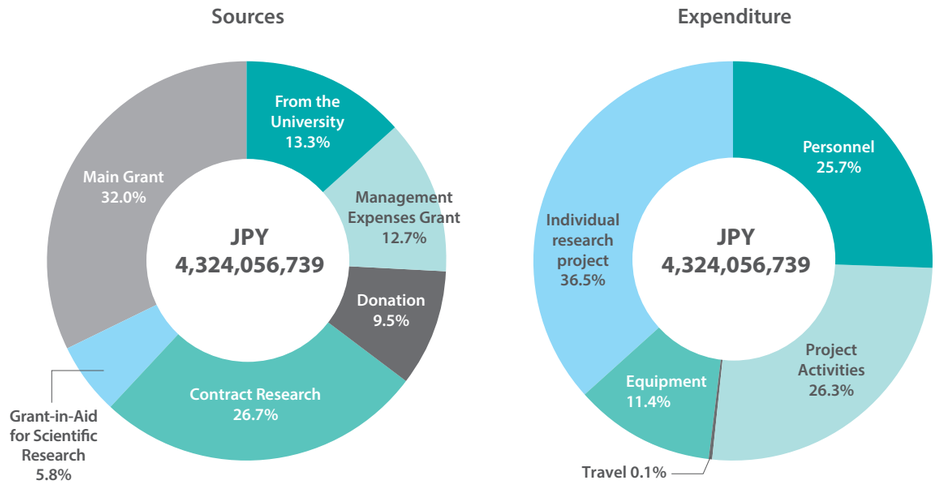


# Composition & Finance

## Composition




## Finance



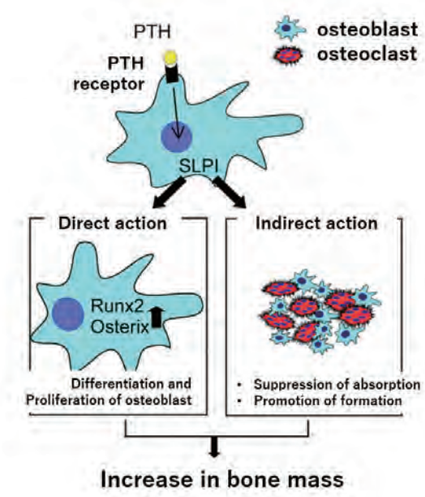
# Selected Articles

## SLPI is a critical mediator that controls PTH-induced bone formation.

Morimoto A, Kikuta J, Nishikawa K, et al.

***Nat Commun.*** 12:2136 (2021).

The research group of Masaru Ishii identified a serine protease inhibitor, secretory leukocyte protease inhibitor (SLPI), as a critical mediator that is involved in the anabolic parathyroid hormone (PTH)-mediated shift to the osteoblastic phase. Slpi is highly upregulated in osteoblasts by PTH, while genetic ablation of Slpi severely impairs PTH-induced bone formation. Slpi induction in osteoblasts enhances its differentiation, and increases osteoblast–osteoclast contact, thereby suppressing osteoclastic function. Intravital bone imaging reveals that thePTH-mediated association between osteoblasts and osteoclasts is disrupted in the absence of SLPI. These results demonstrate that SLPI regulates the communication between osteoblasts and osteoclasts to promote PTH-induced bone anabolism.

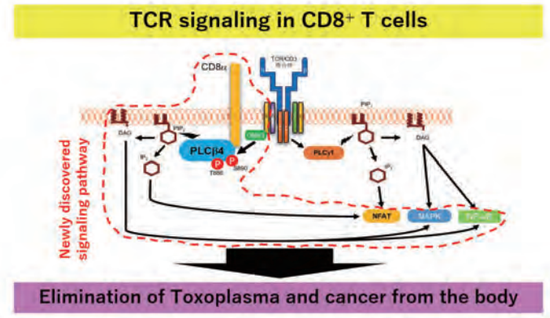



## A novel role of PLCβ4 in selectively mediating TCR signaling in CD8+ but not CD4+ T cells.

Sasai M, Ma JS, Okamoto M, et al.

***J Exp Med.*** 218(7):e20201763 (2021).

Masahiro Yamamoto and his research group showed that TCR signaling in CD8+ T cells is qualitatively different from that in CD4+ T cells, since CD8α ignites another cardinal signaling cascade involving phospholipase C β4 (PLCβ4). TCR-mediated responses were severely impaired in PLCβ4-deficient CD8+ T cells, whereas those in CD4+ T cells were intact. PLCβ4-deficient CD8+ T cells showed perturbed activation of peripheral TCR signaling pathways downstream of IP3 generation. PLCβ4-deficient mice exhibited defective antiparasitic host defense and antitumor immune responses. The authors concluded PLCβ4 differentiates TCR signaling in CD4+ and CD8+ T cells and selectively promotes CD8+ T cell–dependent adaptive immunity.

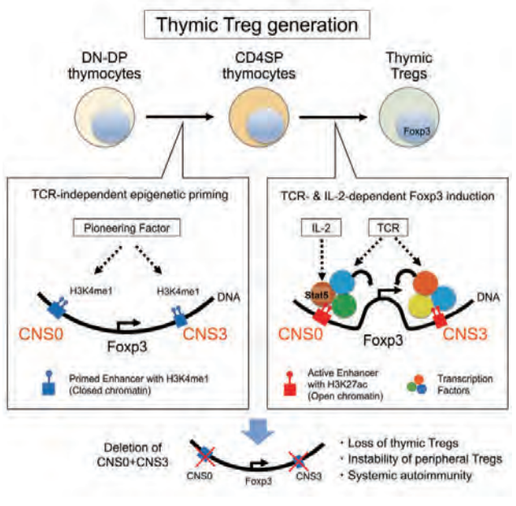



## Distinct Foxp3 enhancer elements coordinate development, maintenance and function of regulatory T cells.

Kawakami R, Kitagawa Y, Chen KY, et al.

***Immunity*** 54:947-961 (2021).

Regulatory T cells (Tregs) are one of the most significant cells in the maintenance of immune tolerance. Tregs work to maintain homeostasis of the body in various situations such as prevention of autoimmune diseases, termination of immune responses and damaging tissue repairing, by braking excessive immune responses. The research group led by Shimon Sakaguchi identified non-coding DNA sequences indispensable for Treg generation. These DNA sequences are distributed into the two separated regions near the Foxp3 gene locus, the master transcription factor of Tregs. Defect of these elements developed severe autoimmune-diseases. Controlling the development and function of Tregs holds promise for the new therapies for immunological diseases.

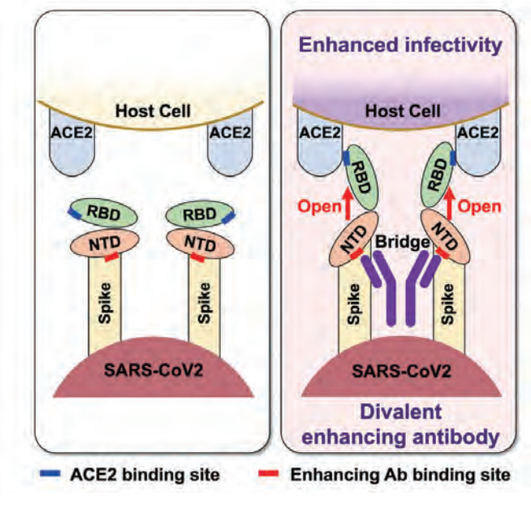



## An infectivity-enhancing site on the SARS-CoV-2 spike protein targeted by antibodies.

Liu Y, Soh WT, Kishikawa J, et al.

***Cell*** 184:3452-3466.e18 (2021).

Antibodies against the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein prevent SARS-CoV-2 infection. However, the effects of antibodies against other spike protein domains are largely unknown. The research group led by Hisashi Arase found that infection with SARS-CoV-2 produces not only neutralizing antibodies that prevent infection, but also infection-enhancing antibodies. They demonstrated that the infection-enhancing antibodies enhance the infectivity of SARS-CoV-2 by modulating the conformation of spike protein of SARS-CoV-2. Furthermore, the infection-enhancing antibodies attenuated the ability of neutralizing antibodies to prevent infection.

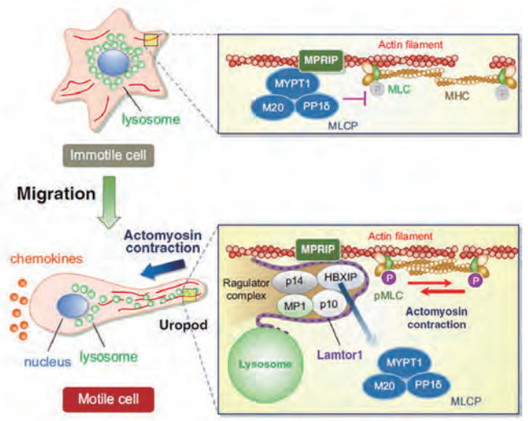


## The lysosomal Ragulator complex plays an essential role in leukocyte trafficking by activating myosin II.

Nakatani T, Tsujimoto K, Park J, et al.

***Nat Commun.*** 12:3333 (2021).

Atsushi Kumanogoh and their research group showed that the lysosomal Ragulator complex plays an essential role in leukocyte migration by activating myosin II. In immotile cells (upper), the Ragulator complex localized to lysosomes is preferentially distributed in the perinuclear region (L), and MPRIP anchors MLCP on myosin–actin bundles by binding MYPT1, a subunit of MLCP, resulting in suppression of MLC phosphorylation (R). In motile cells exposed to chemokines (lower), the lysosomes bearing the Ragulator complex moves to the uropod (L), where the Ragulator complex interacts with MPRIP. This interaction interferes with the interaction between MPRIP and MYPT1 and decreases MLCP activity, thereby increasing MLC phosphorylation. Consequently, cell motility is facilitated (R).

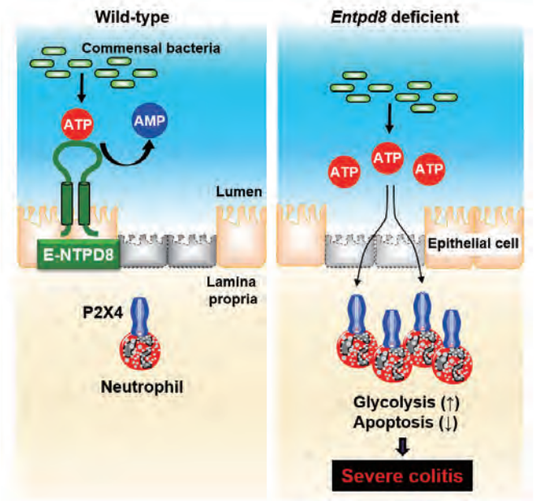



## The ATP-hydrolyzing ectoenzyme E-NTPD8 attenuates colitis through modulation of P2X4 receptor-dependent metabolism in myeloid cells.

Tani H, Li B, Kusu T, et al.

***Proc Natl Acad Sci U S A.*** 118:e2100594118 (2021).

Hisako Kayama, Kiyoshi Takeda and their research group showed that E-NTPD8 is highly expressed in large intestinal epithelial cells and hydrolyzes microbiotaderived luminal ATP. E-NTPD8 in colonic epithelial cells maintains the gut homeostasis through hydrolysis of luminal ATP produced by commensal bacteria (left). An increased level of luminal ATP caused by lack of E-NTPD8 modulates neutrophil physiology, such as prolonged survival, through P2X4-mediated promotion of glycolysis, which links to aggravation of colitis (right).

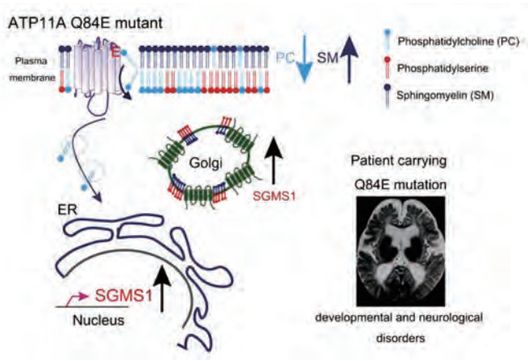



## A sublethal ATP11A mutation causes aberrant phosphatidylcholine flipping in plasma membranes.

Segawa K, Kikuchi A, Noji T, et al.

***J Clin Invest.*** 131:e148005 (2021).

ATP11A translocates phosphatidylserine (PtdSer), but not phosphatidylcholine (PtdCho), from the outer to inner leaflet of plasma membranes, thereby maintaining the asymmetric distribution of PtdSer. Shigekazu Nagata (Biochemistry & Immunology, IFReC), Katsumori Segawa (present: TMDU) and the research group detected a de novo heterozygous point mutation in ATP11A in a patient with developmental delays and neurological deterioration. Mice carrying the corresponding mutation died perinatally or soon after birth with signs of neurological disorders. This mutation caused an amino acid substitution (Q84E) in the first transmembrane segment of ATP11A, and mutant ATP11A flipped PtdCho.

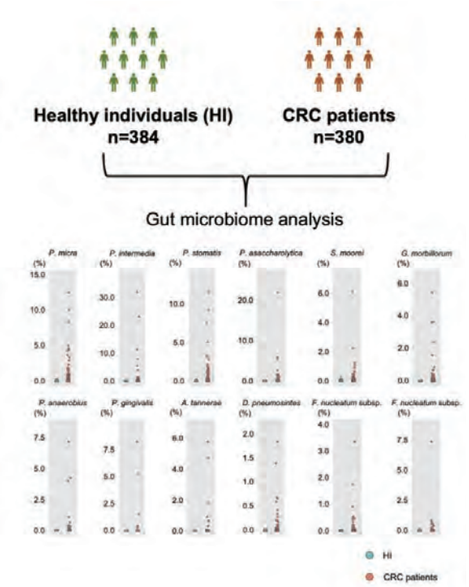



## Gut bacteria identified in colorectal cancer patients promote tumourigenesis via butyrate secretion.

Okumura S, Konishi Y, Narukawa M, et al.

***Nat Commun.*** 12:5674 (2021).

The research group of Eiji Hara showed that 12 faecal bacterial taxa are enriched in CRC patients in two independent cohort studies. Among them, 2 Porphyromonas species are capable of inducing cellular senescence, an oncogenic stress response, through the secretion of the bacterial metabolite, butyrate. Notably, the invasion of these bacteria is observed in the CRC tissues, coinciding with the elevation of butyrate levels and signs of senescence-associated inflammatory phenotypes. Moreover, although the administration of these bacteria into $Apc^{\Delta 14/+}$ mice accelerate the onset of colorectal tumours, this is not the case when bacterial butyrate-synthesis genes are disrupted. These results suggest a causal relationship between Porphyromonas species overgrowth and colorectal tumourigenesis which may be due to butyrate-induced senescence.

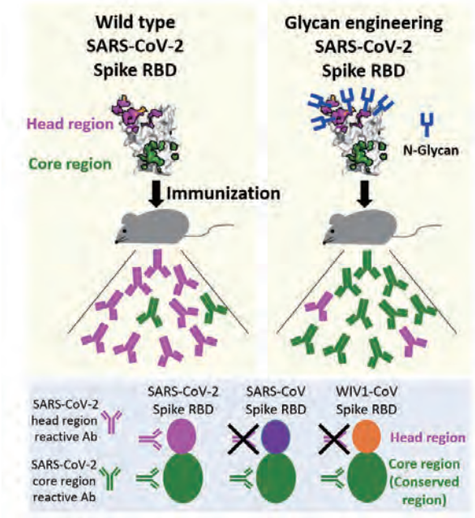


## Glycan engineering of the SARS-CoV-2 receptor-binding domain elicits cross-neutralizing antibodies for SARS-related viruses.

Shinnakasu R, Sakakibara S, Yamamoto H, et al.

***J Exp Med.*** 218:e20211003 (2021).

In order to induce the antibodies predominantly against the regions that are structurally conserved in RBD among SARS-related viruses, the research group of Ryo Shinnakasu, Tomohiro Kurosaki and Shuhei Sakakibara performed glycan engineering to mask the dominant epitope on the RBD head region which is a structurally non-conserved region. When mice are immunized with this modified RBD vaccine, as expected, antibodies that recognize the core-RBD region of not only SARS-CoV-2 but also other SARS-related viruses such as SARS-CoV or WIV1-CoV are predominantly induced. Furthermore, these induced antibodies showed a high protective effect against various SARS-related viruses.

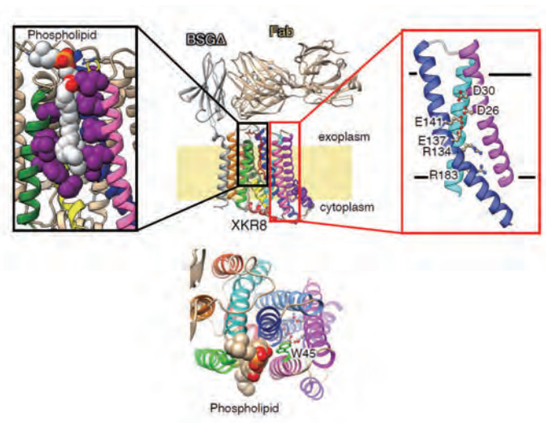



## The tertiary structure of the human Xkr8-Basigin complex that scrambles phospholipids at plasma membranes.

Sakuragi T, Kanai R, Tsutsumi A, et al.

***Nat Struct Mol Biol.*** 28:825-834 (2021).

Xkr8–Basigin is a plasma membrane phospholipid scramblase activated by kinases or caspases. The research group of Takaharu Sakuragi and Shigekazu Nagata combined cryo-EM and X-ray crystallography to investigate its structure at an overall resolution of 3.8 Å. Its membrane-spanning region carrying 22 charged amino acids adopts a cuboid-like structure stabilized by salt bridges between hydrophilic residues in transmembrane helices. Its mutation to alanine made the Xkr8–Basigin complex constitutively active, indicating that it plays a vital role in regulating its scramblase activity. The structure of Xkr8–Basigin provides insights into the molecular mechanisms underlying phospholipid scrambling.





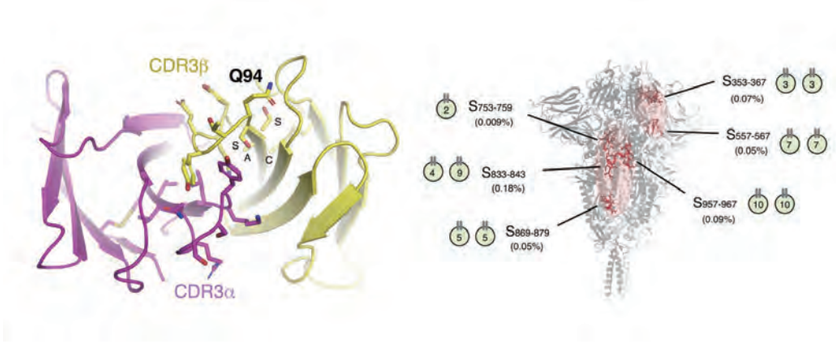

## Identification of conserved SARS-CoV-2 spike epitopes that expand public cTfh clonotypes in mild COVID-19 patients.

Lu X, Hosono Y, Nagae M, et al.

***J Exp Med.*** 218:e20211327 (2021).

Follicular helper T (Tfh) cells are a subset of CD4+ T cells that mediate the production of protective antibodies; however, the SARS-CoV-2 epitopes activating Tfh cells are not well characterized.

Sho Yamasaki and the research group identified and crystallized TCRs of public circulating Tfh (cTfh) clonotypes that are expanded in patients who have recovered from mild symptoms. These public clonotypes recognized the SARS-CoV-2 spike (S) epitopes conserved across emerging variants.

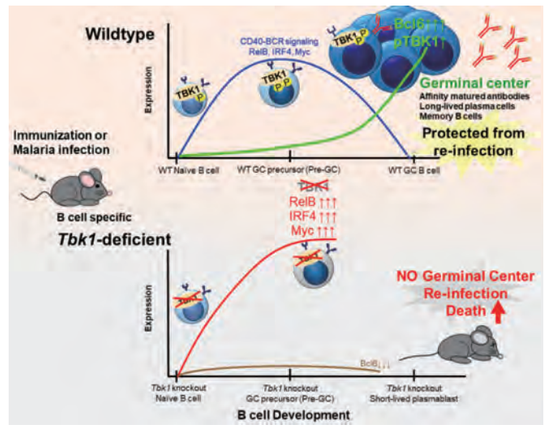



## TBK1 is an essential factor for germinal center formation during infection and vaccination.

Lee MSJ, Inoue T, Ise W, et al.

***J Exp Med.*** 219:e20211336 (2022).

Michelle S. J. Lee (IMS, Univ Tokyo), Cevayir Coban (IMS, Univ Tokyo/IFReC), and their research group identified an unexpected role of Tank-binding kinase-1 (TBK1) as a crucial B cell intrinsic factor for GC formation during infection and vaccination in mice. Wild type mice (top of the figure) and B cell intrinsic TBK1-deficient mice (bottom of the figure) responded differently to vaccination and malaria infection due to distinct B cell fate decision. The horizontal axis represents the stages of germinal center B cell development. The vertical axis represents the expression level of B cell signaling molecules. B cell receptor (BCR) and costimulatory receptor (CD40) engagement activates CD40-BCR signaling to initiate B cell activation. P stands for phosphorylation for TBK1 activation, GC stands for germinal center.

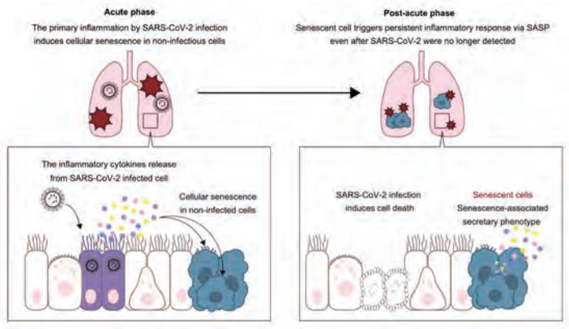


## SARS-CoV-2 infection triggers paracrine senescence and leads to a sustained senescenceassociated inflammatory response.

Tsuji S, Minami S, Hashimoto R, et al.

***Nat Aging*** 2:115-124 (2022).

Reports of "post-acute COVID-19 syndrome," in which the inflammatory response persists even after SARS-CoV-2 has disappeared, are increasing but the underlying mechanisms of post-acute COVID-19 syndrome remain unknown. The research group of Eiji Hara (IFReC/RIMD/CiDER, Osaka University) show that SARS-CoV-2 infected cells trigger senescence-like cell-cycle arrest in neighboring uninfected cells in a paracrine manner via virus-induced cytokine production. The sustained infection-induced paracrine senescence described here may be involved in the long-term inflammation caused by SARS-CoV-2 infection.

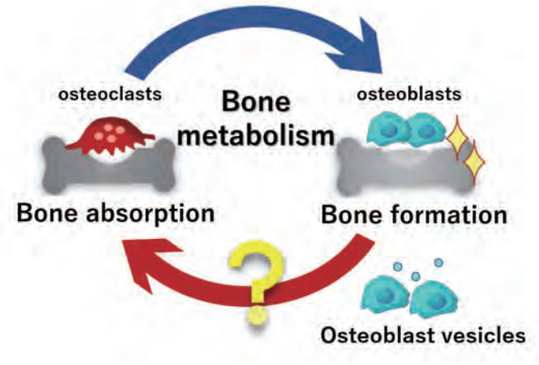



## Osteoblasts Secrete Extracellular Vesicles to Exchange Information.

Uenaka M, Yamashita E, Kikuta J, et al.

***Nat Commun.*** 13:1066 (2022).

The mechanism of the transition from bone formation to bone resorption has been unknown. Using original cutting-edge technology for in vivo bone imaging, Maki Uenaka, Junichi Kikuta, Masaru Ishii and their research group revealed that bone-forming cells (osteoblasts) secrete micro particles (vesicles) outside the cells. These extracellular vesicles secreted from osteoblasts suppress the differentiation of osteoblasts and promote the differentiation of osteoclasts. The regulation of the vesicles is expected to be applied to new therapeutic agents for bone diseases.

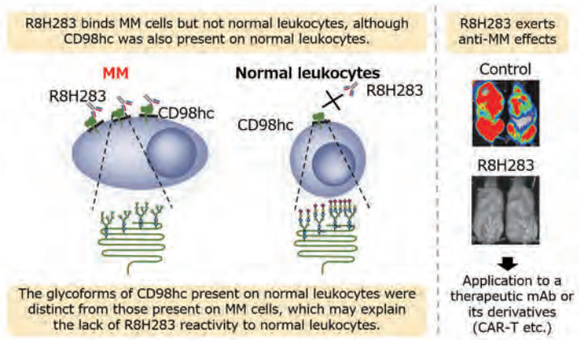



## Selective targeting of multiple myeloma cells with a monoclonal antibody recognizing the ubiquitous protein CD98 heavy chain.

Hasegawa K, Ikeda S, Yaga M, et al.

***Sci Trans Med.*** 14 (2022)

Searching for cancer-specific surface antigens could identify potential targets for cancer therapy that may be missed by -omic approaches. Kana Hasegawa, Naoki Hosen, and their research group screened monoclonal antibody (mAb) clones against patient-derived multiple myeloma (MM) cells, in the process identifying R8H283, an mAb that recognized the CD98 heavy chain protein, which is part of an amino acid transporter. Despite the ubiquitous presence of CD98 heavy chain in normal cells, the mAb only bound to MM cells, and differing glycosylation patterns between normal cells and MM cells contributed to this selectivity. In MM xenograft models in mice, R8H283 injections prolonged survival, suggesting that the mAb should be further assessed for utility in treating MM.

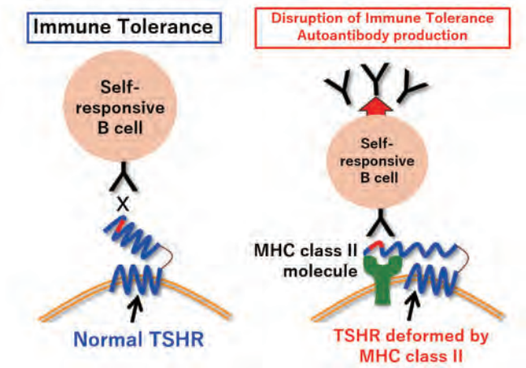



## Abrogation of self-tolerance by misfolded self-antigens complexed with MHC class II molecules.

Jin H, Kishida K, Aease N, et al.

***Sci Adv.*** 8:eabj9867 (2022).

In Graves' disease, one of the autoimmune diseases, autoantibodies against thyroid-stimulating hormone receptors are produced. The research group of Hui Jin and Hisashi Arase found that autoantibodies in patients with Graves' disease preferentially recognize thyroid-stimulating hormone receptor (TSHR) complexed with MHC class II molecules of Graves' disease risk alleles. This suggests that the aberrant TSHR transported by MHC class II molecules is the target of autoantibodies produced in Graves' disease.



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The articles were published between April, 2021 and March, 2022. The data were acquired using Web of Science™ Core Collection on April 28, 2022, and sorted by alphabetical order of the first authors.

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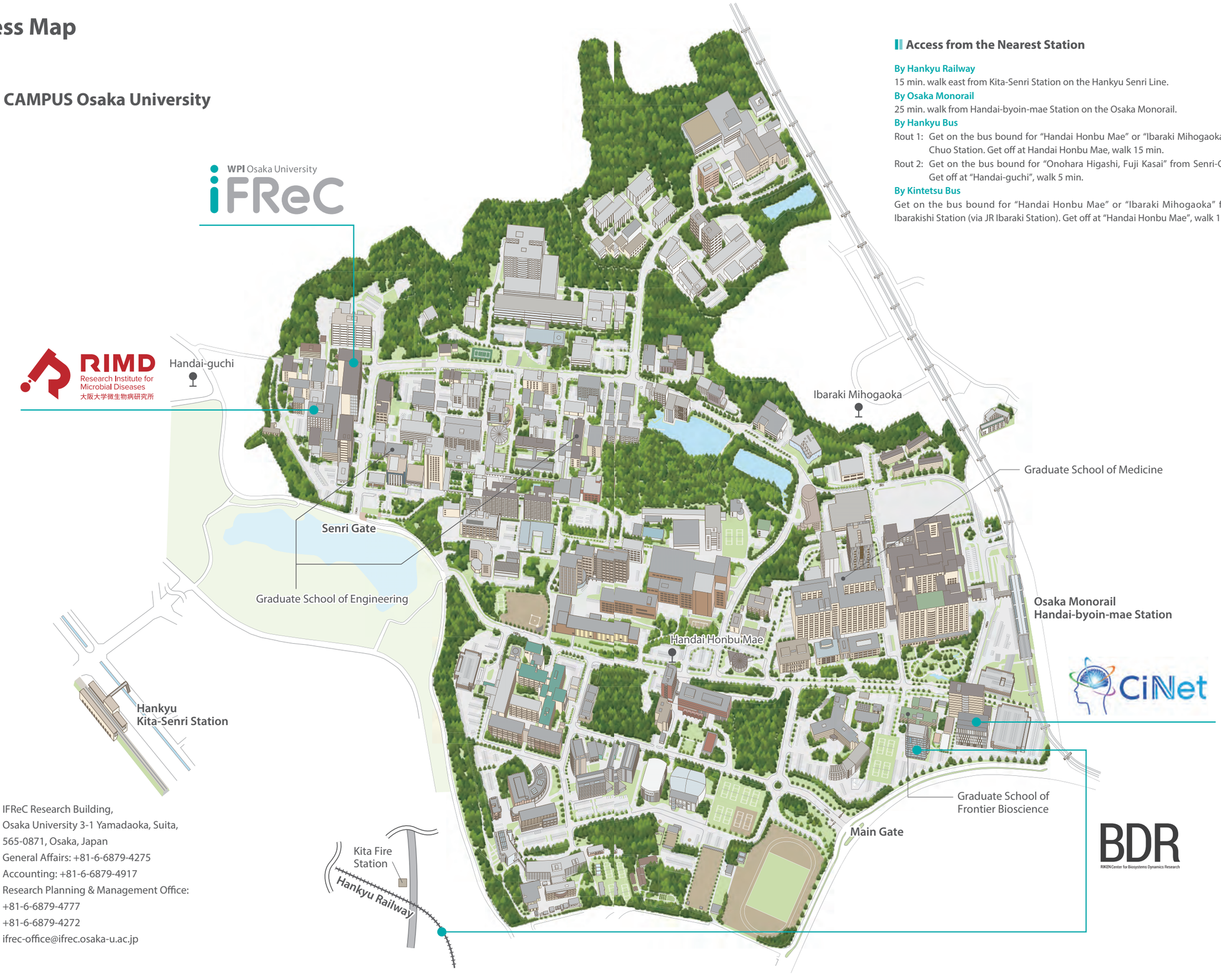


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