World Premier International
Research Center

Osaka University

Immunology Frontier Research Center

Annual Report of IFReC FY 2017

WPI Immunology Frontier Research Center FY2017 Osaka University

Edit : Research Planning & Management Office, IFReC
Published in June, 2018

wpi
World Premier International Research Center Initiative

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iFReC

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Message from the Director

As the Director of the Immunology Frontier Research Center (WPI-IFReC) at Osaka University, I am very pleased to present the IFReC annual report for fiscal 2017.

Since its inception in 2007, IFReC has established itself as a Visible International Research Center of Immunology with the support of many people including the WPI Program Director and the Program Officer.

In the April of 2017, IFReC became one of the first members of the "WPI Academy". Furthermore, IFReC created a new mark in its history with a novel academic-industry partnership agreement. This governance system is an ambitious program without precedent. The researchers at IFReC have been making an increased effort to make this innovative attempt successful in Japan.

Although the governing structure has changed, our most important mission "Constructing a world-top immunology research center" remains the same. We will make unceasing efforts to develop immunology research to ensure translation to medical science.

We are committed to continuing contributions to scientific advances through research and education and evolvement as a world top immunology research center.

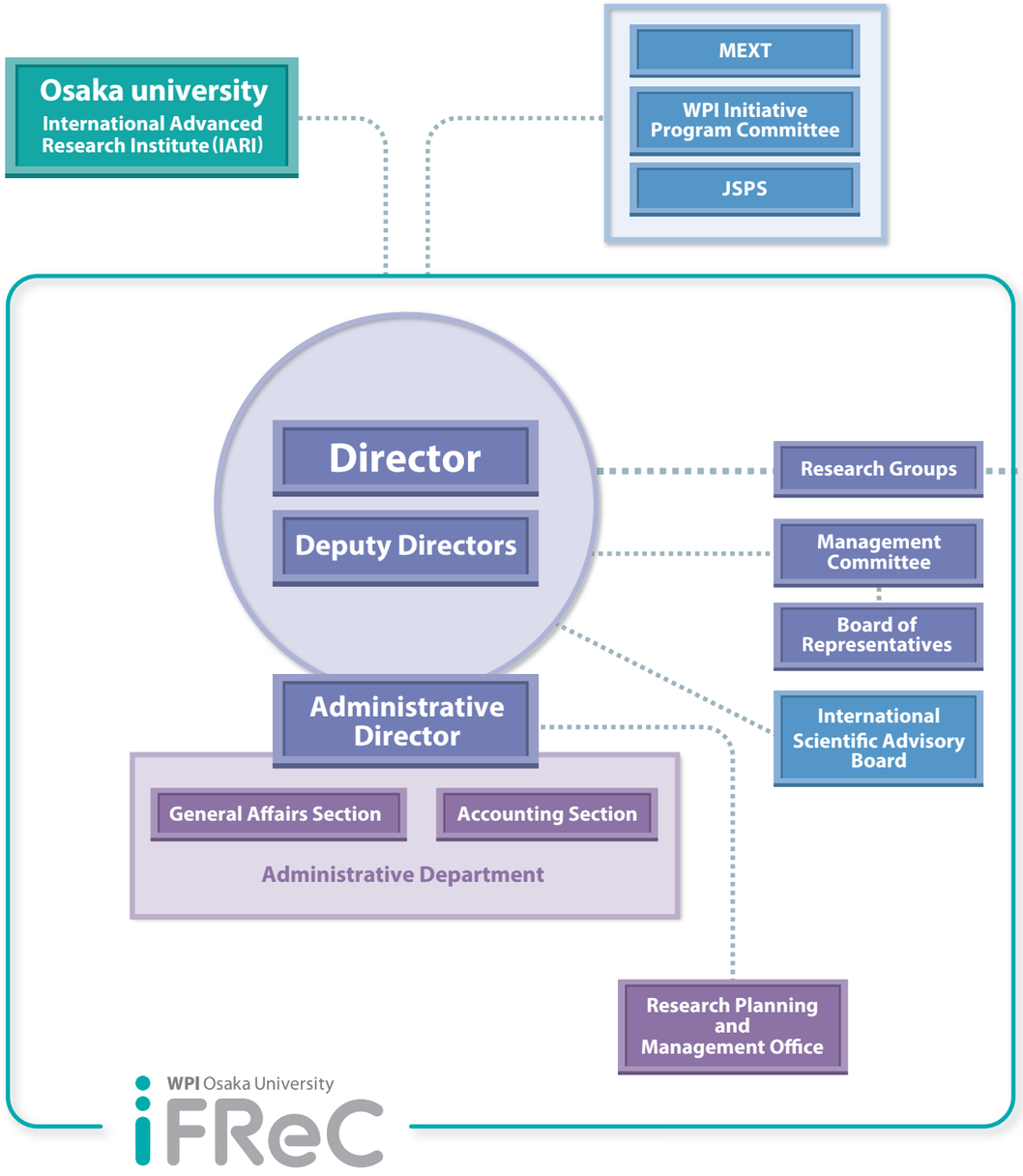
Shizuo Akira

Shizuo Akira, MD, PhD
Director
WPI Immunology Frontier Research Center

Organization

As of March, 2018

Organization Chart



Immunology Group

Host Defense	Shizuo Akira
Immunoglycobiology	Taroh Kinoshita
Immunopathology	Atsushi Kumanogoh
Immunochemistry	Hisashi Arase
Immune Regulation	Tadamitsu Kishimoto
Mucosal Immunology	Kiyoshi Takeda
Immune Regulation	Hitoshi Kikutani
Experimental Immunology	Shimon Sakaguchi
Cell Signaling	Takashi Saito
Lymphocyte Differentiation	Tomohiro Kurosaki
Lymphocyte Development	Fritz Melchers
Malaria Immunology	Cevayir Coban
Vaccine Science	Ken J. Ishii
Immunoparasitology	Masahiro Yamamoto
Biochemistry and Immunology	Shigekazu Nagata
Molecular Neuroscience	Toshihide Yamashita
Molecular Immunology	Sho Yamasaki
Statistical Immunology	Yukinori Okada
Stem Cell Biology and Developmental Immunology	Takashi Nagasawa

Imaging Group

Single Molecule Imaging	Toshio Yanagida / Ben Seymour
Immunology and Cell Biology	Masaru Ishii
Nuclear Medicine	Jun Hatazawa
Chemical Imaging Techniques	Kazuya Kikuchi
Biophotonics	Nicholas Isaac Smith
Immune Response Dynamics	Kazuhiro Suzuki

Informatics Group

Systems Immunology	Daron M Standley

Unit for Combined Research Fields

Quantitative Immunology	Yutaro Kumagai Diego Diez

Joint Research Chair of Innovative Drug Discovery in Immunology

Chugai-pharm	Kunihiro Hattori Ryusuke Omiya Junichi Hata

Common Facilities

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

Cooperative Institutions

Domestic
- Institute for Frontier Life and Medical Sciences, Kyoto University
- RIKEN Center for Integrative Medical Sciences
- National Institute of Biomedical Innovation, Health and Nutrition

Overseas
- Indian Institute of Science Education and Research, India

Committee and Advisory Board for IFReC

World Premier International Research Center Initiative (WPI)

Program Director

As of Mar. 2018

Akira Ukawa	Deputy Director, RIKEN Advanced Institute for Computational Science, Japan

Deputy Program Director

Minoru Yoshida	Chief Scientist, Chemical Genetics Laboratory, RIKEN, Japan

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Michinari Hamaguchi	President, JST, Japan
Hiroshi Matsumoto	President, RIKEN, Japan
Rita Colwell	Distinguished Professor, University of Maryland, USA
Richard Dasher	Consulting Professor, Stanford University, USA
Victor Joseph Dzau	President, National Academy of Medicine, USA
Klaus von Klitzing	Director, Max Planck Institute for Solid State Research, Germany Nobel laureate in Physics (1985)
Chuan Poh Lim	Chairman, Agency for Science, Technology and Research, Singapore
Harriet Wallberg	Former president, Karolinska Institutet, Sweden
Jean Zinn-Justin	Scientific adviser, IRFU/CEA, France

WPI Academy

In FY 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.

The five WPI centers including IFReC are regarded to have achieved "world-premier status", and thus became the initial members of the WPI Academy.

In the decade ahead, the research institutes of WPI and WPI Academy will work together to hold public relations and outreach activities.

Academy Director

Toshio Kuroki	Special Advisor, Research Center for Science Systems, JSPS, Japan

Academy Officer for IFReC

Takehiko Sasazuki	University Professor, Institute for Advanced Study, Kyushu University, Japan

International Scientific Advisory Board

As of Mar. 2018

Jeffrey Ravetch	The Rockefeller University, USA	**Immunology**
Christopher Goodnow	Australian National University, Australia	**Immunology**
Richard Locksley	University of California, San Francisco, USA	**Immunology**
Lewis L. Lanier	University of California, San Francisco, USA	**Immunology**
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Kiyoshi Takatsu	Toyama Prefectural Institute for Pharmaceutical Research, Japan	**Immunology**
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Masamitsu Iino	Nihon University School of Medicine, Japan	**Imaging**
Akinori Kidera	Yokohama City University, Japan	**Informatics**

Administrative Office of IFReC

General Affairs Section

- Employment /acceptance of researchers and staff procedures
- Social insurance (part-time)/ employment insurance
- Management of work hours
- Procedures related to patents
- Issuing various certificates
- Procedures related to international students
- Support for international researchers

Accounting Section

- Budget drafting / implementation / management
- Purchasing procedures
- Acceptance and implementation of third-party funding
- Payment of payroll, travel expense and honorarium
- Health insurance procedures
- Management of buildings and assets
- RI (Radio Isotope) procedures

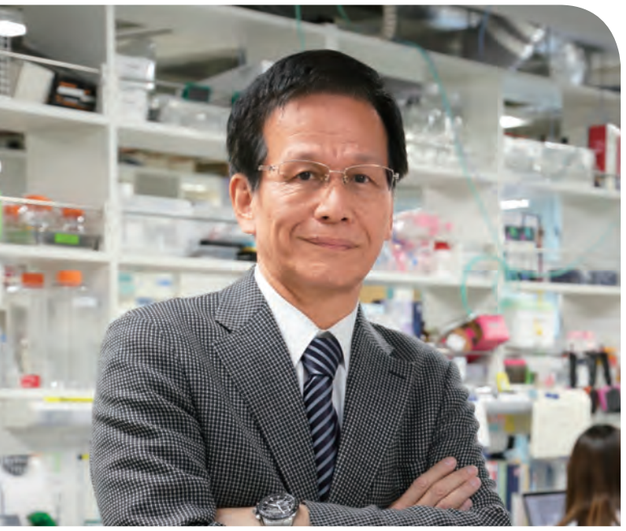
Research Planning and Management Office

- Research Promotion and Support
  (Consultation for grants and patents, Fusion research program, etc.)
- Establishing Research Environments
  (Facility and Safety management, Research agreement, etc.)
- Fostering Young Scientists
  (Winter School, Postdoc program, Orientation, etc.)
- Organizing Scientific Events
  (Symposia, Colloquia, Seminars, etc.)
- Public Relations
  (Publishing, Website, Outreach to citizens, etc.)

As of April, 2018

Laboratories

Host Defense



Shizuo Akira, MD/PhD

Professor	**Shizuo Akira**
Associate Professor	**Kazuhiko Maeda**
Assistant Professor	**Takashi Satoh**
	Hiroki Tanaka
Postdoctoral Fellow	**2**
Research Assistant	**8**
Visiting Scientist	**5**
Support Staff	**8**

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 (PRRs), 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.

Identification of an atypical monocyte and committed progenitor involved in fibrosis, SatM

Macrophages consist of at least two subgroups. M1 macrophages are pro-inflammatory and have a central role in host defense. On the contrary, M2 macrophages are associated with responses to anti-inflammatory reactions, and tissue remodeling. Monocytes and macrophages comprise a variety of subsets with diverse functions. It is thought that these cells play a crucial role in homeostasis of peripheral organs, key immunological processes and development of various diseases. Among these diseases, 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. Recently, we have identified a new macrophage subset in that Ceacam1+Msr1+Ly6C-F4/80-Mac1+ monocytes, which we termed segregated-nucleus-containing atypical monocytes (SatM), share granulocyte characteristics, are regulated by CCAAT/enhancer binding protein beta (C/EBPβ), and are critical for fibrosis. We are further investigating physiological role of SatM and related subsets.

Role of basic leucine zipper transcription factor ATF-like 2 (Batf2) in tumor-associated macrophages

The development of effective treatments against cancers is urgently needed, and the accumulation of CD8+ T cells within tumors is especially important for cancer prognosis. Innate immune cells play an important role in the cancer progression through the production of various cytokines. We found that basic leucine zipper transcription factor ATF-like 2 (Batf2) has an antitumor effect. Subcutaneous inoculated tumor model produced fewer IL-12 p40+ macrophages and activated CD8+ T cells within the tumors of Batf2-deficient mice compared with WT mice. In vitro studies showed that the IL-12 p40 expression was significantly lower in Batf2-deficient macrophages following their stimulation by TLR ligands. Additionally, we found that BATF2 interacts with NF-kB p50/p65 and promotes IL-12 p40 expression. BATF2 has an antitumor effect through the up-regulation of IL-12 p40 in tumor-associated macrophages, which eventually induces CD8+ T-cell activation and accumulation within the tumor. Therefore, BATF2 may be an important target in anticancer treatment with immune checkpoint blockers and TLR agonists.

Regulation of mRNA stability by CCCH-type zinc-finger protein Regnase-1 in immune system

Regnase-1, also known as Zc3h12a, is a member of CCCH-type zinc finger proteins. Conventional Regnase-1 knockout mice developed spontaneous autoimmune diseases accompanied by splenomegaly and lymphadenopathy. 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. Regnase-1 itself is cleaved by Malt1 protease after T cell receptor stimulation and enhances T cell activation. Consistently, the protease activity of Malt1 is involved in control of the mRNA stability of T cell effector genes. This indicates that dynamic control of Regnase-1 expression is critical for modulation of T cell activation. Based on these findings, we promote the understanding of the precious role of Regnase-1 in immune and non-immune cells by using both mutant and tissue-specific Regnase-1 deficient mice. We are 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.

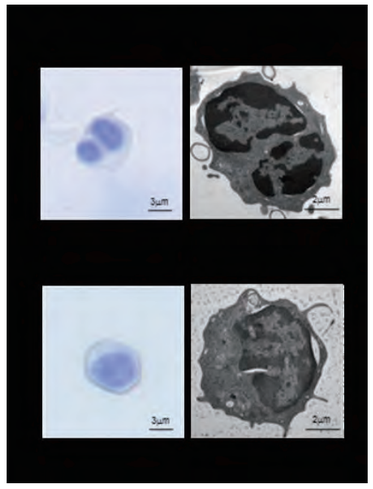


Figure 1.
The morphology of SatM.

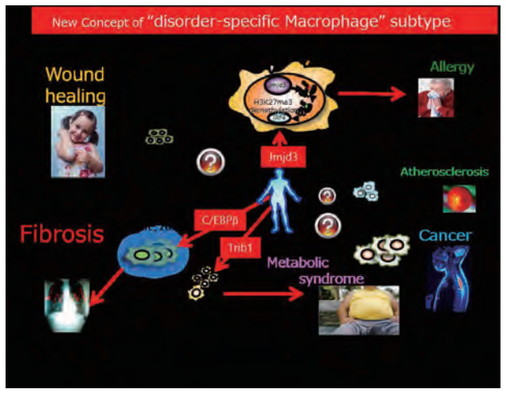


Figure 2.
Concept of disorder specific macrophages.

Recent Publications

- Kanemaru H. et al. BATF2 activates DUSP2 gene expression and upregulates NF-κB activity via phospho-STAT3 dephosphorylation. Int. Immunol. (2018) in press.
- Nakagawa K. et al. Schlafen-8 is essential for lymphatic endothelial cell activation in experimental autoimmune encephalomyelitis. Int. Immunol. 30, 69-78 (2018).
- Kanemaru H. et al. Antitumor effect of Batf2 through IL-12 p40 up-regulation in tumor-associated macrophages. Proc. Natl. Acad. Sci. USA. 114, E7331 (2017).
- Eid MMA. et al. Integrity of immunoglobulin variable region is supported by GANP during AID-induced somatic hypermutation in germinal center B cells. Int. Immunol. 29, 211-220 (2017).
- Maeda K & Akira S. Regulation of mRNA stability by CCCH-type zinc finger proteins in immune cells. Int. Immnunol. 29, 149-155 (2017).

Immunoglycobiology

Taroh Kinoshita, PhD
Yoshiko Murakami, MD/PhD

Professor	Taroh Kinoshita Yoshiko Murakami
Research Assistant	1
Visiting Scientist	3
Support Staff	4

We have been studying biosynthesis, functions and deficiencies of glycosylphosphatidylinositol (GPI) and GPI-anchored proteins (GPI-APs). In 2017, we made the following progress in the studies on GPI biosynthesis and GPI deficiency.

Studies on GPI-AP biosynthesis

GPI-anchors in some mammalian GPI-APs are modified by a side-chain glycan. Biosynthesis and biological functions of the side-chain modification have been unclear. We identified a Golgi-resident N-acetylgalactosamine transferase and termed it PGAP4. PGAP4 transfers N-acetylgalactosamine side-chain to GPI-anchor. This is the 17th step in the GPI-AP biosynthesis pathway occurring after fatty acid remodeling mediated by PGAP3 and PGAP2 (Figure). PGAP4 has a novel structure among many Golgi-resident glycosyltransferases: PGAP4 has three transmembrane domains (TMs) whereas many other glycosyltransferases are type II single membrane pass proteins. PGAP4 has type II membrane protein characteristics in the N-terminal part (short cytoplasmic part, TM1 and stem region) and in the C-terminal portion has tandem transmembrane domains (TM2 and TM3) insertion into a typical glycosyltransferase domain as shown in the 3D structural model (Figure). We propose TM2 and TM3 act to localize a glycosyltransferase domain close to the Golgi membrane where the substrate GPI is inserted (Hirata, T. et al., Nat. Commun., 2018).

We collaborated with Dr. Fujita in Jiangnan University, China, and studied regulation of the first GPI remodeling reaction (Inositol deacylation) that occurs soon after attachment of GPI to proteins. We found that N-glycan attached to nascent GPI-AP regulates GPI-inositol deacylation. Mechanistically, a molecular chaperone calnexin associates with GPI-APs, dependent upon N-glycan and GPI, and facilitates GPI-inositol deacylation. These results indicate that N-glycan participates in quality control of GPI-AP folding before exiting the endoplasmic reticulum (Liu, Y. et al, J. Cell Biol., 2017).

Studies on inherited GPI deficiencies (IGDs)

In collaborations with clinicians and medical geneticists working with IGD caused by PIGO mutations, we clarified genotype - phenotype correlations of PIGO deficiency with variable phenotypes from infantile lethality to mild learning difficulties (Tanigawa, J. et al., Hum. Mutat., 2017). In collaborations with Dr. Campeau group in Canada, who identified the first cases of IGD having mutations in GPAA1 gene, we characterized functional abnormalities caused by those mutations. GPAA1 encodes a subunit of GPI transamidase that transfers GPI to precursor proteins to generate GPI-APs. We found the patients' mutations cause partial losses of function of GPAA1 (Nguyen, T.T.M. et al., Am. J. Hum. Genet., 2017). In collaboration with Dr. Pagnamenta and colleagues in UK, who identified the first case of IGD caused by mutations in PIGH gene, we determined functions of PIGH proteins generated from the mutant gene (Pagnamenta, A.T. et al., Hum. Mutat., 2018). Pathogenic mutations that cause IGDs were found in 18 of 27 genes for biosynthesis and maturation of GPI. Knowledge gained from these studies contributes to our deeper understanding of IGDs and progress in development of better diagnostics of IGDs and effective measures to ameliorate symptoms of IGDs.

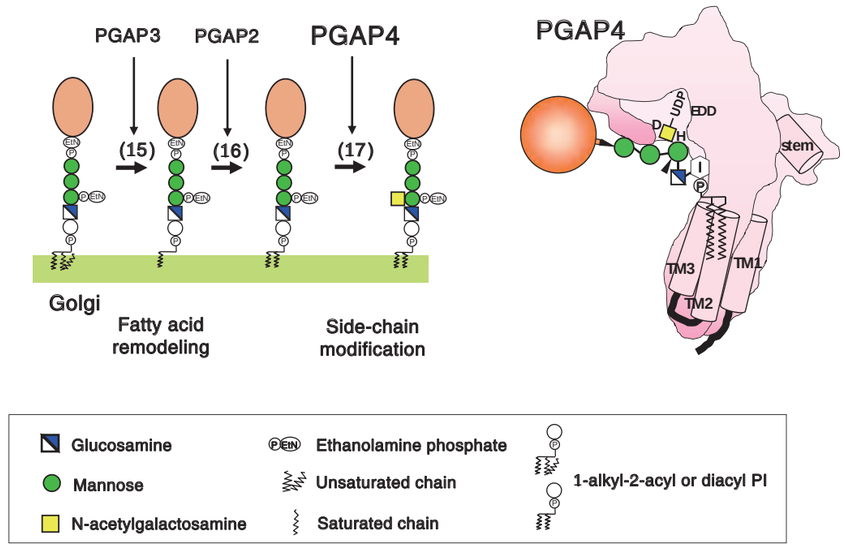


Figure.
PGAP4 transfers N-acetylgalactosamine side-chain to GPI-anchor at the 17th step in GPI-anchored protein biosynthesis pathway (left). PGAP4 has type II membrane protein characteristics in the N-terminal part (short cytoplasmic part, TM1 and stem region) and in the C-terminal portion has tandem transmembrane domains (TM2 and TM3) insertion into a glycosyltransferase domain as shown in the 3D structural model (right).

Recent Publications

- Hirata T, Mishra SK, Nakamura S, Saito K, Motooka D, Takada Y, Kanzawa N, Murakami Y, Maeda Y, Fujita M, Yamaguchi Y & Kinoshita T. Identification of a Golgi GPI-N-acetylgalactosamine transferase with tandem transmembrane regions in the catalytic domain. Nat. Commun. 9, 405 (2018).
- Tanigawa J, Mimatsu H, Mizuno S, Okamoto N, Fukushi D, Tominaga K, Kidokoro H, Muramatsu Y, Nishi E, Nakamura S, Motooka D, Nomura N, Hayasaka K, Niihori T, Aoki Y, Nabatame S, Hayakawa M, Natsume J, Ozono K, Kinoshita T, Wakamatsu N & Murakami Y. Phenotype-genotype correlations of PIGO deficiency with variable phenotypes from infantile lethality to mild learning difficulties. Hum. Mutat. 38, 805-815 (2017).
- Lee G-H, Fujita M, Takaoka K, Murakami Y, Fujihara Y, Kanzawa N, Murakami K, Kajikawa E, Takada Y, Saito K, Ikawa M, Hamada H, Maeda Y & Kinoshita T. A GPI processing phospholipase A2, PGAP6, modulates Nodal signaling in embryos by shedding CRIPTO. J. Cell Biol. 215, 705-718 (2016).
- Makrythanasis P, Kato M, Zaki M, Saitsu H, Nakamura K, Santoni F, Miyatake S, Nakashima M, Issa MY, Guipponi M, Letourneau A, Logan C, Roberts N, Parry DA, Johnson CA, Matsumoto N, Hamamy H, Sheridan E, Kinoshita T, Antonarakis SE & Murakami Y. Pathogenic variants in PIGG cause intellectual disability with seizures and hypotonia. Am. J. Hum. Genet. 98, 615-626 (2016).
- Hirata T, Fujita M, Nakamura S, Gotoh K, Motooka D, Murakami Y, Maeda Y & Kinoshita T. Post-Golgi anterograde transport requires GARP-dependent endosome-to-TGN retrograde transport. Mol. Biol. Cell. 26, 3071-3084 (2015).

Immunopathology

Atsushi Kumanogoh, MD/PhD

Professor	**Atsushi Kumanogoh**
Assistant Professor	**Shohei Koyama**
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 focus here on the functions of Semaphorin 6D (Sema6D) in neuronal, immune and metabolic systems.

Polarization of macrophages into the pro-inflammatory M1 or alternative M2 states has distinct metabolic requirements. Emerging evidence points to a critical role of mTORC (mechanistic target of rapamycin complex) pathway in macrophage polarization. However, it remains unclear how metabolic status regulates macrophage activation state. In this study, we found that following exposure to exogenous stimuli, mTORC activity–dependent Sema6D expression plays an essential role in M2 polarization via regulation of fatty acid uptake and metabolism. Loss of Sema6D in macrophages was refractory to Th2 cytokine–induced M2 macrophage activation, but promoted pro-inflammatory cytokine production. Moreover, Sema6D-mediated PPARγ expression is indispensable not only for optimal M2 macrophage polarization, but also for uptake of fatty acid and reprogramming of related metabolism. Specifically, we found that upon IL-4 stimulation, association with the tyrosine kinase c-Abl via the SH3 domain of Sema6D contributes to PPARγ expression through reverse signaling. Furthermore, we found that the Sema6D–PPARγ signaling axis governs the anti-inflammatory function of intestinal resident CX3CR1^{hi} macrophages and prevents development of colitis in vivo and in vitro. Collectively, these findings highlight a crucial role for Sema6D reverse signaling in modulating macrophage polarization, and provide mechanistic insights into metabolic reprogramming with implications for macrophage homeostasis.

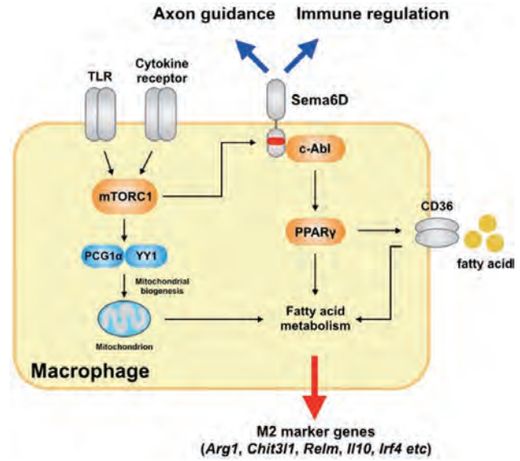
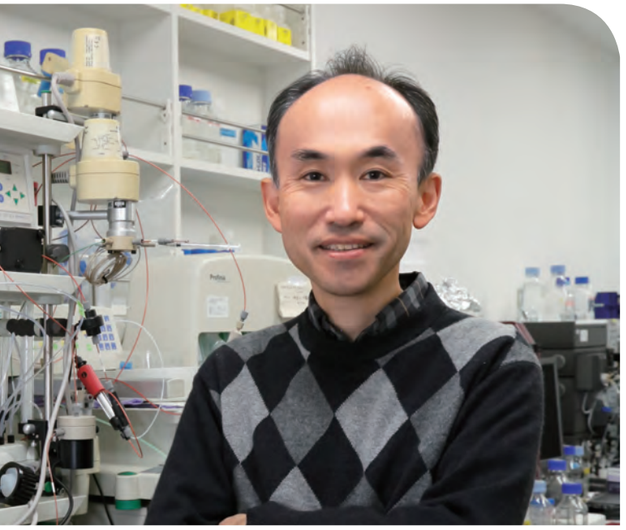


Figure.
During IL-4 induced M2 activation, mTORC1 is activated and upregulates Sema6D, that in turn activates c-Abl kinase to PPARγ expression. Activated PPARγ mediates fatty acid uptake and metabolic reprogramming, which are essential events for fully M2 activation. This Sema6D- PPARγ axis signaling confers anti-inflammatory properties on CX3CR1^{hi} intestinal macrophage and prevent development of colitis.

Recent Publications

- 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, Takagi J, Toyofuku T and Kumanogoh A. Semaphorin 6D reverse signaling controls macrophage lipid metabolism and anti-inflammatory polarization. Nat. Immunol. (2018) in press.
- Nishide M and Kumanogoh A. The role of semaphorins in immune responses and autoimmune rheumatic diseases. Nat. Rev. Rheumatol. 14, 19-31 (2018).
- Hosen N, Matsunaga Y, Hasegawa K, Matsuno H, Nakamura Y, Makita M, Watanabe K, Yoshida M, Satoh K, Morimoto S, Fujiki F, Nakajima H, Nakata J, Nishida S, Tsuboi A, Oka Y, Manabe M, Ichihara H, Aoyama Y, Mugitani A, Nakao T, Hino M, Uchibori R, Ozawa K, Baba Y, Terakura S, Wada N, Morii E, Nishimura J, Takeda K, Oji Y, Sugiyama H, Takagi J and Kumanogoh A. The activated conformation of integrin β7 is a novel multiple myeloma-specific target for CAR T cell therapy. Nat. Med. 23, 1436-1443 (2017).
- Kimura T,Nada S, Takegahara N, Okuno T, Nojima S, Kang S, Ito D, Morimoto K, Hosokawa T, Hayama Y, Mitsui Y, Sakurai N, Sarashina-Kida H, Nishide M, Maeda Y, Takamatsu H, Okuzaki D, Yamada M, Okada M and Kumanogoh A. Polarization of M2 macrophages requires Lamtor1 that integrates cytokine and amino-acid signals. Nat. Commun. 7, 13130 (2016).

Immunochemistry



Hisashi Arase, MD/PhD

Professor	**Hisashi Arase**
Associate Professor	**Tadahiro Suenaga**
Assistant Professor	**Masako Kohyama**
	Kouyuki Hirayasu
Postdoctoral Fellow	4
Research Assistant	3

We have been working on the interactions between pathogens and various paired receptors. In addition, we have found that MHC class II molecules function as molecular chaperons to transport cellular misfolded proteins to the cell surface. Analyses of misfolded proteins transported to the cell surface revealed that these proteins are involved in autoimmune diseases as a target for autoantibodies.

Host pathogen interaction mediated by paired receptor

Paired receptors are composed with activating and inhibitory receptors. PILRα is one of paired inhibitory receptors that are expressed on various immune cells. We have shown that PILRα plays an important role in the regulation of immune response (Wang et al. *Nat. Immunol. 2012*; Kishida et al. *Int. Immunol*. 2015; Kohyama et al. *Eur. J. Immunol*. 2016). We also found that PILRα associates with glycoprotein B (gB), an envelope protein of herpes simplex virus-1 (HSV-1), and the interaction between PILRα and gB is involved in membrane fusion during HSV-1 infection (Satoh et al. *Cell* 2008; Wang et al. *J. Virol*. 2009). Similarly, Siglec-4 (MAG, myelin associated glycoprotein), one of paired receptors, associates with varicella zoster virus (VZV) gB and mediates VZV infection (Suenaga et al. *Proc. Natl. Acad. Sci. USA*. 2010; Suenaga et al. *J. Biol. Chem*. 2015). These findings suggested that paired receptors are involved in viral infection.

LILR is another type of paired receptor family. We found that activating LILRA2 recognizes abnormal immunoglobulins cleaved by microbial proteases but not normal immunoglobulins. LILRA2 seems to be a sensor to detect immunoglobulin abnormalities in microbial infection (Hirayasu et al. *Nature Microbiology* 2016). On the other hand, we found that RIFINs, products of the multigene family of *Plasmodium falciparum*, bind to inhibitory LILRB1 and downregulate immune response. Furthermore, expression of RIFINs was associated with severe malaria. These findings suggest that binding of RIFIN to LILRB1 plays an important role in immune evasion by *Plasmodium falciparum* (Figure 1. Saito et al. Nature 2017).

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 (Jiang et al. *Int. Immunol*. 2013). Furthermore, we found that misfolded proteins complexed with MHC class II molecules are targets for autoantibodies in autoimmune disease patients (Jin et al. *Proc. Natl. Acad. Sci. USA*. 2014; Tanimura et al. Blood. 2015). In addition, we could detect autoantibodies against β2GPI/HLA class II complex in the patients with refractory cutaneous ulcers (Arase et a. *Br. J. Dermatol*. 2017). Similarly, we also found that myeloperoxidase/HLA class II complex is a target for autoantibodies in ANCA-associated vasculitis (Hiwa et al. *Arthritis. Rheumatol*. 2017). Autoantibody binding to misfolded proteins transported to the cell surface by MHC class II molecules was strongly correlated with susceptibility to autoimmune disease. This suggested that misfolded proteins, which normally would not be exposed to the immune system, can be targets for autoantibodies as 'neo self' antigens, which are involved in the pathogenicity of autoimmune diseases (Figure 2).

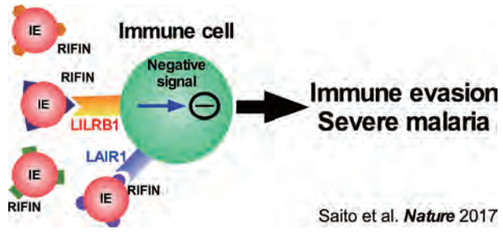


Figure 1. Immune evasion of Plasmodium falciparum by RIFIN via inhibitory receptors.
Plasmodium falciparum **induces the expression of RIFINs on the surface of infected erythrocytes. Individual RIFINs may have evolved to target host inhibitory receptors, thus facilitating escape from host immune systems, which may lead to inefficient development of immunity against malaria parasites (Satio et al.** ***Nature*** **2016).**

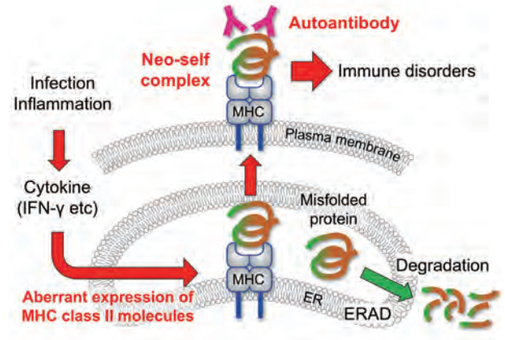


Figure 2. Misfolded proteins transported to the cell surface by MHC class II molecules are targets for autoantibodies.
Cellular misfolded proteins are generally degraded in the cells and are not transported to outside the cells. Therefore, misfolded proteins transported to the cell surface by MHC class II molecules may be recognized as 'neo-self' antigens by the immune system, which might initiate aberrant immune response to self-antigens (Jiang et al. ***Int. Immunol***. **2013; Jin et al.** ***Proc. Natl. Acad. Sci. USA***. **2014; Tanimura et al.** ***Blood*** **2015, Arase** ***Adv. Immunol***. **2016; Arase et al.** ***Br. J. Dermatol***. **2017; Hiwa et al.** ***Arthritis Rheumatol***. **2017).**

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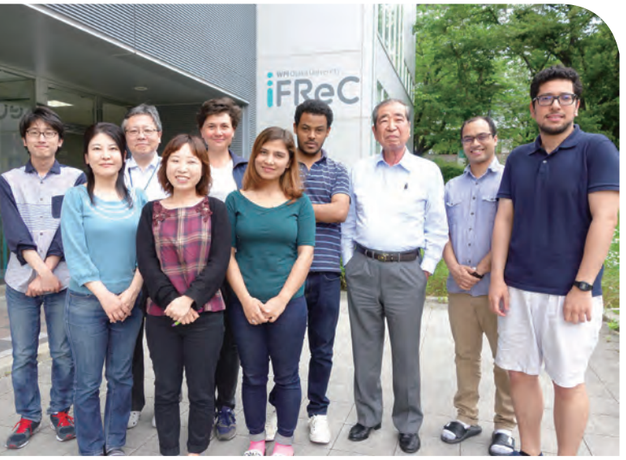
Immune Regulation



Tadamitsu Kishimoto, MD/PhD

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Associate Professor	**Shigeru Hashimoto**
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Postdoctoral Fellow	**1**
Research Assistant	**3**
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Support Staff	**3**

Introduction

Interleukin-6 (IL-6) is an important cytokine in the early phase of acute immune responses, as well as activation of lymphocytes, hematopoietic cells and vascular endothelial cells to protect the body against invasion of pathogens. On the other hand, persistent production of IL-6 leads to development of various chronic diseases such as rheumatoid arthritis (RA) and juvenile idiopathic arthritis (JIA). The significance of IL-6 in the pathogenesis of chronic diseases, has been successfully proven by tocilizumab, a humanized anti-IL-6 receptor antibody, when used for those chronic immune disorders. In addition, a high level of IL-6 expression in the sera of patients has also been responsible for the induction of systemic inflammatory responses syndrome (SIRS) and cytokine release syndrome (CRS) To pursue the molecular mechanisms of overproduction of IL-6, we focused on IL-6 gene expression in activated macrophages under TLR signaling. IL-6 mRNA is tightly regulated by RNA binding proteins, the dysfunction of which leads to sustained IL-6 production. Previously, we identified an RNA-binding protein, AT-rich interactive domain 5a (Arid5a), which binds to the 3'UTR of IL-6 mRNA. Arid5a is highly expressed in macrophages in response to lipopolysaccharide (LPS) and stabilizes Il6 mRNA by binding to its 3'-UTR. Additionally, Arid5a also stabilizes either Stat3 or T-bet mRNAs in T cells and is involved in T cell-fate regulation. Moreover, Arid5a-deficient (Arid5a$^{-/-}$) mice are highly resistant to endotoxic shock, experimental autoimmune encephalomyelitis (EAE), and bleomycin-induced lung injury. These findings suggest that Arid5a plays important roles in post-transcriptional regulation and inflammatory disease control.

TLR4-induced NF-κB and MAPK signaling regulate IL-6 mRNA-stabilizing protein Arid5a (published)

Arid5a mRNA expression is induced in response to LPS stimulation, however, the mechanisms of the Arid5a protein during stabilization of IL-6 mRNA has remained unclear. We identified that NF-κB and an NF-κB-triggered IL-6-positive feedback loop activate Arid5a gene expression, increasing IL-6 expression via stabilization of the IL-6 mRNA during the early phase of TLR4 stimulation. Subsequently, AU-rich element RNA-binding protein 1 binds to the Arid5a mRNA 3'-UTR to destabilize it, which involves MAPK phosphatase-1. During the late phase of TLR4-stimulation, p38 phosphorylates Arid5a, leading to the degradation of Arid5a through the E3 ubiquitin ligase, WWP1. Inhibition of Arid5a phosphorylation increased IL-6 expression. Thus, the regulation of Arid5a by NF-κB and MAPK signaling is required to maintain the balance of IL-6 mRNA expression.

Arid5a stabilizes OX40 mRNA in murine CD4+ T cells by recognizing a stem-loop structure in its 3'-UTR (published)

The roles of Arid5a in Th17 cells and its association with autoimmunity remain unknown.

Deficiency of Arid5a in T cells decreased OX40 expression levels and repressed IL-17 production upon OX40 ligation. Notably, Arid5a stabilized OX40 mRNA by recognizing the alternative decay element (ADE)-like stem-loop (SL) in the 3′-UTR. Arid5a$^{-/-}$ mice showed resistance to EAE by either decreased OX40 expression in CD4^{+} T cells or reduction of CD4^{+}CD45^{+} T cell numbers in the CNS. Thus, our findings suggest that Arid5a-OX40 axis in Th17 cells plays important roles in the pathogenesis of EAE.

Regulation of inflammatory responses by dynamic subcellular localization of RNA-binding protein Arid5a (published)

Although Arid5a plays important roles in immune regulation, how its subcellular localization impacts immune regulation has remained unclear. We found that IL-6 was substantially produced by LPS stimulation in Arid5a overexpressing-transgenic mice, but not in an unstimulated condition, indicating that the dynamics and subcellular localization of Arid5a might be important for its function in the immune response. In addition, Arid5a was imported into the nucleus via a classical importin-α/β1-mediated pathway. On stimulation, nuclear Arid5a levels decreased whereas cytoplasmic Arid5a levels increased. Moreover, Arid5a was associated with up-frameshift protein 1, and its nuclear export was regulated by a nuclear export receptor, chromosomal region maintenance 1. Taken together, our results suggest the importance of the nucleocytoplasmic localization of Arid5a in inflammation through a change in the Arid5a:Regnase-1 ratio (Figure 1).

Analysis of anti-inflammatory effects of lenalidomide (ongoing)

Lenalidomide is an immunomodulatory drug (IMiD) having a therapeutic action in several autoimmune/inflammatory diseases. However, it is difficult to study the mechanism of IMiD action in murine disease models because murine cereblon—the substrate receptor for IMiDs action—is resistant to some of IMiDs' therapeutic effects. To overcome this difficulty, we generated humanized cereblon mice, thereby providing a novel animal model to unravel complex IMiD mechanisms of action in mouse physiological setup. In our current study, we investigated potential IMiD degradative effects towards Ikaros family zinc finger protein 1/3 (IKZF1/3), a potential target substrate of lenalidomide. Unlike wild type mice, which were resistant to Lenalidomide, T lymphocytes from humanized cereblon mice responded with a higher degree of IKZF1/3 protein degradation. Furthermore, IMiD degradation of IKZF1/3 resulted in an increase in intereukin-2 among humanized cereblon mice but not in the wild type group. Additionally, we assessed the potential therapeutic effects of IMiDs in dextran sulfate sodium (DSS) induced colitis. In both WT and humanized mice lenalidomide showed a significant therapeutic effect in DSS model of colitis, while the effect of pomalidomide was less pronounced. We conclude that lenalidomide offers therapeutic opportunities against inflammatory diseases independent of cereblon whereas its effect on IKZF1/3 and interleukin-2 is totally dependent of cereblon.

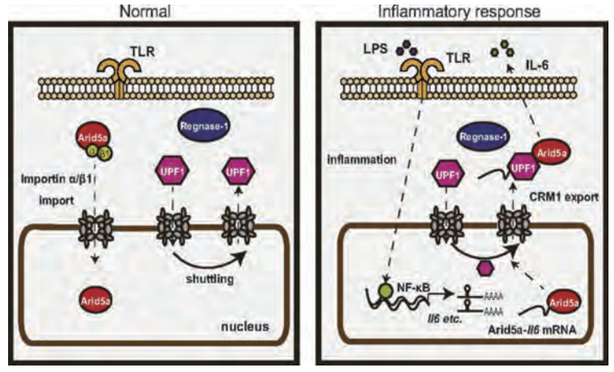

Figure.
Model of inflammatory response by Arid5a/Regnase-1/UPF1.

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Mucosal Immunology

Kiyoshi Takeda, MD/PhD

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Regulation of protozoa-induced immune response by BATF2

Chagas' disease, characterized by chronic cardiomyopathy, is caused by infection with the intracellular protozoan parasite *Trypanosoma cruzi*. Although IL-17 confers protection against lethal *T. cruzi* infection, inappropriate IL-17 responses are implicated in chronic tissue inflammation. IL-23 contributes to *T. cruzi*-specific IL-17 production, but the molecular mechanisms underlying the regulation of the IL-23/IL-17 axis during *T. cruzi* infection are poorly understood. The Toll-like receptor (TLR)–MyD88/TRIF adaptor molecule pathways initiate both innate and adaptive immune responses and thus contribute to the resistance of the host to *T. cruzi*. In addition, the mechanisms underlying the TLR-independent host defense against *T. cruzi* infection have recently been demonstrated. We found that transcription factor BATF2 is induced in Mϕ and DCs in a TLR-independent and IFN-γ-dependent manner. Therefore, we analyzed the roles of IFN-γ-inducible BATF2 in *T. cruzi*-infected innate immune cells. In the spleens and livers of *T. cruzi*-infected *Batf2*$^{-/-}$ mice, IL-17, but not IFN-γ, was more highly produced by CD4^{+} T cells compared to those of wild-type mice. In this context, Batf2$^{-/-}$ mice showed severe multi-organ pathology, despite reduced levels of parasitemia. In Batf2$^{-/-}$ innate immune cells, *T. cruzi*-induced production of IL-23, but not IL-6, was increased. The *T. cruzi*-induced enhanced Th17 response was abrogated in *Batf2*$^{-/-}$*Il23a*$^{-/-}$ mice. The interaction of BATF2 with c-JUN prevented c-JUN/ATF-2 complex formation, thereby inhibiting *Il23a* expression. Taken together, IFN-γ-inducible BATF2 expressed in the innate immune cells suppresses IL-23 production by preventing the formation of the c-JUN/ATF-2 heterodimer, resulting in the inhibition of excessive Th17 responses during *T. cruzi* infection (Fig. 1).

Intestinal metabolites that regulate mucosal inflammation

Pro-inflammatory CD4^{+} T effector (Teff) cells continually circulate in intestinal tissue to provide immune surveillance, as intestinal mucosal is constantly exposed to potentially pathogenic stimuli. However, an excess of pro-inflammatory cytokines is implicated in the pathogenesis of inflammatory bowel diseases including Crohn's disease and ulcerative colitis. Therefore, Teff cells are tightly regulated by innate myeloid cells, such as CD103^{+} DCs and CX$_3$CR1^{+} phagocytes, and microbiota in the intestine. But whether intestinal T cells interface with host-derived metabolites is less clear. Mucosa-associated taurine-/glycine-conjugated bile acids (CBAs) in the ileum are present at high micro-molar concentrations. We found that the xenobiotic transporter, Mdr1, is expressed in mouse Teff cells at steady-state, where it is highest in the ileum. In addition, CD103^{+} dendritic cells in the small intestine induced Mdr1 expression in Teff cells. Wild-type Teff cells upregulated Mdr1 in the ileum, but those lacking Mdr1 displayed mucosal dysfunction and induced Crohn's disease-like ileitis in naïve T cells-transferred *Rag1*$^{-/-}$ mice. Both in vitro and in the small intestine in vivo, Mdr1 mitigated oxidative stress and pro-inflammatory cytokines expression and then enforced homeostasis in Teff cells during CBAs exposure. Blocking ileal CBA reabsorption in *Rag1*$^{-/-}$ mice lacking an apical sodium-dependent bile acid transporter Asbt, which is expressed in epithelial cells, restored Mdr1-deficient Teff cell homeostasis and attenuated ileitis. Furthermore, a subset of ileal Crohn's disease patients exhibited MDR1loss of function. Together, these results suggest that coordinated interaction between Teff cells and CBAs in the ileum regulates intestinal immune homeostasis (Fig. 2).

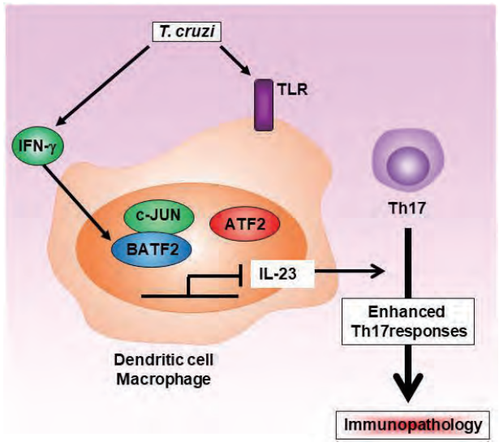

Figure 1.
Transcription factor BATF2 induced by IFN-γ in Mϕ and DCs prevents Th17-mediated multi-organ pathology by suppressing IL-23 production through prevention of the c-JUN/ATF-2 heterodimer formation during *T. cruzi* infection.

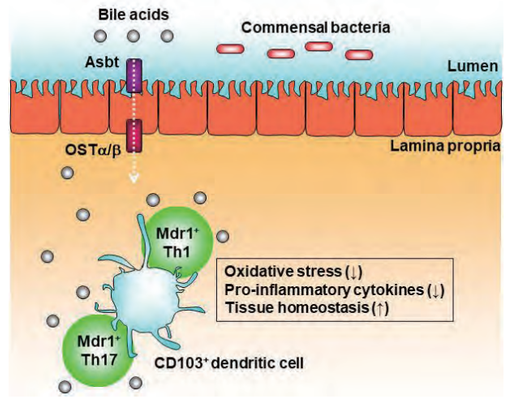

Figure 2.
CD4^{+} T effector (Teff) cells in the ileum upregulates Mdr1 expression in a CD103^{+} DCs-dependent mechanism. Mdr1 protects Teff cells from bile acid-driven oxidative stress and pro-inflammatory cytokines expression in the ileum.

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Immune Regulation

Hitoshi Kikutani, MD/PhD

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Structural basis for antigen recognition of anti-DNA antibody in systemic lupus erythematosus (SLE)

Anti-DNA antibody is the established criterion for SLE diagnosis and its contribution to clinical symptoms in SLE has been widely accepted. However, it is still unclear how anti-DNA antibodies exert pathogenicity. This may be due to a lack of precise structural studies of anti-DNA antibodies. In the current study, we isolated high-affinity anti-DNA monoclonal antibodies (mAbs) from acute SLE subjects. Unlike previously reported anti-DNA mAbs obtained from SLE model mice, they exhibited nano-molar KD in surface plasmon resonance (SPR). In collaboration with Prof. Junichi Takagi (Institute of Protein Research, Osaka Univ), the crystallography of ligand-bound Fab of one mAb clone (designated as 71F12) was solved (Protein Data Bank [PDB] ID: 5GKR). The structural analysis clearly demonstrated a contribution of somatic hypermutation (SHM) to the antigen recognition, in which nucleobases are rigidly grabbed by the Fab through the characteristic stacking interaction. The structure revealed that this antibody recognizes thymine-containing oligonucleotides in a manner that is only compatible with ssDNA unlike previously proposed models. We speculate that high affinity anti-dsDNA antibodies target "loosened" single-stranded segments, which may be present in a released dsDNA upon cell death.

Characterization of low-affinity progenitor B cells for pathogenic anti-dsDNA antibody-producing cells derived from SLE

Our previous study has demonstrated that SLE ANA are generated through rigorous clonal expansion and affinity maturation, in which low-affinity anti-ssDNA B cells can acquire high-affinity to both ss- and dsDNA by only one or two mutations (Figure 2). Now, the findings raise the questions of how low-affinity anti-ssDNA precursor B cells escape from tolerance checkpoints and in what conditions they undergo clonal expansion and affinity mutation to differentiate into high affinity anti-dsDNA antibody-producing cells. To address these questions, we generated a site-directed knock-in (KI) mouse line, G9gl, which carries unmutated IgH and L chains derived from one of the SLE anti-DNA antibody clones. The number of G9gl-expressing B cells was reduced in blood and lymphoid organs of the KI mice. In the G9gl heterozygotes, about 60% of B cells expressed endogenous BCR and their KI heavy chain alleles were inactivated via aberrant receptor editing. Although G9gl^{+} B cells displayed reduced levels of surface BCR, they were not functionally anergic and could respond efficiently to LPS, anti-CD40 or BCR crosslinking. Moreover, ssDNA could induce vigorous activation and proliferation in G9gl B cells. A fraction of G9gl mice exhibited high titer of serum autoantibodies such as anti-DNA IgG with spontaneous germinal center formation in spleen. Taken together, low affinity precursors of SLE-derived anti-DNA B cells can escape from tolerance check point during early B cell development and retain the capability to trigger self-reactive germinal center reaction.

Tracking allergy-related IgE in chronic rhinosinusitis with nasal polyposis (CRSwNP)

CRSwNP is characterized by eosinophilic inflammation and nasal polyposis. Nasal polyps (NPs) of CRSwNP patients contain a high concentration of IgE presumably originated from infiltrating B cells. We sought to understand the development pathway of IgE-producing B cells in NPs. We first determined antigens of IgE produced in NPs by using antibody cloning and expression of monoclonal IgE antibodies. The majority of isolated mAbs appeared to be specific to bacteria which normally inhabit the sinonasal cavity, such as *Streptococcus pyogenes*. Next, to comprehend the development pathway of the antigen-specific IgE, deep sequencing of NP-associated BCR repertoires was performed (Figure 3). It showed that major clonal lineages of IgE were often found in IgG or IgA$_1$ clonal lineages, indicative of sequential class switch to IgE in NPs. Taken together, CRSwNP is derived from protective immune response against nasal bacteria, in which unnecessary class switching to IgE takes place concomitantly. Immune cells involved in IgE production remains to be elucidated.

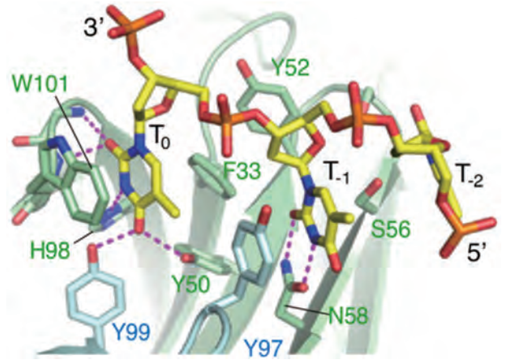

Figure 1. X-ray crystallography of anti-DNA antibody 71F12.
The Fab binds to thymines through a stacking interaction (W101 of the heavy chain) and several hydrogen bonds. It should be noted that mutated F33 of the heavy chain locates at the antigen recognition site.

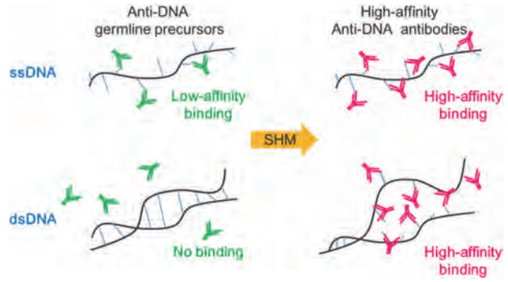

Figure 2.
Working model of generation process of anti-dsDNA antibodies in SLE. Unmutated low-affinity anti-ssDNA precursors cannot bind to dsDNA. After SHM, high-affinity anti-DNA clones acquire binding capability to both ss and dsDNA at high affinity.

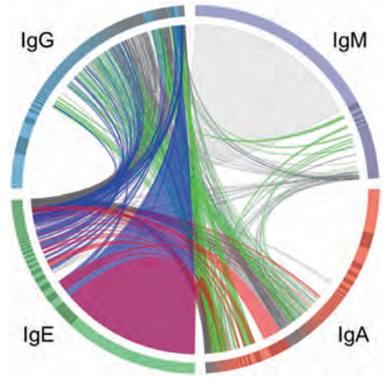

Figure 3. Clonal connectivity between isotypes in NP-associated immunoglobulins.
Deep sequencing analysis of expressed BCR repertoires unraveled the clone distribution across immunoglobulin isotypes. Large IgE clones were strongly connected with IgG and IgA lineages.

Recent Publications

- Sakakibara S. et al. Clonal evolution and antigen recognition of anti-nuclear antibodies in acute systemic lupus erythematosus. Sci Rep 7(1), 16428 (2017).

Experimental Immunology

Shimon Sakaguchi, MD/PhD

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Support Staff	**5**

This laboratory studies: (i) the cellular and molecular basis of immunologic self-tolerance, in particular the roles of regulatory T cells; (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. In the previous years, we studied how Treg cells controlled humoral immune responses and showed that CTLA-4-expressing T follicular regulatory (Tfr) cells suppressed B cell expression of CD80 and CD86, which was essential for T follicular helper (Tfh) cell formation and germinal center (GC) development (Wing and Sakaguchi, Int. Immunol. 2013; Wing et al., Immunity 2014). This year, we have further explored which type of Tfr cells controls Tfh cells and germinal center formation (Wing et al., PNAS, 2017). Tfh cells differentiate through a multistep process, culminating in GC-localized GC-Tfh cells that provide support to GC B-cells. Tfh are known to be inhibited by IL-2 which suppresses expression of the canonical Tfh transcription factor BCL6. On the other hand, we initially identified Tregs on the basis of their expression of the IL-2 receptor alpha chain (CD25) and broadly speaking Treg survival and proliferation is dependent on IL-2 (Sakaguchi et al., J. Immnol, 1995). Since Tfr are a type of Treg but must also express BCL6 this puts them in a unique position of needing to balance the IL-2 dependent Treg identify with IL-2 inhibited BCL6 expression. While investigating this issue we have found a novel $CD25^-$ negative subpopulation within both murine and human $PD1^+CXCR5^+Foxp3^+$ Tfr cells. Downregulation of CD25 appears to be a marker of Tfr development with $CD25^+$Tfr forming initially before later formation of more highly differentiated $CD25^-$Tfr. During the response to protein vaccination $CD25^+$Tfr formation occurs earlier at around days 3-5 whereas $CD25^-$Tfr form later at around day 6-7 and coincide with peak germinal center formation. Microscopic analysis of Tfr in the spleens and draining lymph nodes of vaccinated mice reveals that while the majority of Tfr resident in the follicle and near the T-B border express CD25, almost all germinal center resident Tfr lack CD25 expression. Accordingly, detailed analysis of chemokine receptor and cell adhesion molecules demonstrates that, in keeping with their germinal enter localization, $CD25^-$Tfr express significantly increased levels of CXCR5, while reducing expression of molecules responsibly for maintain localization in the T-cell zones such as CCR7 and PSGL-1. Further characterization by flow cytometry and RNA-sequencing shows that while $CD25^+$Tfr are more similar to effector Tregs, $CD25^-$Tfr have shifted their gene expression signature to a point equidistant between Tfh and effector Tregs displaying an extreme level of flexibility in their phenotype. Despite this they retain stable expression of Foxp3, maintain a characteristic Treg epigenetic signature and express key Treg suppressive molecules such as CTLA-4, allowing them to suppress both T-cells and B-cells during in vitro co-culture. We found that circulating $CD45RO^+$ Tfr in human blood also downregulate CD25, while in tonsils a fraction of $BCL6^{hi}CXCR5^{hi}Foxp3^+$ Tfr could be found that lacked CD25 expression. Surprisingly, a significant fraction of $CD45RA^+$ Tregs in human blood also express CXCR5 a point that we are now investigating in more detail.

We suggest that, similar to Tfh cells, Tfr cells follow a differentiation pathway generating a mature GC localized subpopulation, $CD25^-$Tfr cells. This represents a significant split from previously described BLIMP-1 dependent effector Tregs (eTregs). Tfr and eTregs are both highly activated but have distinct effector markers (PD1 and ICOS for Tfr), CD103 and KLRG1 for eTregs (Figure 1). From these findings we also infer that $CD25^+$Tfr located at the T-B border and $CD25^-$Tfr located in the germinal center may have different roles in the control of Tfh responses at each phase of the Tfh formation process. These findings can be exploited for effective control of vaccine-induced humoral immune responses and also treatment of autoantibody-dependent autoimmune diseases.

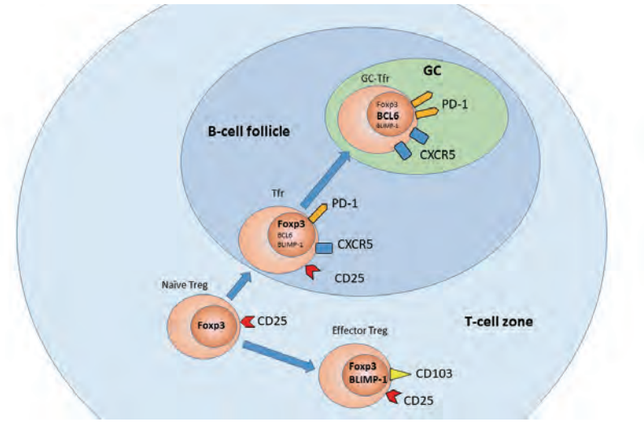

Figure.
Upon activation Naïve $CD25^+$ Tregs bifurcate into either IL-2 dependent BLIMP1 positive effector Tregs in the T-cell zone or non-lymphoid tissues or early follicular resident $CD25^+$Tfr. These can them downregulate CD25 expression causing the loss of BLIMP1 expression and higher level BCL6 and CXCR5 expression, allowing these $CD25^-$Tfr to travel to the germinal center. Adapted from Wing et al. Clin. Immunol. and Allergology. 2018.

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- Maeda Y, Nishikawa H, Sugiyama D, Ha D, Hamaguchi M, Saito T, Nishioka M, Wing JB, Adeegbe D, Katayama I, Sakaguchi S. Detection of self-reactive $CD8^+$ T cells with an anergic phenotype in healthy individuals. Science. 346, 1536-1540 (2014).
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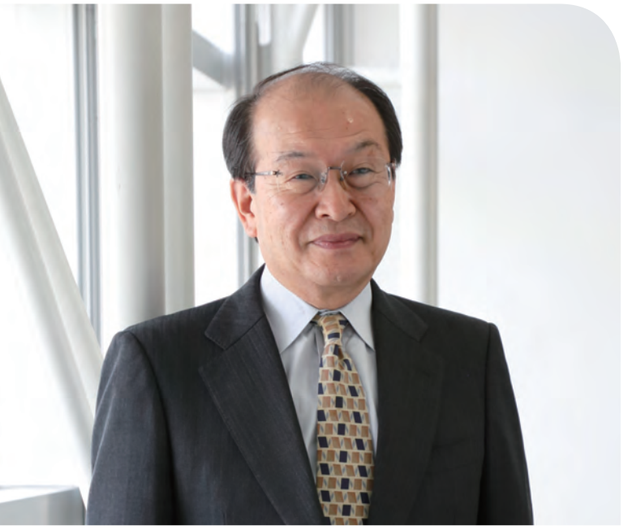
Cell Signaling



Takashi Saito, PhD

Professor	Takashi Saito

T cells play central roles in immune regulation. They initiate immune responses, induce activation and generate various effector T cells, which protect against infection and oncogenesis. Aberrancy of T cell function results in infectious and autoimmune diseases and cancer. Our laboratory aims to determine the molecular mechanism of T cell activation, differentiation and homeostasis, particularly from a signaling perspective.

T cell activation is induced through TCR-microclusters (MC), the signaling clusters generated by recruiting TCR and proximal signaling molecules. We have analyzed function and dynamics of TCR-MC and downstream signal molecules such as a downstream adaptor molecule CIN85. Analysis of T cell-specific CIN85 deficient mice revealed that CIN85 functions in negatively regulating TCR signals by recruiting several negative regulators including Sts-2 phosphatase as a feedback regulation of activation.

We have also investigated the regulation of T cell activation by innate signals. We have already analyzed the function of TLRs and nucleic acid recognition in T cells. Since we found that STING, a major intracellular DNA sensor in innate immune cells, is highly expressed in T cells, the function of STING in T cell activation was analyzed. STING activation in T cells induced growth arrest and production of type I-interferon (IFN), in mTORC1-dependent manner. The level of type I-IFN produced in effector T cells was much higher than innate immune cells. Together with our observation that STING in T cells contributes to anti-tumor immune response, STING activation in T cells can be utilized for modulating proliferation of T cells as well as anti-tumor immunity (Figure).

One of the final aims of our diverse approaches is to elucidate the mechanisms of inducing autoimmune diseases through aberrant T cell function in order to be able to modulate them and inhibit/prevent autoimmunity and allergic inflammation. We have analyzed the regulation of T cell function by phosphatases (PTPN22, PTPN2), whose deficiency is related to the induction of autoimmune diseases. We generated and analyzed T cell-specific deficient mice of these phosphatases, and the function of these phosphatases in T cell activation by analyzing associated proteins and imaging analysis. The onset of autoimmunity will be eventually analyzed in these mouse models.

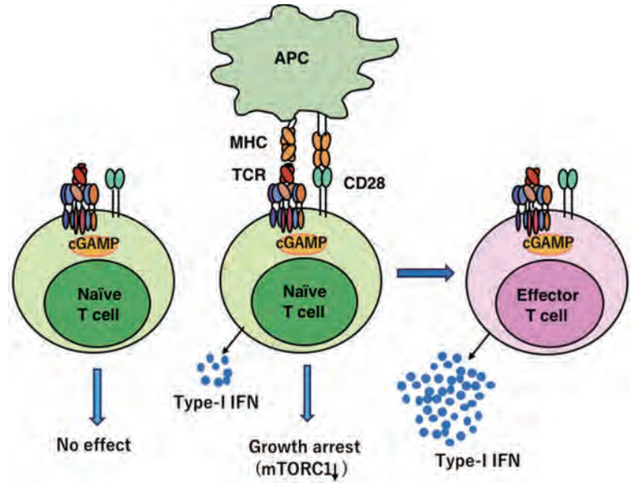


Figure. STING stimulation induces growth arrest of and type I-IFN production in T cells.
Stimulation of T cells through TCR (antigen) and STING (STING ligand such as cGAMP) induces growth arrest of T cells by inhibiting mTORC1 and simultaneously produces type-I IFN from T cells. Effector T cells such as Th1 cells and activated CD8 T cells produce high level of type-I IFN, which may contribute to anti-tumor immunity and protection from viral infection.

Recent Publications

- Hayashi M. et al. Advax, a Delta Inulin Microparticle, Potentiates In-built Adjuvant Property of Co-administered Vaccines. EBioMedicine 15, 127-136 (2017).
- Hashimoto-Tane A, Yokosuka T & Saito T. Analyzing the Dynamics of Signaling Microclusters. Methods Mol. Biol. 1584, 51-64 (2017).
- Takeuchi A & Saito T. CD4 CTL, a Cytotoxic Subset of CD4+ T Cells, Their Differentiation and Function. Front. in Immunol. 8,194 (2017).
- Hashimoto-Tane A. et al. Micro adhesion-ring surrounding TCR microclusters are essential for T cell activation. J. Exp. Med. 213, 1609-1625 (2016).
- Takeuchi A. et al. CRTAM determines the CD4+ cytotoxic T lymphocyte lineage. J. Exp. Med. 213, 123-138 (2016).

Lymphocyte Differentiation

Tomohiro Kurosaki, MD/PhD

Professor	**Tomohiro Kurosaki**
Associate Professor	**Wataru Ise**
	Takeshi Inoue
Assistant Professor	**Ryo Shinnakasu**
Research Assistant	**2**
Support Staff	**3**

The epigenetic modifications, such as chromatin modification and DNA metylation, influence the transmission of genetic information and regulate cellular differentiation and proliferation.

Action mechanisms of DNA demethylases, Tet2 and Tet3, on B cell tolerance

As B cell intrinsic tolerance mechanisms, it is known that even modest alterations in B cell signaling thresholds can break tolerance, promoting autoimmunity in the appropriate environmental settings. In addition to the B cell signaling components, the contribution of epigenetic factors has been proposed. One of the previously observed epigenetic abnormalities associated with autoimmune diseases is altered DNA metylation, prompting us to look for the roles of Tet proteins in B cell tolerance. Ten-eleven translocation proteins (Tet1, Tet2, and Tet3) were recently discovered to act as DNA demetylase by catalyzing the conversion of 5-methylcytosine (5mC) in DNA to 5-hydroxymethylcytosine (5hmc) and other intermediate products in the DNA metylation pathway.

We found that specific deletion of both Tet2 and Tet3 in B cells (using CD19-Cre) resulted in spontaneous hyper T cell activity, autoantibody production, and lupus nephritis. Treatment of anti-CD20 or anti-CD4 depletion antibodies ameliorated aberrant activation of $CD4^+$ T cells or B cells, respectively, suggesting that a positive feed-forward loop between activated B and T cells is operating. Mechanistically, by employing the hen egg lysozyme Ig transgene model system, we demonstrated that self-tolerant B cells express low levels of CD86 (B7.2), whereas ablation of Tet2/Tet3 rendered upregulation of CD86, thereby switching the B cell fate from peripheral tolerance to proliferation through T-B interaction. We also found that Tet2 and Tet3 recruited HDAC2, thereby repressing transcription of CD86 in self-tolerant B cells. Together, our data demonstrate that Tet2/Tet3 contributes to peripheral B cell tolerance by suppressing CD86 expression in a chromatin-modified manner.

Roles of DNA demetylases, Tet2 and Tet3, in foreign-antigen induced immune responses

As mentioned above, chronic depletion of Tet2/Tet3 in B cells resulted in breaking B cell tolerance and the resultant T cell hyper-activation. Therefore, to address the function of Tet2 and Tet3 in immune responses, we acutely deleted Tet2/Tet3 in B cells. We found that initial proliferation took place normally even in the absence of Tet2 and Tet3, whereas the deficient B cells could not differentiate into germinal center (GC) B cells or plasma cells. Because a transcription factor Bcl6 or IRF4 is known to be critical for GC B cell or plasma cell differentiation, respectively, our data demonstrate the importance of Tet2/Tet3 in induction of Bcl6 and IRF4. We are now analyzing how Tet2/Tet3 regulates transcription of Bcl6 and IRF4.

Roles of a Src-family regulator Csk in B cell development and function

The Src family kinases (SFKs) play key roles in regulating signal transduction by a diverse set of surface receptors, and their activation is negatively regulated by C-terminal Src Kinase (Csk). However, the physiological significance of SFK regulation by Csk in vivo remains unclear. To define this, we generated and analyzed Csk kinase-dead knock-in mutant mice. Histological examination of multiple tissues revealed the smaller splenic white pulp in mutant mice, and flow cytometric analysis showed significant decrease of mature B cells, most notably marginal zone (MZ) B cells, in mutant spleen. In addition, the mutant mice showed impaired GC and memory B cell generation upon immunization with TD antigen. These results suggested that the regulation of SFK activity by Csk plays important roles in B cell development and function.

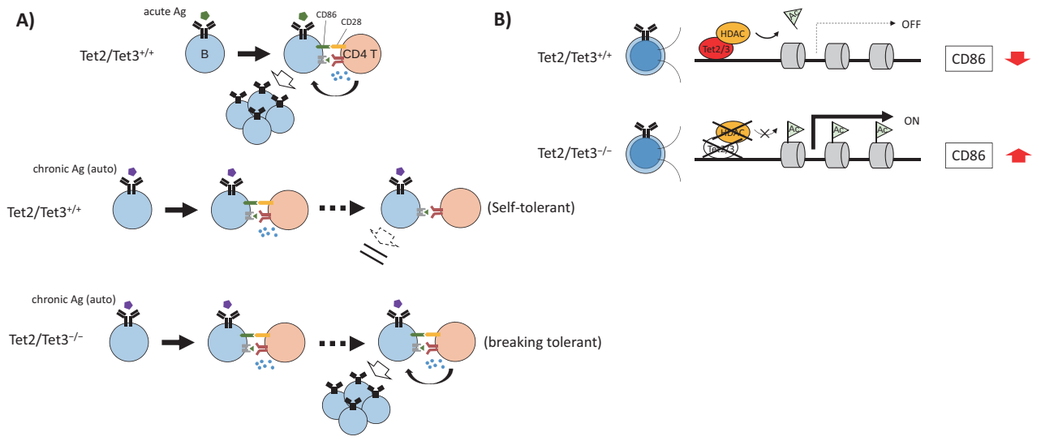


Figure. Tet2 and Tet3 contribute to peripheral tolerance of B cells.
(A)In the presence of Tet2 and Tet3, acute antigen stimulation induces a co-stimulatory molecule, CD86, which in turn, evokes cytokine production by T cells, thereby B cell proliferation (upper panel).
When this antigen stimulation continues like auto-antigen stimulation, such CD86 upregulation ceases. Therefore, T cell activation and subsequent B cell proliferation stop (self-tolerant state).
But in the absence of Tet2 and Tet3, such chronic antigen stimulation cannot downregulate CD86 expression, thereby changing the B cell fate from peripheral tolerance to proliferation.
(B)Tet2 and Tet3 recruit HDAC to the CD86 locus in the chronic state, thereby dampening transcription of CD86.

Recent Publications

- Inoue T, Moran I, Shinnakasu R, Phan TG, Kurosaki T. Generation of memory B cells and their reactivation. Immunol. Rev. 283(1):138-149. (2018).
- Ise W, Fujii K, Shiroguchi K, Ito A, Kometani K, Takeda K, Kawakami E, Yamashita K, Suzuki K, Okada T, Kurosaki T. T Follicular Helper Cell-Germinal Center B Cell Interaction Strength Regulates Entry into Plasma Cell or Recycling Germinal Center Cell Fate. Immunity. 48(4), 702-715.e4. (2018).
- Herndler-Brandstetter D, Ishigame H, Shinnakasu R, Plajer V, Stecher C, Zhao J, Lietzenmayer M, Kroehling L, Takumi A, Kometani K, Inoue T, Kluger Y, Kaech SM, Kurosaki T, Okada T, Flavell RA. KLRG1+ Effector CD8+ T Cells Lose KLRG1, Differentiate into All Memory T Cell Lineages, and Convey Enhanced Protective Immunity. Immunity. 48(4), 716-729.e8. (2018).
- Inoue T, Shinnakasu R, Ise W, Kawai C, Egawa T, Kurosaki T. The transcription factor Foxo1 controls germinal center B cell proliferation in response to T cell help. J. Exp. Med. 214(4), 1181-1198 (2017).
- Shinnakasu R, Inoue T, Kometani K, Moriyama S, Adachi Y, Nakayama M, Takahashi Y, Fukuyama H, Okada T, Kurosaki T. Regulated selection of germinal center cells into the memory B cell compartment. Nat. Immunol. 17(7), 861-869 (2016).

Malaria Immunology

Cevayir Coban, MD

Professor	Cevayir Coban
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Postdoctoral Fellow	**2**
Research Assistant	**1**
Visiting Scientist	**2**
Support Staff	**1**

Research in our laboratory focuses on the elucidation of the mechanisms involved in the host and *Plasmodium* parasites. Malaria is still an important disease and continues to kill half-a million people every year. Why and how malaria infection causes sudden and life-threatening complications such as cerebral malaria, has not been fully clarified and is extensively investigated in our lab. However, after an initial systemic infection, most malaria patients develop only partial immunity over the years and suffer from mild symptoms with low parasitemia. Hence, the 'chronic' illness caused by incomplete immunity to malaria may cause long-term 'hidden' pathologies such as Burkitt lymphoma, physical growth retardation in children and a detrimental effect on the bone tissue environment (Nature Reviews Immunology, 2018; International Immunology, 2018).

In the above mentioned context, we have investigated the pathology of cerebral malaria by using an experimental cerebral malaria model. Deep investigation of the brain by ultra-high field MRI showed that the olfactory bulb is physically and functionally damaged by *Plasmodium* parasites. Live multiphoton imaging of the olfactory bulb confirmed that the complex trabecular small capillaries comprising the olfactory bulb show parasite accumulation and cell occlusion followed by micro-bleeding, events associated with high fever and cytokine storm. With these findings, we provided evidence that olfactory functional impairment (loss of smell) could be a valuable early diagnosis marker for cerebral malaria (Cell Host Microbe, 2014).

There is increasing compelling evidence that malaria survivors experience long-term 'hidden' pathologies. Using well-established mouse models mimicking various aspects of human *Plasmodium* infection, we recently showed that infection causes significant and long term bone loss in adult mice, and growth retardation in young mice. Acute malaria infection severely suppresses bone homeostasis, but sustained accumulation of *Plasmodium* products in the bone marrow niche induces MyD88-dependent inflammatory responses in osteoclast and osteoblast precursors, leading to increased RANKL expression and overstimulation of osteoclastogenesis favoring bone resorption. Infection with a mutant parasite with impaired hemoglobin digestion that produces little hemozoin, a major *Plasmodium* by-product, did not cause bone loss. Importantly, supplementation of alfacalcidol, a vitamin D3 analog, could prevent the bone loss. These results highlight the risk of bone loss in malaria-infected patients and the potential benefits of coupling bone therapy with anti-malarial treatment (Figure 1, Science Immunology, 2017).

These recent studies from our lab have led us to hypothesize that systemic infection by *Plasmodium* parasites causes local, but tissue specific immunopathology during blood stage of infection, mainly via the manipulation of inter-tissue interactions between the blood and other host tissues that often result in dysfunction of certain organs (Nature Reviews Immunology, 2018). Understanding these pathologies might be critical for the development of novel adjunct therapies to be used in combination with current anti-malarials.

The final goal of our research is to translate our understanding of host-*Plasmodium* interactions into vaccines or drugs to treat malaria. Our strategy is to focus on the adjuvants, because if rationally designed, adjuvants improve vaccine efficacy. We have developed a new adjuvant called synthetic hemozoin, a synthetic analog of *Plasmodium*-produced hemozoin, and completed its preliminary GLP non-clinical safety and toxicology studies in several animals and infection models (Vaccine, 2016).

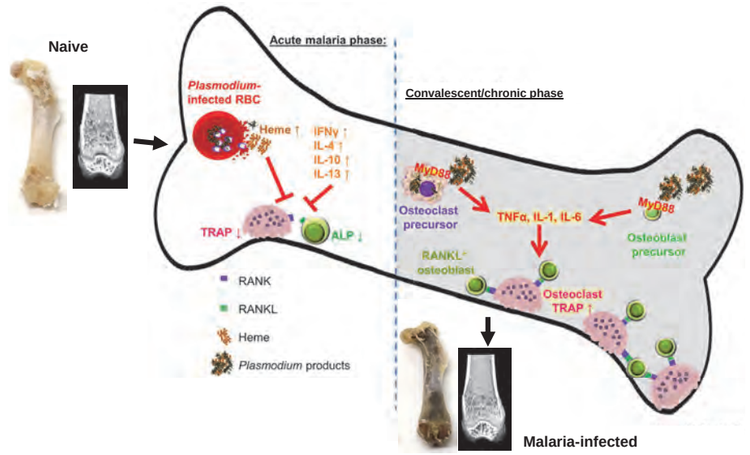

Figure. Malaria induces chronic bone loss due to sustained accumulation of *Plasmodium* products even after the cure of malaria disease (Science Immunology, 2017).
Although blood stage *Plasmodium* infection causes deadly complications, most people develop partial immunity and suffer from mild symptoms. Recent evidence suggests that the incomplete recovery from infection causes chronic illnesses such as bone loss or growth retardation.
During acute malaria infection, heme is one of the factors released abundantly due to hemolysis of infected and non-infected red blood cells and strongly inhibits both osteoclasts and osteoblasts. Cytokines such as IL-4, IL-10, IL-13 and IFNγ released from activated T cells in response to *Plasmodium* infection, disrupt the signaling in osteoclastogenesis. During convalescence and chronic phase of *Plasmodium* infection, although parasites are cleared systemically, parasite products remain and gradually accumulate abundantly in the bone marrow for long periods leading to MyD88-dependent chronic inflammation in immature and mature osteoclasts and osteoblasts. *Plasmodium* product-induced inflammatory responses upregulate RANKL expression on osteoblasts. The inflammatory cytokines (IL-1α, IL-1β, IL-6 and TNFα) and RANKL synergistically induce osteoclastogenesis. The robust activation of OCs after clearance of parasites skews bone remodeling toward bone resorption and eventually leads to bone loss.

Recent Publications

- Coban C, Lee MSJ, Ishii KJ. Tissue-specific immunopathology during malaria infection. Nat. Rev. Immunol., doi:10.1038/nri.2017.138 (2018).
- Lee MSJ, Maruyama K, Fujita Y, Konishi A, Lelliott PM, Itagaki S, Horii T, Lin JW, Khan SM, Kuroda E, Akira S, Ishii KJ, Coban C. *Plasmodium* products persist in the bone marrow and promote chronic bone loss. Sci. Immunol. 2(12), pii: eaam8093 (2017).
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Vaccine Science

Ken J. ISHII, MD/PhD

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Associate Professor	**Etsushi Kuroda**
	Norifumi Iijima
Assistant Professor	**Natsuko Kishishita**
Postdoctoral Fellow	**4**
Research Assistant	**4**
Visiting Scientist	**6**
Support Staff	**2**

The 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.

An Antigen-Free, Plasmacytoid Dendritic Cell-Targeting Immunotherapy To Bolster Memory CD8(+) T Cells in Nonhuman Primates

The priming, boosting, and restoration of memory cytotoxic CD8+ T lymphocytes by vaccination or immunotherapy in vivo is an area of active research. Particularly, nucleic acid-based compounds have attracted attention due to their ability to elicit strong Ag-specific CTL responses as a vaccine adjuvant. Nucleic acid-based compounds have been shown to act as anticancer monotherapeutic agents even without coadministration of cancer Ag(s); however, so far they have lacked efficacy in clinical trials. We recently developed a second-generation TLR9 agonist, a humanized CpG DNA (K3) complexed with schizophyllan (SPG), K3-SPG, a nonagonistic Dectin-1 ligand. K3-SPG was previously shown to act as a potent monoimmunotherapeutic agent against established tumors in mice in vivo. In this study we extend the monoimmunotherapeutic potential of K3-SPG to a nonhuman primate model. K3-SPG activated monkey plasmacytoid dendritic cells to produce both IFN-α and IL-12/23 p40 in vitro and in vivo. A single injection s.c. or i.v. with K3-SPG significantly increased the frequencies of activated memory CD8+ T cells in circulation, including Ag-specific memory CTLs, in cynomolgus macaques. This increase did not occur in macaques injected with free CpG K3 or polyinosinic-polycytidylic acid. Injection of 2 mg K3-SPG induced mild systemic inflammation, however, levels of proinflammatory serum cytokines and circulating neutrophil influx were lower than those induced by the same dose of polyinosinic-polycytidylic acid. Therefore, even in the absence of specific Ags, we show that K3-SPG has potent Ag-specific memory CTL response-boosting capabilities, highlighting its potential as a monoimmunotherapeutic agent for chronic infectious diseases and cancer.

Essential Role of CARD14 in Murine Experimental Psoriasis

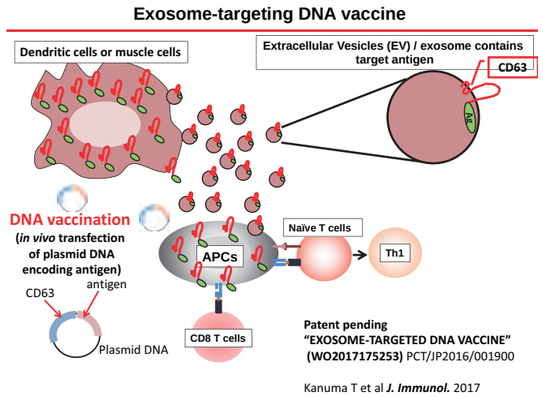
Caspase recruitment domain family member 14 (CARD14) was recently identified as a psoriasis-susceptibility gene, but its immunological role in the pathogenesis of psoriasis in vivo remains unclear. In this study, we examined the role of CARD14 in murine experimental models of psoriasis induced by either imiquimod (IMQ) cream or recombinant IL-23 injection. In all models tested, the psoriasiform skin inflammation was abrogated in Card14-/- mice. Comparison of the early gene signature of the skin between IMQ-cream-treated Card14-/- mice and Tlr7-/-Tlr9-/- mice revealed not only their similarity, but also distinct gene sets targeted by IL-23. Cell type-specific analysis of these mice identified skin Langerinhigh Langerhans cells as a potent producer of IL-23, which was dependent on both TLR7 and TLR9 but independent of CARD14, suggesting that CARD14 is acting downstream of IL-23, not TLR7 or TLR9. Instead, a bone marrow chimera study suggested that CARD14 in radio-sensitive hematopoietic cells was required for IMQ-induced psoriasiform skin inflammation, controlling the number of Vγ4+ T cells producing IL-17 or IL-22 infiltrating through the dermis to the inflamed epidermis. These data indicate that CARD14 is essential and a potential therapeutic target for psoriasis.

CD63-Mediated Antigen Delivery into Extracellular Vesicles via DNA Vaccination Results in Robust CD8+ T Cell Responses

DNA vaccines are attractive immunogens for priming humoral and cellular immune responses to the encoded Ag. However, their ability to induce Ag-specific CD8+ T cell responses requires improvement. Among the strategies for improving DNA vaccine immunogenicity are booster vaccinations, alternate vaccine formulations, electroporation, and genetic adjuvants, but a few, such as extracellular vesicles (EVs), target natural Ag delivery systems. By focusing on CD63, a tetraspanin protein expressed on various cellular membranes, including EVs, we examined whether a DNA vaccine encoding an Ag fused to CD63 delivered into EVs would improve vaccine immunogenicity. In vitro transfection with plasmid DNA encoding an OVA Ag fused to CD63 (pCD63-OVA) produced OVA-carrying EVs. Immunizations with the purified OVA-carrying EVs primed naive mice to induce OVA-specific CD4+ and CD8+ T cells, whereas immunization with EVs purified from cells transfected with control plasmids encoding OVA protein alone or a calnexin-OVA fusion protein delivered into the endoplasmic reticulum failed to do so. Vaccinating mice with pCD63-OVA induced potent Ag-specific T cell responses, particularly those from CD8+ T cells. CD63 delivery into EVs led to better CD8+ T cell responses than calnexin delivery into the endoplasmic reticulum. When we used a mouse tumor implantation model to evaluate pCD63-OVA as a therapeutic vaccine, the EV-delivered DNA vaccination significantly inhibited tumor growth compared with the control DNA vaccinations. These results indicate that EV Ag delivery via DNA vaccination offers a new strategy for eliciting strong CD8+ T cell responses to the encoded Ag, making it a potentially useful cancer vaccine.

Future prospect

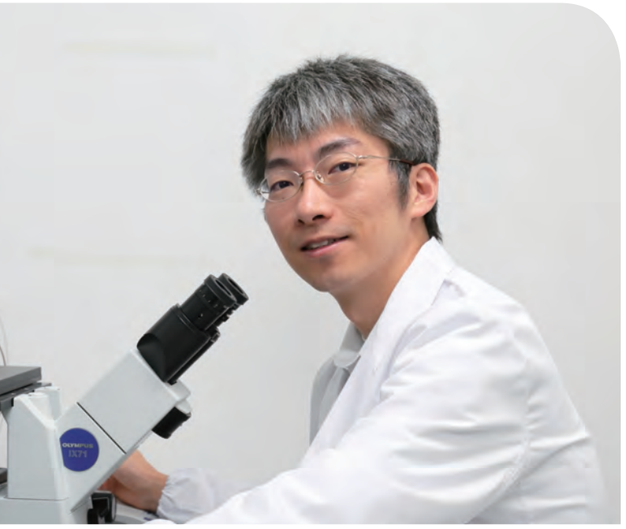
Vaccine target diseases are now not only restricted to a framework of infectious diseases but include a broad range of diseases such as cancer, allergy, Alzheimer's disease, and many other life-style-related diseases. We will continue innovative research and development vaccines against these diseases together closely with National Institute of Biomedical Innovation, Health and Nutrition (NIBIOHN) accompanying active exchanges of researchers.



Recent Publications

- Temizoz B, Kuroda E, Ishii KJ. Combination and inducible adjuvants targeting nucleic acid sensors. Curr Opin Pharmacol. 2018 Jun 2;41:104-113.
- Masuta Y, Yamamoto T, Natsume-Kitatani Y, Kanuma T, Moriishi E, Kobiyama K, Mizuguchi K, Yasutomi Y, Ishii KJ. An Antigen-Free, Plasmacytoid Dendritic Cell-Targeting Immunotherapy To Bolster Memory CD8(+) T Cells in Nonhuman Primates. J. Immunol. 200(6), 2067-2075 (2018).
- Tanaka M, Kobiyama K, Honda T, Uchio-Yamada K, Natsume-Kitatani Y, Mizuguchi K, Kabashima K, Ishii KJ. "Essential Role of CARD14 in Murine Experimental Psoriasis."J. Immunol. 200(1), 71-81 (2018).
- Kanuma T, Yamamoto T, Kobiyama K, Moriishi E, Masuta Y, Kusakabe T, Ozasa K, Kuroda E, Jounai N, Ishii KJ. CD63-Mediated Antigen Delivery into Extracellular Vesicles via DNA Vaccination Results in Robust CD8(+) T Cell Responses. J. Immunol. 198(12), 4707-4715 (2017).
- Hayashi M, Aoshi T, Haseda Y, Kobiyama K, Wijaya E, Nakatsu N, Igarashi Y, Standley DM, Yamada H, Honda-Okubo Y, Hara H, Saito T, Takai T, Coban C, Petrovsky N, Ishii KJ. Advax, a Delta Inulin Microparticle, Potentiates Inbuilt Adjuvant Property of Co-administered Vaccines. EBioMedicine. 15, 127-136 (2017).

Immunoparasitology



Masahiro Yamamoto, PhD

Professor	**Masahiro Yamamoto**
Associate Professor	**Miwa Sasai**
Assistant Professor	**Jisu Ma**
	Youngae Lee
Postdoctoral Fellow	**1**

Macroautophagy (herein, autophagy) is a fundamental eukaryotic cellular system to sustain homeostasis to degrade old/damaged cytoplasmic components or unwanted pathogens in double membranous autophagosome and autolysosome involving autophagy-related (Atg) proteins. Although yeast has only one Atg8, which is essential for autophagy, mammals harbor at least five Atg8 orthologues consisting of LC3 and Gabarap subfamilies: LC3a, LC3b, LC3C, Gabarap, Gabarapl1 and Gate-16 (also known as Gabarap2). Autophagic stimuli, such as nutrient starvation, increase LC3 lipidation in an Atg3/Atg5/Atg7/Atg16L1-dependent manner and induce accumulation of the lipidated LC3 on autophagosomal membrane, which is a widely accepted marker for autophagy activity assessment[7]. LC3 subfamily members play an important role in the elongation of phagophore membrane. In contrast, Gabarap subfamily members are required for autophagosomal maturation, suggesting that both LC3 and Gabarap Atg8 subfamilies differentially participate in autophagy. Furthermore, LC3C plays an important role in anti-bacterial autophagy (xenophagy) in human cells. Thus, conserved canonical roles of Atg8 in eukaryotic autophagy have been established so far.

Noncanonical functions of Atg8 have been recently reported. LC3 is detected on single membranous phagosome, whose process is known as LC3-associated phagocytosis (LAP) and is involved in autoimmunity and host defense against fungus and bacteria. Although LAP requires the LC3 conjugation machinery such as Atg5, Atg7 and Atg16L1, it does not require Atg14, another essential canonical autophagy regulator. In addition, maturation and functions of osteoclasts are associated with LC3. Although formation of the ruffled border by osteoclasts and their secretory functions in bone resorption requires Atg5, Atg7 and lapidated LC3, the maturation of osteoclasts is not associated with autophagic activity.

An unconventional Atg8 conjugation system is also reported in cell-autonomous host defense stimulated by interferons (IFNs). Many intracellular pathogens create pathogen-containing vacuoles (PCVs), which protect the microbe from sensing by cytoplasmic innate receptors and allows efficient proliferation. Against infection by vacuolar pathogens, the host immune system generates IFNs, which further stimulate production of immunity-related GTPases called IFN-inducible GTPases. These GTPases are accumulated on PCV membranes, leading to the PCV disruption, death of vacuolar pathogens and subsequent various inflammatory responses. The mechanism by which type II IFN (IFN-γ) stimulation induces recruitment of IFN-inducible GTPases to PCV of *Toxoplasma gondii*, an obligatory protozoan pathogen that causes life-threatening toxoplasmosis in human and animals, has been extensively studied and shown to require Atg3, Atg5, Atg7 and Atg16L1 but not Atg9a, Atg14 and ULK1/2, indicating the unconventional usage of the Atg8 conjugation system other than canonical autophagy. In particular, *T. gondii* PCV is highly decorated with LC3 as well as IFN-inducible GTPases[26], suggesting the major function. However, physiological relevance of the Atg8 conjugation system and the biological significance of LC3 in the IFN-γ-induced response are not fully understood.

Here we have analyzed mice lacking individual Atg8 family members and found that, although LC3 subfamily members are unexpectedly dispensable, Gate-16 uniquely and critically participates in IFN-inducible GTPase-dependent host defense against vacuolar pathogens. The mechanism of action of Gate-16 involves Arf1 activation and distribution of IFN-γ-induced GTPases throughout the cytosol and subsequently to the vacuole containing the intracellular pathogen.

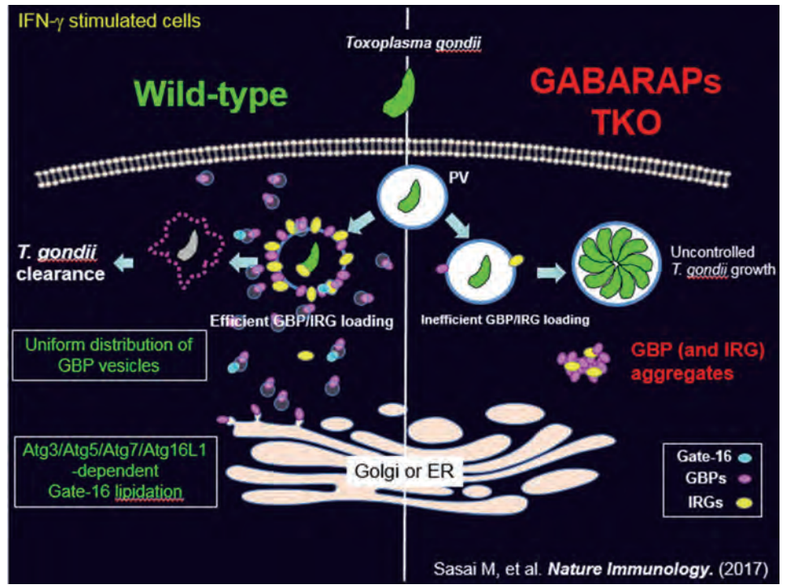
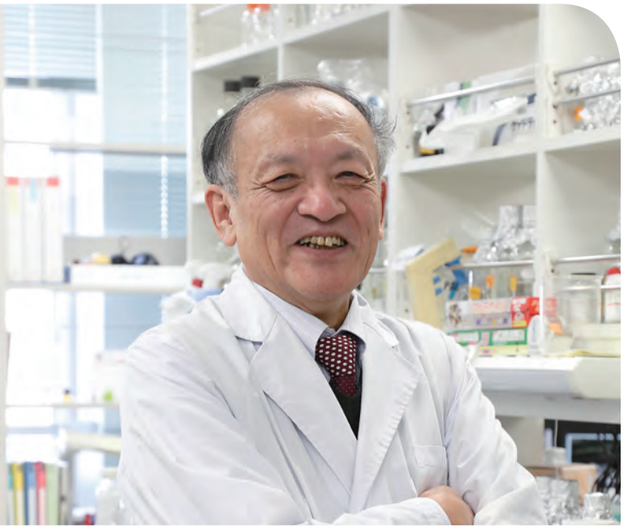


Figure.
GABARAP subfamily of mammalian ATG8 plays a critical role in IFN-γ-induced cell-intrinsic immunity.

Recent Publications

- Sasai M, Sakaguchi N, Ma JS, Nakamura S, Kawabata T, Bando H, Lee Y, Saitoh T, Akira S, Iwasaki A, Standley DM, Yoshimori T, Yamamoto M. Essential role for GABARAP autophagy proteins in interferon-inducible GTPase-mediated host defense. Nat. Immunol. 18, 899-910 (2017).

Biochemistry and Immunology



Shigekazu Nagata, PhD

Professor	Shigekazu Nagata
Associate Professor	**Katsumori Segawa**
Postdoctoral Fellow	**4**
Research Assistant	**3**
Visiting Scientist	**2**
Support Staff	**1**

Phospholipids in plasma membranes are asymmetrically distributed between inner and outer leaflets. Phosphatidylserine (PtdSer), one of most abundant phospholipids in eukaryotic plasma membranes, is exclusively localized in the inner leaflet. This asymmetrical distribution of phospholipids is maintained by an ATP-dependent phospholipid flippases, that translocates PtdSer and phosphatidylethanolamine from outer to inner leaflets. When cells undergo apoptosis, or platelets are activated, the asymmetrical distribution of phospholipids is disrupted by a scramblase(s) that non-specifically scramble phospholipids between the inner and outer leaflets of plasma membranes, leading to PtdSer-exposure. The PtdSer, thus exposed to the cell surface, works as an "eat me" signal for apoptotic cells, and as a scaffold on the activated platelets for blood clotting factor. The PtdSer-exposure is also observed in activated lymphocytes, capacitated sperm, aged erythrocytes, exosomes, and enveloped virus.

We identified three P4-type ATPases (ATP8A2, ATP11A and ATP11C) and their subunit CDC50A as flippases that actively translocate PtdSer from outer to inner leaflets of the plasma membrane. Among these three flippases, ATP8A2 is specifically expressed in brains, while ATP11A and ATP11C are ubiquitously expressed in various cells including lymphocytes and hepatocytes. ATP11A and ATP11C contain two or three caspase-recognition sites in the middle of molecules, and their flippase activity is destroyed during apoptosis. The CDC50A works as a chaperone for ATP11A and ATP11C to escort them from ER to plasma membranes, and is required for their flippase activity at plasma membranes.

There are two families of membrane proteins that support non-specific scrambling of phospholipids at plasma membranes. Five members (TMEM16C, 16D, 16F, 16G and 16J) of the TMEM16 family, that contain 10 transmembrane segments, function as Ca^{2+}-dependent scramblases (Figure 1). In a microarray system of membrane bilayers in which phospholipids are asymmetrically localized, we recently showed that a single dimeric molecule of TMEM16F can scramble phospholipids between two membrane bilayers.

TMEM16F is ubiquitously expressed in various cells, while other members are specifically expressed in the brains or intestines. Human and mouse platelets express only TMEM16F as a Ca^{2+}-dependent scramblase. The TMEM16F-/- activated platelets cannot expose PtdSer, leading to the reduced ability to produce thrombin for blood clotting. In fact, human patients of Scott syndrome, a rare congenital bleeding disorder, were found to carry a loss of function mutation in TMEM16F.

Three members (Xkr4, Xkr8 and Xkr9) of the Xkr family that are supposed to carry 10 transmembrane segments support scrambling phospholipids during apoptosis. These members contain a caspase-recognition site in the C-terminal tail region, and are cleaved by caspase during apoptosis. Thus, when cells undergo apoptosis, caspase cleaves and irreversibly inactivates ATP11A and ATP11C flippases, and cleaves and activates Xkr4, Xkr6, and Xkr9 scramblases, to quickly and irreversibly expose PtdSer (Figure 2).

The PtdSer on the dead cell's surface is recognized by macrophages for engulfment. Xkr4 and Xkr9 are rather tissue-specifically expressed in the brains and intestines, respectively. Whereas, Xkr8 is ubiquitously expressed in various tissues. Mouse thymocytes, lymphocytes, and neutrophils express only Xkr8, and when they undergo apoptosis, they expose PtdSer in an Xkr8-dependent manner. Thus, Xkr8-null thymocytes and neutrophils are not efficiently engulfed by macrophages. The Xkr8-null female mice develop systemic lupus erythematosus-type autoimmune disease. It is possible that unengulfed dead cells undergo secondary necrosis, and intracellular materials released from dead cells activate the immune system.

We are studying the molecular mechanism of how flippases and scramblases flip and scramble phospholipids at plasma membranes, and also the physiological and pathological roles of the flippases and scramblases.

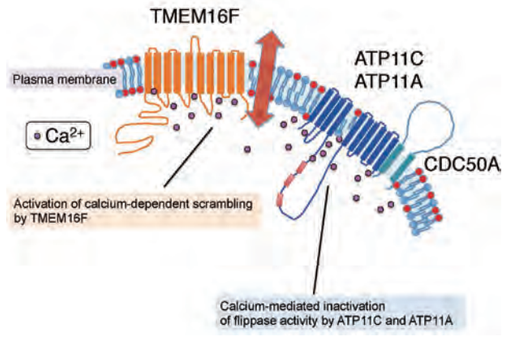


Figure 1. The molecular mechanism for PtdSer exposure in cells with high Ca^{2+}-concentration.
A flippase comprised of P4-ATPase (ATP11A or ATP11C) and CDC50A, and a Ca^{2+}-dependent scramblase (TMEM16F) is schematically shown. In activated platelets, the intracellular Ca^{2+} concentration increases and activates TMEM16F to scramble phospholipids, while it inactivates P4-ATPases and reduces their flipping activity. When the Ca^{2+} concentration returns to normal level, TMEM16F stops scrambling phospholipids, while P4-ATPases resume flipping PtdSer and PtdEtn. Thus, PtdSer is only transiently exposed to the cell surface in this process, and likely depends on the intracellular concentration of ATP and Ca^{2+}. The constant flipping of PtdSer prevents the PtdSer-exposing cells from being engulfed by macrophages.

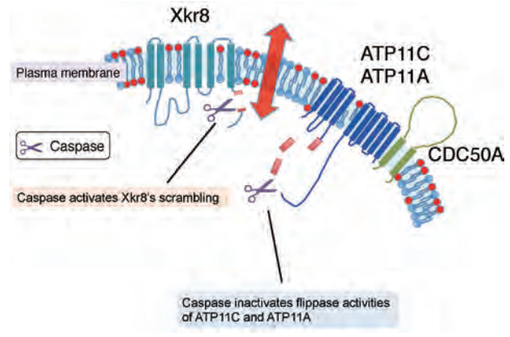


Figure 2. The PtdSer-exposure in apoptotic cells.
A caspase-dependent phospholipid scramblase of Xkr8, and flippase (ATP11A/ATP11C associated with CDC50A) are schematically shown. When cells undergo apoptosis, caspase 3 or caspase 7 in the downstream of the caspase-cascade cleaves Xkr8 to activate its scramblase activity, while the same caspases cleave and inactivate ATP11A and ATP11C. This is an irreversible process, and the PtdSer exposed on the cell surface is recognized by macrophages for engulfment.

Recent Publications

- Nagata S. Apoptosis and clearance of apoptotic cells. Annu. Rev. Immunol. 36, 489-517 (2018).
- Watanabe R, Sakuragi T, Noji H and Nagata S. Single-molecule analysis of phospholipid scrambling by TMEM16F. Proc. Natl. Acad. Sci. USA 115, 3066-3071 (2018).
- Kawano M and Nagata S. Lupus-like autoimmune disease caused by a lack of Xkr8, a caspase-dependent phospholipid scramblase. Proc. Natl. Acad. Sci. USA 115, 2132-2137 (2018).
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Molecular Neuroscience

Toshihide Yamashita, MD/PhD

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Associate Professor	**Rieko Muramatsu**
Assistant Professor	**Yuki Fujita**
	Shogo Tanabe
	Hiroshi Tsujioka
	Ken Matoba
Postdoctoral Fellow	**3**
Research Assistant	**3**
Support Staff	**4**

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 control mechanism exerted by the associations among the nervous system, immune system, and vascular system. Additionally, we aim to elucidate the principles involved in the operation 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 perceive the central nervous system as a single organ within a biological system; further, studies from the perspective of the involvement of the entire biological system in disorders and recovery of neural networks are scarce. By perceiving disorders in the 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 create a new and never-seen-before trend in Life Sciences.

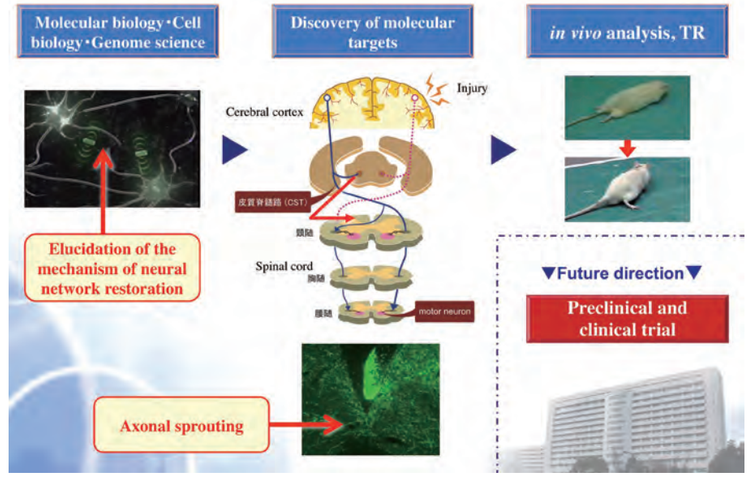

Figure 1.
The mechanism of spontaneous functional recovery.

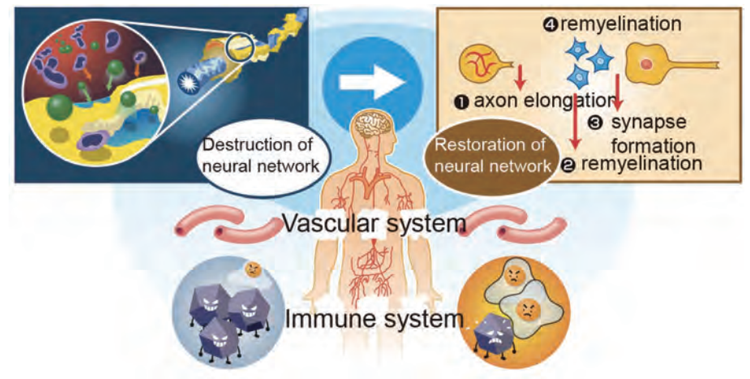

Figure 2.
Biological systems that regulate rewiring of neural network after CNS injury.

Recent Publications

- Tanabe S & Yamashita T. B-1a lymphocytes promote oligodendrogenesis during brain development. Nat. Neurosci. (2018) in press.
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Molecular Immunology

Sho Yamasaki, PhD

Professor	**Sho Yamasaki**
Assistant Professor	**Eri Ishikawa**
	Chihiro Motozono
Research Assistant	**1**
Support Staff	**2**

Our bodies are continuously exposed to external and internal insults caused by infections and stresses, most of which are primarily sensed by immune receptors to maintain tissue homeostasis. However, the molecular mechanism by which these receptors discriminate diverse insults to elicit suitable immune responses remains elusive. To illustrate the molecular basis behind this regulation, our laboratory is focusing on the following areas; 1) immune sensing of insults via C-type lectin receptors, 2) T cell responses induced by self-peptides, and 3) innate T cell subsets related to autoimmune diseases.

Immune sensing of pathogens and damaged-self via C-type lectin receptors

C-type lectin receptors (CLRs) comprise a large family of proteins that share a common structural motif and are involved in various immune responses. Among them, ITAM-coupled CLRs are recently identified as pattern recognition receptors (PRRs) for pathogens. We found that macrophage-inducible C-type lectin (Mincle/Clec4e) is an FcRγ-coupled activating receptor for *Mycobacterium tuberculosis*. A widely-known mycobacterial adjuvant, cord factor (also called trehalose-6,6′-dimycolate, TDM), was identified as a Mincle ligand. Another CLR, MCL (Clec4d), also recognizes mycobacterial cord factor and promotes Mincle expression. Indeed, innate and acquired immune responses induced by TDM were abrogated in both Mincle- and MCL-deficient mice. In addition, we found that another CLR Dectin-2 (Clec4n) also recognizes mycobacteria through a hydrophilic lipoglycan, lipoalabinomannan (LAM). This interaction triggered unique adjuvant activities that are distinct from those induced by TDM-Mincle/MCL axis. Furthermore, DCAR (Clec4b1), a CLR located in the same gene cluster, was identified as a receptor for phosphatidylinositol mannosides of mycobacteria. DCAR was selectively expressed in monocyte-derived inflammatory cells and triggered Th1 protective immunity against mycobacteria. Thus, these four CLRs clustered on the same chromosome are found to be activating receptors for mycobacteria (Figure 1). In addition to mycobacteria, Mincle also recognized pathogenic fungi and gram-negative bacteria that lack TDM. Instead of TDM, several novel glycolipids, glycosylglycerolipids and glycosyldiacylglycerols were identified as Mincle ligands from these pathogens. These ligands possessed adjuvant activity in mice, as the injection of the purified ligands augmented T cell responses. These findings shed light on CLRs as an emerging immune receptor family for wide spectrum of pathogens, and thus CLRs could be potential targets for the development of a novel adjuvant.

Sensing tissue damage is a crucial function of pattern recognition receptors (PRRs). However, endogenous ligand recognition by PRRs is not well documented. We have previously reported that Mincle recognizes damaged cells. To elucidate endogenous ligands derived from damaged cells, we fractionated supernatants from damaged cells and identified β-glucosylceramide (GlcCer). β-GlcCer induced inflammatory and acquired immune responses via Mincle on myeloid cells. Accumulation of β-GlcCer leads to Gaucher disease, a disorder characterized mainly by systemic inflammation. In a Gaucher model in which mice are deficient in the β-GlcCer–degrading enzyme, further deletion of the Mincle gene attenuated inflammatory responses. These results suggest that β-GlcCer is an endogenous Mincle ligand and acts as an immunostimulatory factor upon cell damage (Figure 2).

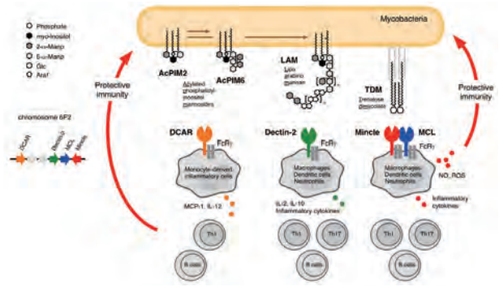

Figure 1.
Protective immunity against mycobacteria initiated through C-type lectin receptors. Mycobacterial components initiate host immune responses through CLRs. CLRs trigger signal cascades via immune-receptor tyrosine-based activation motifs (ITAMs) by directly recognizing multivalent glycolipids.

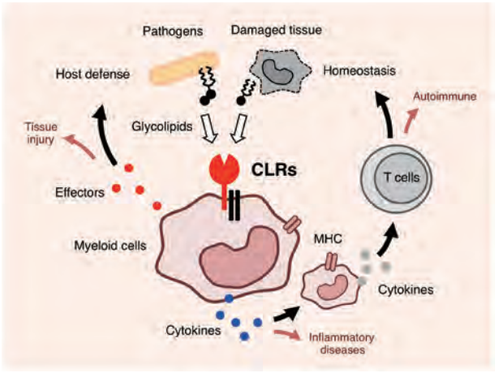

Figure 2.
Sensing pathogens and damaged tissue by CLRs. Mincle and related CLRs expressed on myeloid cells can sense ligands come from pathogens and damaged self. Myeloid cells are activated upon ligand binding, leading to the secretion of cytokines as well as antigen presentation by MHC class II, and consequently activate adaptive immunity. On the other hand, activated myeloid cells could also secrete some effectors. Through these responses, Mincle functions in host defense and dead tissue clearance. These procedures are precisely controlled in a healthy immune system; however, the overreaction of any step may induce harmful responses to our body, generating disorders such as inflammatory diseases, autoimmune or tissue injury.

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- Yonekawa A, Saijo S, Hoshino Y, Miyake Y, Ishikawa E, Suzukawa M, Inoue H, Tanaka M, Yoneyama M, Oh-hora M, Akashi K, Yamasaki S. Dectin-2 is a direct receptor for mannose-capped lipoarabinomannan of mycobacteria. Immunity 41, 402-13 (2014).
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Statistical Immunology

Yukinori Okada, MD/PhD

Professor	**Yukinori Okada**
Research Assistant	1
Visiting Scientist	1
Support Staff	1

Goal of our laboratory

The genetic backgrounds of individuals have a substantial impact on the risk for 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. Recent developments in genome sequencing technologies have provided the human genome data of millions of subjects, and successfully identified comprehensive catalogues of genetic risk loci of immune-related diseases. However, little is known regarding how to develop methodology to integrate large-scale human genome data with diverse biological and immunological resources. The goal of our laboratory is to develop such methods and apply them to the latest large-scale disease genome and multi-layer omics data.

Identification of causal immune cells on human complex traits using trans-layer multi-omics analysis

We conducted large-scale genome-wide association studies (GWAS) of >160,000 Japanese subjects on 58 clinical measurements, including hematological measurements (e.g., counts of red blood cells, white blood cell subtypes, and platelets), biochemical measurements (blood sugar, serum lipids, liver functions, kidney functions, and electrolytes), and clinical tests (e.g., blood pressure and echocardiography). Our study identified 1,400 genetic variants associated with these phenotypes, of which half were novel findings. We further obtained additional GWAS results of 30 human diseases including immune-related diseases such as rheumatoid arthritis and Graves' disease. We conducted trans-layer omics analysis to integrate the GWAS results of human complex traits with the epigenetic signature of promoter and enhancer information from >200 cell types. We adopted the newly developed statistical method named "linkage disequilibrium score regression (LDSC)" which quantifies genetic correlation among the traits according to each of the cell types. Our study successfully elucidated a hidden network among complex human traits and cell types (Figure 1). For example, the most relevant human diseases to the regulatory T cells was Graves' disease and autoimmune thyroiditis. This finding should support the original feature of the regulatory T cells in mice that depletion causes autoimmune thyroiditis. Our *in silico* study successfully extracted the biological pathophysiology of human immune-related diseases by integrating genetic and epigenetic data (Kanai M et al. *Nat Genet*, 2018).

In silico drug repositioning based on the large-scale human disease genome study

Utilization of large-scale human disease genetic studies for novel drug discovery is one of the most featured topics in the pharmaceutical field. We previously developed a statistical method to screen novel candidate drugs for drug repositioning by connecting genetic information with drug databases (e.g., repositioning of CDK4/6 inhibitors for treatment of rheumatoid arthritis). Through international collaboration with trans-ethnic genetic studies on stroke comprising 520,000 individuals, we newly developed a method to additionally integrate the disease and drug classification curated by WHO Anatomical Therapeutic Chemical (ATC) Classification System. Application of genetic risk variants on stroke to the newly developed *in silico* drug repositioning pipeline successfully identified a strong network with antithrombotic therapy as the most strongly associated clinical indication (Figure 2). This result is considered as evidence that utilization of genetic risk variants should contribute to novel drug discovery (Rainer M et al. Nat Genet 2018).

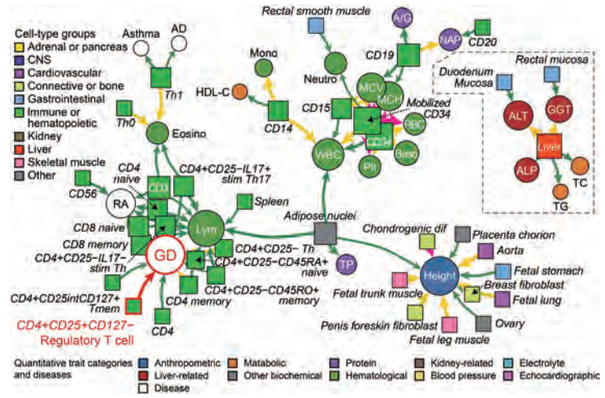

Figure 1.
Network among human complex traits and cell types identified by trans-layer omics analysis. The most relevant human disease with regulatory T cells was Graves' disease (GD; human autoimmune thyroiditis).

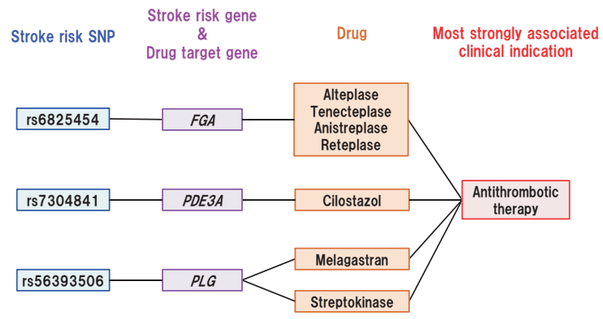

Figure 2.
Application of *in silico* drug repositioning method identified network between human genetic variants with stroke risk and drugs currently used for antithrombotic therapy.

Recent Publications

- Malik R. et al. Tyrosine kinases Multiancestry genome-wide association study of 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes. Nat. Genet. doi:10.1038/s41588-018-0058-3 (2018).
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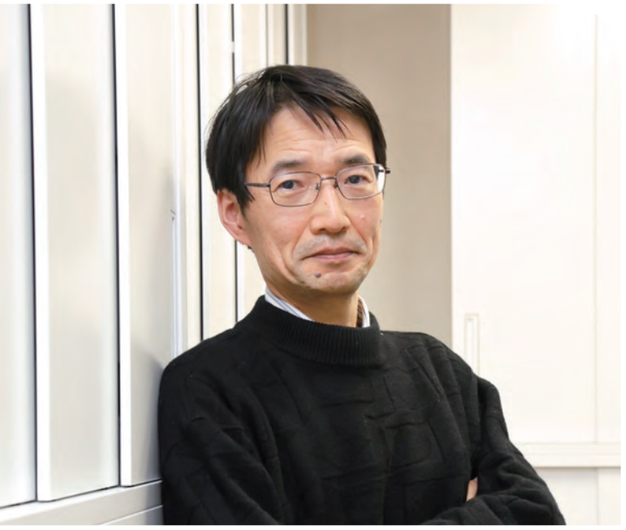
Stem Cell Biology and Developmental Immunology



Takashi Nagasawa, MD/PhD

Professor	**Takashi Nagasawa**
Associate Professor	**Tatsuki Sugiyama**
Assistant Professor	**Yoshiki Omatsu**
Support Staff	**1**

Hematopoietic stem cells (HSCs), which give rise to all blood cells, including immune cells, are maintained by special microenvironments known as niches 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 hematopoietic stem cells (HSCs)(Nagasawa et al. Nature 1996; Ara et al. Immunity 2003), maintenance of a pool of HSCs in bone marrow (Sugiyama et al. Immunity 2006), and development of immune cells, including B cells, plasmacytoid dendritic cells (pDCs) and NK cells (Nagasawa. Nat. Rev. Immunol. 2006) as well as vascular formation and cardiogenesis (Tachibana et al. Nature 1998). Subsequently, we identified a population of reticular cells expressing CXCL12 at high levels, termed CXCL12-abundant reticular (CAR) cells within bone marrow (Sugiyama et al. Immunity 2006) and indicated that CAR cells are adipo-osteogenic progenitors and the major producer of CXCL12 and SCF, creating the special microenvironment (niche) for HSCs and B cells (Omatsu at al. Immunity 2010). Furthermore, we found that the transcription factor Foxc1 was preferentially expressed in CAR cells in the marrow, enhancing CXCL12 and SCF expression and was essential for inhibiting adipogenic processes in CAR cell progenitors, and development and maintenance of niches for HSCs and immune cells (Omatsu et al. Nature 2014). We are studying the roles of CXCL12-CXCR4 signaling and CAR cells in the spatiotemporal regulation of lymphohematopoiesis during homeostasis and diseases.

Hematopoietic stem cell niche-specific Ebf3 maintains the bone marrow cavity

Bone marrow is the tissue filling the space between bone surfaces. CAR cells, which are a major cellular component of HSC niches, give rise to osteoblasts in bone marrow. However, it had remained unclear how osteogenesis is prevented in most CAR cells to maintain HSC niches and marrow cavities. Recently, we find that the transcription factor Ebf3 is preferentially expressed in CAR cells and that Ebf3-expressing cells are self-renewing mesenchymal stem cells, which give rise to all osteoblasts and adipocytes in adult bone marrow, using lineage-tracing. When Ebf3 was deleted in CAR cells, HSC-niche function was severely impaired and bone marrow was osteosclerotic with increased bone in aged mice (Figure 1). In mice lacking Ebf1 and Ebf3, CAR cells exhibiting a normal morphology were abundantly present but their niche function was markedly impaired with depleted HSCs in infant marrow. Subsequently, the mutants become progressively more osteosclerotic, leading to the complete occlusion of marrow cavities in early adulthood. CAR cells differentiate into bone-producing cells with reduced HSC-niche factor expression in the absence of Ebf1/Ebf3. Thus, HSC cellular niches express Ebf3 that is required to create HSC niches, to inhibit their osteoblast differentiation, and to maintain spaces for HSCs (Seike et al. Genes Dev. 2018).

Seike, M. *et al.*, *Genes Dev* 2018

Figure 1. A progression of occlusion of the marrow cavities in the absence of Ebf3
Femurs in aged Lepr-Cre; EBF3$^{f/f}$ mice revealed that the bone marrow spaces were filled with bone (Seike et al. Genes Dev. 2018).

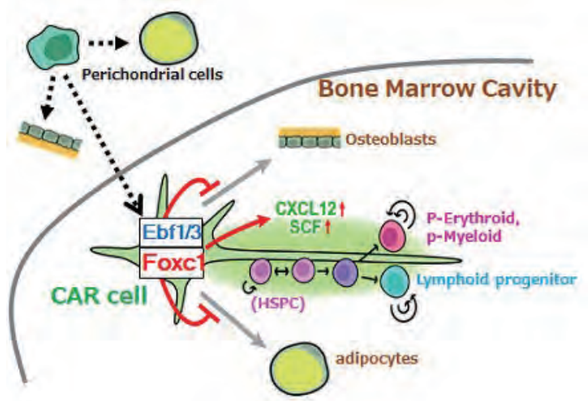


Figure 2. The development and functions of CAR cells.
The transcription factors Foxc1 and Ebf3, and HSC niche factors CXCL12 and SCF were preferentially and abundantly expressed in CAR cells within the bone marrow.

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- Shimba A, Cui G, Tani-Ichi S, Ogawa M, Abe S, Okazaki F, Kitano S, Miyachi H, Yamada H, Hara T, Yoshikai, Y, Nagasawa T, Schütz G, Ikuta K. Glucocorticoids Drive Diurnal Oscillations in T Cell Distribution and Responses by Inducing Interleukin-7 Receptor and CXCR4. Immunity 48(2), 286-298 (2018).
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Single Molecule Imaging (Yanagida – Seymour Group)

Toshio Yanagida, PhD
Ben Seymour, MD/PhD

Professor	**Toshio Yanagida Ben Seymour**
Visiting Scientist	1
Support Staff	1

Recent advances in imaging methodologies provide a unique opportunity to interrogate biological process at multiple levels. Our goal is to integrate imaging methods across levels and species, with new intelligent approaches to data analysis, to provide translational insight into the multi-system interactions in the body that support physiological and pathophysiological functions.

A particular interest of our lab is pain and fatigue, which are the two dominant symptoms of immune diseases such as inflammatory arthritis. Their relative resistance to treatment by disease modifying drugs such as immunologics represents a primary limitation to the clinical efficacy of these drugs. Our research aims to identify the adaptive changes that occur in the brain that allow these symptoms to develop and persist after peripheral inflammation, using brain imaging in rodent, human pre-clinical and clinical models of pain and inflammation. In particular, we combine data-driven and hypothesis-driven analyses to understand the complex brain changes in inflammation. Data-driven methods utilize machine learning and deep learning tools to interrogate classifiable changes in complex brain networks, and the use of graph theory and other network topological analyses to characterize differences between brain states. Hypothesis-driven analyses use computational models of how behavior changes after peripheral injury, to probe the normative basis for illness behavior ('cognitive neuroimmunology').

We know from our previous research that inducing changes in the function of the sensorimotor cortex can modulate pain following nerve injury in human patients [5], but we haven't known if significant sensorimotor reorganization actually occurs in the brain after injury. Now, we have been able to identify and characterize substantial reorganization of the sensorimotor cortices in chronic musculoskeletal back pain, using a new method that identifies changes in the brain modular architecture [1]. In particular, these changes correlate with activity in a specific brain region - the pregenual anterior cingulate cortex (pgACC). This is significant as in another study, using a model of heat injury in healthy humans, we have shown that pgACC modulates pain when people try to engage in behavior to protect potentially inflamed skin: specifically, it appears to modulate incoming sensory signals, and control outgoing autonomic signals, to optimize behavioral responses [2]. The reason this is significant is because it provides a mechanistic link between the modulation of pain and the injured state, reflecting a process that could in principle cause pain to develop in the transition from acute to chronic inflammation. We have now started a large translational project to address this in inflammatory arthritis - in both humans (rheumatoid arthritis) and rodent models (CFA joint injection) [5], using combined molecular and brain imaging methods. In summary, this research suggests that pgACC may represent a new target to understand how pain and fatigue develop in chronic inflammatory disease, potentially providing a way to predict symptomatic response to treatment, and innovate new treatments that could modulate pain and fatigue as an adjunct to immune modulators.

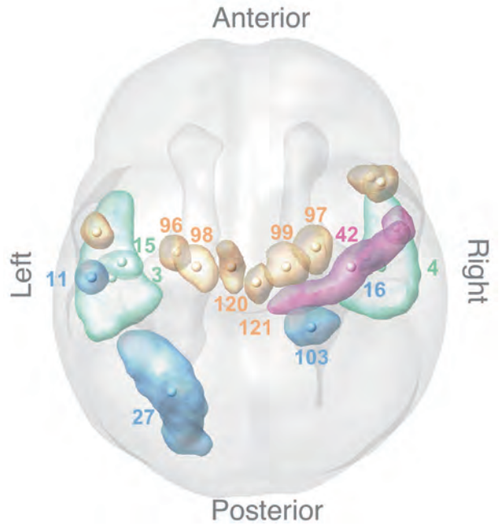

Figure 1.
Sensorimotor brain regions showing modular reorganization in chronic musculoskeletal back pain.

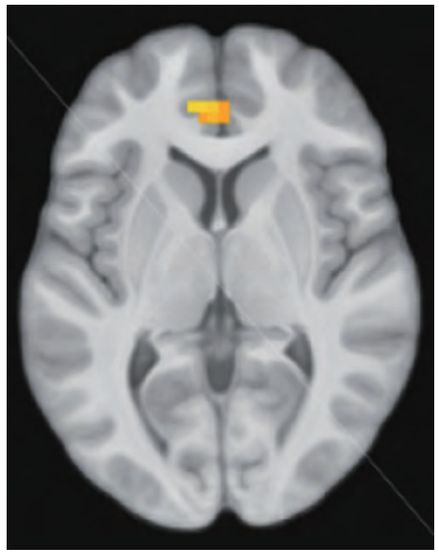

Figure 2.
Localization of pregenual anterior cingulate cortex (pgACC), which supports behavioral 'resilience' after injury.

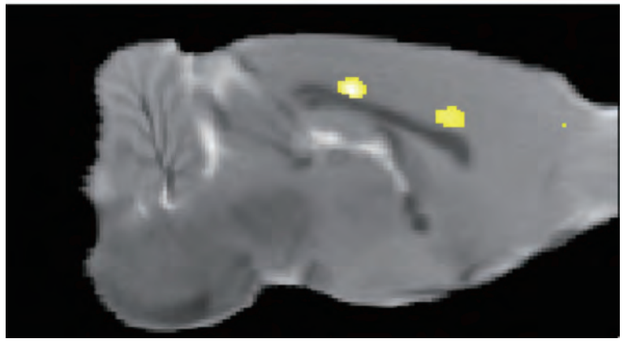

Figure 3.
Structural images showing brain changes in anterior cingulate cortex after joint inflammation in rodents.

Recent Publications

- Mano H, Kotecha G, Leibnitz K, Matsubara T, Nakae A, Shenker N, Shibata M, Voon, Yoshida W, Lee M, Yanagida T, Kawato M, Rosa M, Seymour B. Classification and characterization of brain network changes in chronic back pain: A multicenter study. Wellcome Open Research 3:19 (2018).
- Zhang S, Mano H, Lee M, Yoshida W, Robbins T, Kawato M, Seymour B. The Control of Tonic Pain by Active Relief Learning. eLife 2018;7:e31949 (2018).
- Morris L, Sprenger C, Koda K, de la Mora M, Yamada T, Mano H, Kashiwagi Y, Yoshioka Y, Morioka Y, Seymour B. Anterior cingulate cortex connectivity is associated with suppression of behaviour in a rat model of chronic pain. Brain and Neuroscience Advances (2018) in press.
- Fujita K, Iwaki M, Yanagida T. Transcriptional bursting is intrinsically caused by interplay between RNA polymerases on DNA. Nat. Commun. 7, 13788 (2016).
- Yanagisawa T, Fukuma R, Seymour B, Hosomi K, Kishima H, Shimizu T, Yokoi H, Hirata M, Yoshimine T, Kamitani Y, & Saitoh Y. Induced sensorimotor brain plasticity controls pain in phantom limb patients. Nat. Commun. 7, 13209 (2016).

Immunology and Cell Biology

Masaru Ishii, MD/PhD

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Assistant Professor	**Junichi Kikuta**
	Hiroki Mizuno
	Takao Sudo
	Takahiro Matsui
	Szandor Simmons
	Yutaka Uchida
Research Assistant	**2**
Visiting Scientist	**1**
Support Staff	**2**

The major aim of our laboratory is to understand the fundamental principle controlling cellular dynamics in various kinds of tissues and organs in vivo, by means of advanced imaging techniques. Recent advances in optical imaging technology have enabled us to visualize the dynamic nature of different cell types in a spatiotemporal manner (see the Figure).

Intravital bone imaging revealing bone cell dynamics in vivo

By using intravital multiphoton microscopy, we have originally elaborated the novel imaging system for visualizing inside the bones. We previously succeeded in visualizing the function of fully differentiated osteoclasts adhering to bone surfaces in vivo (J Clin Invest 2013). This novel visualization identified two distinct mature osteoclast functional states; i.e., bone-resorbing (R) osteoclasts firmly adhering to bones and devouring the bone matrix by secreting acids, and non-resorbing (N) osteoclasts relatively loosely attached and wriggling along the bone surface. In order to further analyze the actual event of bone resorption in vivo, in collaboration with Dr. Kikuchi in the frame of IFReC, we have developed a new chemical probe for detecting proton secretion in bone resorption by osteoclasts (Nat Chem Biol, 2016).

Furthermore, we could detect an in vivo mode of dynamic communication between mature osteoblasts (mOBs) and mature osteoclasts (mOCs), and found that the mOBs and mOCs were distributed mainly in a segregated fashion, although some direct cell-to-cell contact was detected between mOBs and mOCs in spatiotemporally limited areas. A pH-sensing fluorescence probe we developed revealed that mOCs secreted protons for bone resorption when they were not in contact with mOBs, whereas mOCs contacting mOBs were non-resorptive, suggesting that mOBs could inhibit the bone resorption activity of mOCs by direct cell–cell contact. This study is the first to use intravital imaging techniques to reveal spatiotemporal intercellular interactions between mOBs and mOCs, thus contributing to our understanding of bone homeostasis in vivo (Nat Commun, 2018).

Moreover, we have shown that our intravital imaging technology turned out to be a powerful tool for dissecting in vivo pharmacological actions of various drugs. For example, of the biologic agents used in clinic for treating rheumatoid arthritis, anti-IL-6R and anti-TNFα mAbs affected mature osteoclasts and switched bone-resorbing osteoclasts to non-resorbing cells. CTLA4-Ig had no action on mature osteoclasts but mobilized osteoclast precursors, eliminating their firm attachment to bone surfaces. In agreement with these results, CD80/86 (the target molecules of CTLA4-Ig) were prominently expressed only in osteoclast precursor cells, being suppressed during osteoclast maturation. Intravital imaging revealed that various biologic DMARDs acted at specific therapeutic time-points during osteoclastic bone destruction, with different efficacies. These results enable us to grasp the real modes of action of drugs, optimizing the usage of drug regimens (Ann Rheum Dis, 2018).

Application of intravital imaging techniques for dissecting human immunology

Intravital imaging with multi-photon 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), and we will be able to dissect human immunology in future.

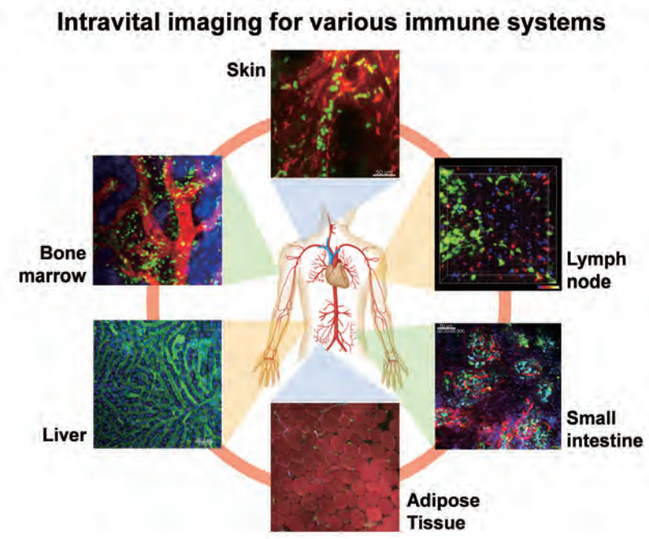

Figure. Intravital imaging for various immune systems.
Immune cells are high dynamic and interconnecting various tissues and organs, by forming a 'soft-wired' network. We are elucidating the basic principle controlling the dynamic nature of immune cells by visualizing in vivo behaviors with advanced imaging techniques.

Recent Publications

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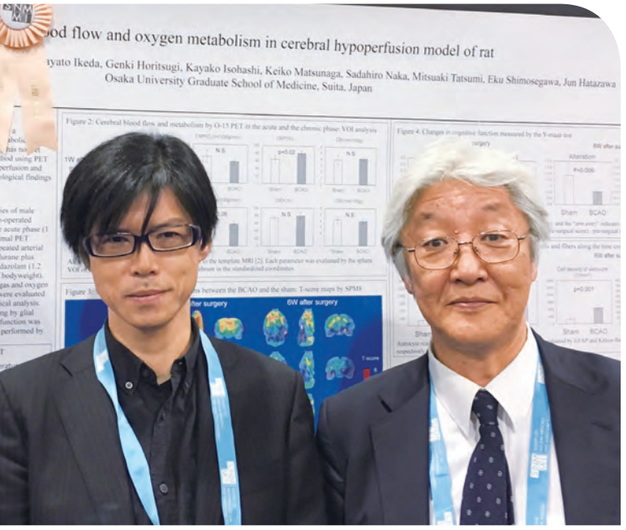
- Maeda H. et al. Real-time intravital imaging of pH variation associated with cell osteoclast activity and motility using designed small molecular probe. Nat. Chem. Biol. 12, 579-85 (2016).
- Iwamoto Y. et al. Intercellular communication between keratinocytes and fibroblasts induces local osteoclast differentiation: a mechanism underlying cholesteatoma-induced bone destruction. Mol. Cell Biol. 6, 1610-20 (2016).
- Nishikawa K. et al. Dnmt3a regulates osteoclast differentiation by coupling to an S-adenosyl methionine-producing metabolic pathway. Nat. Med. 21, 281-287 (2015).

Nuclear Medicine



Jun Hatazawa, MD/PhD

Professor	**Jun Hatazawa**
Associate Professor	**Hiroki Kato**
Assistant Professor	**Tadashi Watabe**
Research Assistant	**2**
Support Staff	**2**

The aim of our lab is to visualize and quantify in-vivo dynamics using molecular imaging techniques and eventually lead to the proper management and treatment of the patients. Theranostics is now focusing attention on the oncology field and tumor selective targeting is essential using the same molecule for both diagnostic imaging and targeted alpha or beta therapy. It is also important to evaluate the resistant factors for the chemo-radiation therapy against cancer such as low perfusion and hypoxia to improve personalized precision medicine.

^{18}F-FBPA as a tumor-specific probe of L-type amino acid transporter 1 (LAT1)

L-type amino acid transporter 1 (LAT1) is the major amino acid transporter which is strongly expressed in many cancer cells while being expressed at lower levels in normal tissues. Inhibition of LAT1 was associated with an antitumor effect in non-small cell lung cancer, suggesting possible application of LAT1 inhibitors to cancer therapy. ^{18}F-fluorodeoxyglucose (^{18}F-FDG) is a major PET tracer used worldwide for the evaluation of cancer patients. However, the glucose transporter is up-regulated not only in cancer cells, but also in inflammatory cells, which may lead to false-positive findings, such as non-specific accumulation in the mediastinal lymph nodes. ^{18}F-FBPA was shown to have low physiological uptake in most normal organs, except the kidney, which is a great advantage to use it as a reliable tracer for cancer diagnosis. We evaluated the in-vitro cellular uptake of ^{18}F-FBPA focusing on its selectivity for LAT1 and the usefulness of ^{18}F-FBPA as a tumor-specific tracer in rat xenograft and inflammation models. As a result, the cellular analyses revealed the higher selectivity of ^{18}F-FBPA for LAT1 and in-vivo ^{18}F-FBPA PET showed the high uptake in the glioma tumor and low uptake in the inflammatory lesion (Figure 1). We could demonstrate the utility of ^{18}F-FBPA as a tumor-specific probe of LAT1 with low accumulation in the inflammatory lesions.

The relationship among intratumoral hypoxia, blood flow and oxygen metabolism

Intratumoral hypoxia is one of the resistant factors in radiotherapy and chemotherapy for cancer. Although it is detected by ^{18}F-fluoromisonidazole (FMISO) PET, the relationship between intratumoral hypoxia and oxygen metabolism has not been studied. We evaluated the intratumoral perfusion and oxygen metabolism in hypoxic regions using the rat xenograft model. Co-registered images of ^{18}F-FMISO and tumoral blood flow showed that intratumoral hypoxic regions with FMISO uptakes were located as lining the inside of the high blood flow area and there was no accumulation in the central area inside the FMISO uptake, suggesting a necrotic region (Figure 2). Quantitative analysis revealed that there were significant negative correlations between ^{18}F-FMISO SUV and tumoral blood flow. In comparison between moderate and severe hypoxia, significant differences were observed in oxygen extraction fraction and tumoral blood volume. Oxygen extraction and vascular density were considered as key factors to define the severity of intratumoral hypoxia. Using the in-vivo molecular PET imaging technique, we showed that intratumoral hypoxic regions showed decreased blood flow with increased oxygen extraction, suggesting the need for a treatment strategy to normalize the blood flow for oxygen-avid active tumor cells in hypoxic regions.

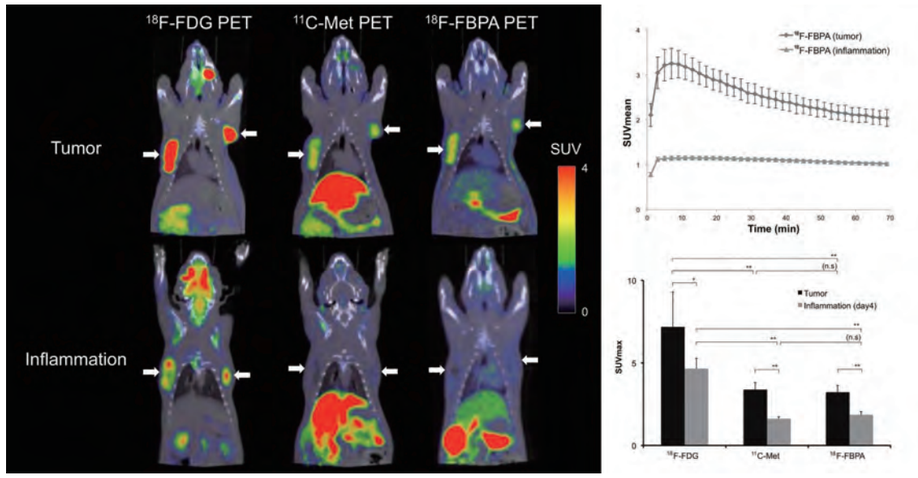


Figure 1.
Static PET images of ^{18}F-FDG, 11C-Methionine (Met), and ^{18}F-FBPA. All tracers showed high uptakes in the tumors on the PET images. In contrast, low uptakes were observed in the inflammatory lesions for ^{18}F-FBPA and 11C-Met PET, whereas the uptake values of ^{18}F-FDG PET remained high in the inflammatory lesions as well.

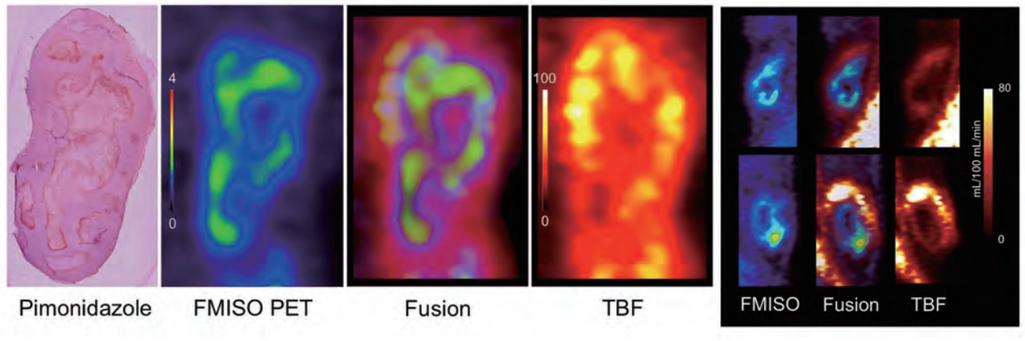
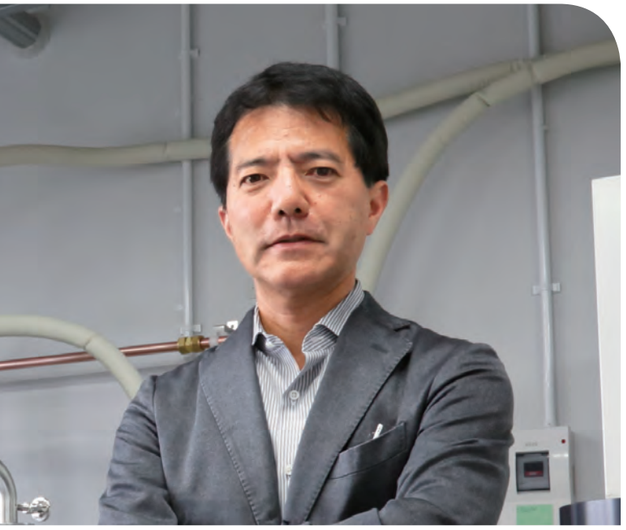


Figure 2.
Comparison among pimonidazole staining, ^{18}F-FMISO PET, tumoral blood flow (TBF), and fusion image. Pimonidazole staining corresponded to ^{18}F-FMISO uptake regions, surrounded by blood flow preserved regions.

Recent Publications

- Horitsugi G. et al. Oxygen-15 labeled CO2, O2, and CO PET in small animals: evaluation using a 3D-mode microPET scanner and impact of reconstruction algorithms. EJNMMI Res. 7, 91 (2017).
- Watabe T. et al. Quantitative evaluation of oxygen metabolism in the intratumoral hypoxia: (18)F-fluoromisonidazole and (15)O-labelled gases inhalation PET. EJNMMI Res. 7, 16 (2017).
- Watabe T. et al. (18)F-FBPA as a tumor-specific probe of L-type amino acid transporter 1 (LAT1): a comparison study with (18)F-FDG and (11)C-Methionine PET. Eur. J. Nucl. Med. Mol. Imaging 44, 321-331 (2017).

Chemical Imaging Techniques



Kazuya Kikuchi, PhD

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Associate Professor	**Yuichiro Hori**
Assistant Professor	**Masafumi Minoshima**
Postdoctoral Fellow	**1**
Research Assistant	**2**
Support Staff	**1**

Intracellular protein-labeling probes for multicolor single-molecule imaging of immune receptor-adaptor molecular dynamics

Multicolor single-molecule imaging (SMI) enables tracking of hundreds or thousands of individual molecules over a period of several seconds, and provides direct information about molecular dynamics and interactions in the cellular context, and consequently, about dynamic and kinetic parameters of biological reactions in living cells. Genetically encoded fluorescent proteins (FPs) are widely used as fluorescence reporters. However, the ability to use FPs for SMI and other photon-intensive imaging applications is limited because of their poor photostability. Moreover, FPs have common limitations in strictly controlling their expression levels. Dual-color SMI using two FPs is particularly challenging, as it requires optimization of the expression levels of both FPs to determine conditions under which FPs display similar fluorescence intensities within a cell. To address this issue, we developed near-infrared (NIR) fluorescent probes for protein-labeling, which specifically binds to a mutant β-lactamase tag (BL-tag). Although NIR fluorophores have many advantages in biological applications such as their low spectral overlap with cellular autofluorescence, the intrinsic high hydrophobicity of the dyes can interfere SMI due to the significant background signals.

Here, we developed NIR fluorescent probes conjugated with a cell-permeable BL-tag ligand for intracellular protein-labeling and application to multicolor SMI. We selected SiR-Me and SiR-carboxyl as the NIR fluorophores, which possess excellent brightness, photo-stability, and cell permeability, and bacampicillin as the BL-tag ligand for intracellular protein-labeling. In addition, novel bacampicillin-based labeling probes by introducing a hydrophilic oligoethylenoxy linker, increasing hydrophilicity and the distance between the fluorescent dye and the BL-tag ligand in a stepwise manner: SiRcB(*n*) (*n* = 2, 4, 6) (Figure 1a). To evaluate its capabilities for intracellular SMI, we used SiRcB(*n*) (*n* = 2, 4, 6) for SMI of the BL-tag anchored to the inner leaflet of the plasma membrane. Tracking analysis revealed that a significantly larger number of fluorescent spots with higher diffusion coefficients was detected upon labeling with SiRcB(*n*) (*n* = 2, 4, 6) than with SiRcB not possessing a hydrophilic linker. Considering the cell permeability and the minimized non-specific binding, we selected SiRcB4 as an NIR-fluorescent probe for multicolor SMI experiments in combination with a HaloTag-based red-fluorescent probe.

In the multicolor SMI study, we focused on the receptor-adaptor interaction between Toll-like receptor 4 (TLR4) and Toll-interleukin-1 receptor (TIR) domain-containing adaptor protein (TIRAP) upon lipopolysaccharide (LPS) stimulation to analyze their molecular dynamics upon innate immune response in living cells. TIRAP was selected as the target adaptor protein because it localizes in the plasma membrane, where it interacts with TLR4. Thus, the dynamic interactions between TLR4 and TIRAP occur at the appropriate depth for SMI using total internal reflection fluorescence microscopy (TIRFM). To analyze the TLR4-TIRAP dynamics, the BL-tag protein-labeling system was used in combination with a commercial HaloTag system. BL-tag and Halo-Tag were attached to the N-terminus of TIRAP (BL-TIRAP) and the C-terminus of TLR4 (TLR4-Halo), respectively (Figure 1d).

We used SiRcB4 and the HaloTag-TMR ligand to perform multicolor SMI in living cells expressing BL-TIRAP and TLR4-Halo, after stimulation with 10 μg/mL LPS, and quantitatively analyzed the protein dynamics by statistical analysis. Analysis of protein dynamics showed an increase in the population of slow-moving TLR4 and TIRAP upon ligand stimulation by LPS (Figure 1e-f). This result indicates that the mobility of both proteins was decreased by their interaction. Another SMI experiment using TIR-domain lacking TLR4 mutant (ΔTIR) revealed that the relative ratio of slow-moving TIRAP molecules did not change even after ligand stimulation (Figure 1g-i), indicating that since TIRAP did not interact with TLR4(ΔTIR), binding of TIRAP to TLR4 through the TIR domain is essential for changing its mobility. This result is consistent with the current model of TLR4 dynamics in response to ligand stimulation, which demonstrates the reliability of our imaging system for detecting protein-protein interactions. Our multicolor SMI system enabled quantitative analysis of LPS-induced TLR4-TIRAP interactions in living cells at the single-molecule level for the first time. This system may also be useful for quantitative analysis of other receptors in response to various PAMPs and investigation of the immune response of downstream adaptors localized at the cytoplasmic membrane.

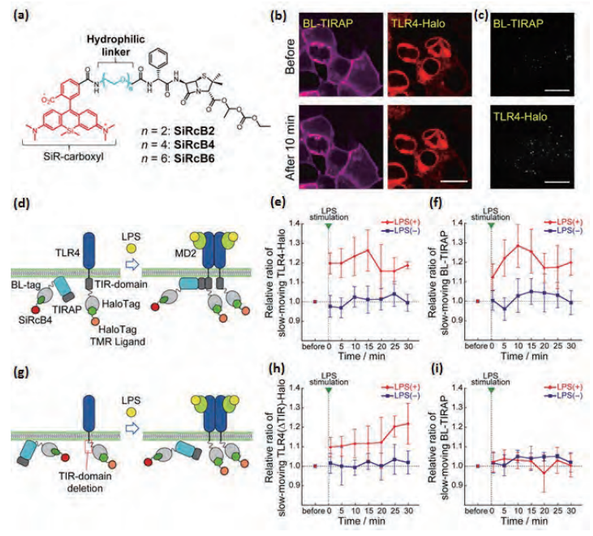

Figure.
(a) Structures of bacampicillin-based probes. SiRcB2, SiRcB4, and SiRcB6 contain di-, tetra-, and hexa-ethylene glycol, respectively. (b) Imaging of HEK293T cells expressing TLR4-Halo and BL-TIRAP by confocal microscopy. The cells were incubated with 100 nM HaloTag-TMR Ligand and 500 nM SiRcB4 at 37 °C for 30 min. Images were acquired before and after 10 min stimulation with 10 μg/mL of LPS. Scale bar: 20 μm. (c) Multicolor SMI of HEK293T cells expressing TLR4-Halo and BL-TIRAP. The cells were incubated with 50 pM HaloTag-TMR Ligand and 100 pM SiRcB4 for 30 min at 37 °C. Scale bar: 5 μm. (d, e) Fluorescent labeling of TLR4 and TIRAP (d), and TLR4(ΔTIR)-Halo and BL-TIRAP (e) with the HaloTag-TMR ligand and SiRcB4, respectively. (f–i) Transient response of the diffusion of TLR4-Halo (f), and BL-TIRAP (g) in TLR4-Halo and BL-TIRAP expressing cells, and TLR4(ΔTIR)-Halo (h), and BL-TIRAP (i) in TLR4(ΔTIR)-Halo and BL-TIRAP expressing cells, upon LPS stimulation (10 μg/mL). N > 140,000 (5 cells).

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- Hori Y, Otomura N, Nishida A, Nishiura M, Umeno M, Suetake I & Kikuchi K. Synthetic-Molecule/Protein Hybrid Probe with Fluorogenic Switch for Live-Cell Imaging of DNA Methylation. J. Am. Chem. Soc. 140, 1686-1690 (2018).
- Matsui Y, Mizukami S & Kikuchi K. Ratiometric Imaging of Intracellular Mg^{2+} Dynamics Using a Red Fluorescent Turn-off Probe and a Green Fluorescent Turn-on Probe. Chem. Lett. 47, 23-26 (2018).
- Sato R, Kozuka J, Ueda M, Mishima R, Kumagai Y, Yoshimura A, Minoshima M, Mizukami S & Kikuchi K. Intracellular protein-labeling probes for multicolor single-molecule imaging of immune receptor-adaptor molecular dynamics. J. Am. Chem. Soc. 139, 17397-17404 (2017).
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- Matsui Y, Sadhu K K, Mizukami S & Kikuchi K. Highly Selective Tridentate Fluorescent Probes for Visualizing Intracellular Mg^{2+} Dynamics without Interference from Ca^{2+} Fluctuation. Chem. Commun. 53, 10644-10647 (2017).

Biophotonics

Nicholas Isaac Smith, PhD

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Assistant Professor	**Alison Hobro**
	Nicolas Pavillon

Our research is aimed at developing label-free imaging methods which can be used to help understand the immune response. In particular, single-cell level analysis is a main aim, coinciding with recent increased awareness of the importance of cell-to-cell diversity. The development of label-free single cell analytic tools means that immune cell type and function can be characterized without any modification of the cells of interest. For T and B cells, the discrimination of the cell type is usually performed by labelling the proteins expressed on the surface. If we can instead develop tools to optically measure the features that best characterize lymphocyte type without labelling, it provides several advantages. One is that the variability in the labelling process itself is removed, and another is that it becomes possible to characterize more of the cell features that emerge during differentiation or activation. In essence, the process may then measure the upstream events that occur before the expression of the surface markers. Along this direction, we successfully used Raman scattering to discriminate between numerous lymphocyte cell lines (Hobro et al 2016), where the identification of a cell line could be performed in a fully label-free optical measurement.

In many types of immunofluorescence imaging, fixation and permeabilization is required. Our lab interests are in non-invasive, non-fixed methods. Nevertheless, since Raman imaging measures the molecular contents of the cell without a-priori knowledge or labelling, it is possible to use Raman imaging to ascertain the changes that occur during fixation to provide insight for other studies that do require fixation. Figure 1 shows data from this project. The Raman mapping of composition of individual cells was performed using line-scanning Raman imaging and principal component analysis (PCA) so that each cell is directly comparable in terms of components to the others. The results show that even common cross-linking fixatives such as paraformaldehyde, although assumed to maintain overall cell structure and contents, actually show a reduction in intensity in the Raman image generated from the first two principal components, showing that the process reduces the components in the cell (Figure 1). See Hobro et al 2017 for details.

As well as characterizing phenotype or for studying large-scale biochemical changes in the cells, we also explored the use of two different label-free techniques for characterizing macrophage activation. Using Raman spectroscopy as well as quantitative phase imaging, we studied the potential for both of these label-free modes to evaluate the changes occurring in macrophages when stimulated by lipopolysaccharides. The quantitative phase imaging mode provides the spatial distribution of refractive index in the cell. Following stimulation, macrophage morphology was observed to change sufficiently so that it is possible to evaluate the activation state of a single cell using only the phase information. In addition to this, the Raman modality gives us quantitative information on changes in cellular composition. Measured Raman data, as well as the quantitative phase, could both be used to generate feature vectors that relate to the activation. We validated these modalities by comparing the Raman and phase information with the signals of inducible NOS in the cell, which is known to be correlated with activation. The results showed that the label-free methods could provide an accurate estimation of activation. In fact, the separation of the two classes (activated vs non-activated) was found to be superior in some cases to the fluorescent measurements. See Figure 2, where the discrimination of LPS-exposed macrophages against control cells is shown, using a fluorescence iNOS signal, compared to the Raman and phase measurements. See Pavillon et al 2018 for details of the project. While this study initially used cell lines, we are now extending the work using primary-cultured murine macrophages.

An interesting finding was that although both the Raman and phase modalities could detect activation, they are based on very different physical features of the cell. The independent measurements based on composition and morphology, respectively, gave highly correlated indicators of activation in paired measurements on the same single cells. When activation was inhibited using progesterone, the samples comprised four different classes of cells: control, LPS-exposed, progesterone-exposed, and LPS + progesterone-exposed. The Raman measurements could successfully distinguish all four classes, implying that the effects of progesterone on resting macrophages creates a sufficient difference in molecular composition of the cell to be discriminated. In contrast, the phase imaging modality could not reliably distinguish between control cells and progesterone exposed cells, indicating that the cellular changes due to progesterone are mostly in the overall composition of the cell, and that there are insufficient downstream effects to exhibit in the cell morphology. This illustrates how a multimodal approach to single-cell analysis can be highly useful, providing insights that are difficult to achieve with other techniques.

As well as the main targets of label-free imaging and analysis, we also aimed to expand the applications of these technologies to the spectroscopic analysis of structure and composition of smaller targets such as RNA, virii, and blood/serum samples.

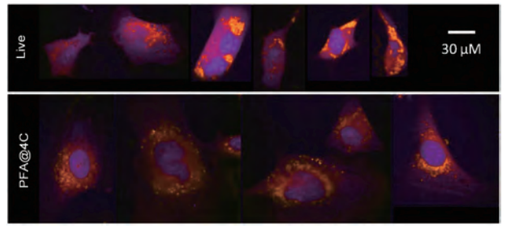

Figure 1.
Use of Raman imaging and principal component analysis to evaluate changes occurring during fixation. Cultured MEF cells were imaged live, then fixed with paraformaldehyde at 4 degrees C. The principal component analysis was performed on the whole dataset so that comparison of changes can be made between the groups of cells. The color channels are generated from the first two spectral principal components. See Hobro et al. 2017 for original figure and details.

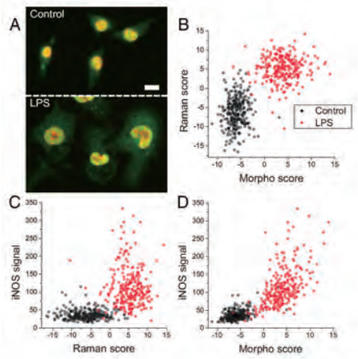

Figure 2.
Evaluating the changes in Raw264 cells under LPS stimulation. Cells were first measured live, without modification in Raman and phase ("morph" denotes morphology measured by phase imaging), and then immunolabelled for iNOS, allowing us to compare our methods to more typical protocols in immunology. Panel A shows a comparison between different cells in iNOS. Panels B, C, D show the combined predictions of Raman and phase measurements for the macrophage state, and comparisons between the Raman, phase, and iNOS signal measurements. Control cells are in black, and LPS-exposed cells are in red. The separation of the two groups shows that the label-free methods are highly effective at estimating macrophage activation.

Recent Publications

- N. Pavillon, A. J. Hobro, S. Akira and N. I. Smith, "Noninvasive detection of macrophage activation with single-cell resolution through machine learning". Proc. Natl. Acad. Sci. USA 115(12), pp. E2676-E2685 (2018).
- A. J. Hobro and N. I. Smith, "Vibrational spectroscopic imaging of pathogens, microorganisms, and their interactions with host systems". Opt. Commun. (2018) in press.
- N. Pavillon and N. I. Smith, "Compressed sensing laser scanning microscopy". Opt. Express 24(26), pp. 30038-300052 (2016).
- A. J. Hobro and N. I. Smith, "An evaluation of fixation methods: spatial and compositional cellular changes observed by Raman imaging". Vib. Spectrosc. 91, pp. 31-45 (2017).
- A. J. Hobro, Y. Kumagai, S. Akira and N. I. Smith, "Raman spectroscopy as a tool for label-free lymphocyte cell line discrimination". Analyst 141, pp. 3756-3764 (2016).

Immune Response Dynamics

Kazuhiro Suzuki, MD/PhD

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Assistant Professor	**Akiko Nakai**
Research Assistant	**2**
Support Staff	**2**

During the last century, extensive studies were performed on individual organ systems and yielded huge progress to biomedical science. However, because the organ systems in our body mutually communicate to cross-regulate their functions, it is nearly impossible to precisely predict responses of one organ system without knowing the states of other organ systems. Thus, the importance of studies that clarify interconnections among multiple organ systems has been increasingly recognized. In this regard, recent studies that have revealed the cellular and molecular basis for connections between the nervous and immune systems represent the new trend. Studies during the last decade have demonstrated that the autonomic and somatosensory nervous systems regulate both the development and functions of immune cells through multiple modes of action.

Adrenergic nerves constitute the efferent arc of the sympathetic nervous system and produce noradrenaline that induces cellular responses through α_1, α_2, β_1, β_2 and β_3 adrenergic receptors (ARs). Like other vital organs, lymphoid organs receive a rich supply of adrenergic nerves. However, it had been unclear how the inputs from adrenergic nerves affect lymphocyte migration among lymphoid organs. Therefore, we have been studying the role of adrenergic nerves in the control of lymphocyte trafficking and adaptive immune responses.

After spending several hours in a lymph node (LN), lymphocytes exit from the LN into lymph, return to blood flow, and travel to other lymphoid organs to continue antigen surveillance. We demonstrated that inputs from adrenergic nerves control lymphocyte egress from LNs through β_2ARs. Activation of lymphocyte β_2ARs enhances the responsiveness of CCR7 and CXCR4, chemokine receptors that promote LN retention of lymphocytes, and consequently inhibits their LN egress (Fig. 1). Additionally, in murine models of inflammatory diseases, including multiple sclerosis and allergic dermatitis, we showed that activation of β_2ARs ameliorates the diseases, which is accompanied by reduced LN egress of pathogenic T cells and their recruitment to inflammatory sites (Nakai et al., 2014).

The activity of adrenergic nerves displays a circadian rhythm that is synchronized with the rest-activity cycle of the species. The noradrenaline release from adrenergic nerves increases during the daytime in humans, whereas it reaches a peak at night in rodents. Indeed, the noradrenaline content in LNs was elevated toward the night time in mice. The night time surge of adrenergic nerve activity in LNs was found to cause restricted LN egress of lymphocytes and an increase of their numbers in LNs. The accumulation of lymphocytes in LNs during the night time was accompanied by more robust humoral immune responses than those induced in the daytime. The diurnal variation of humoral immune responses was dependent on β_2AR-mediated neural signals and was diminished when lymphocyte recirculation through LNs was stopped. These findings suggest that the β_2AR-mediated control of lymphocyte trafficking contributes to the daily rhythm of adaptive immune responses (Suzuki et al., 2016). The time-dependent differences in the magnitude of adaptive immune responses may have evolved to maximize the efficiency of host defense when encounters with pathogens are more likely to occur (Fig. 2).

Our findings have added a novel layer of immune regulation by the nervous system. However, some questions remain unsolved. First, the molecular mechanism of the crosstalk between β_2ARs and chemokine receptors is unclear. Second, it remains to be visualized how adrenergic nerves interact with lymphocytes and influence their behaviors in LNs. Third, the effect of β_2AR activation on other lymphocyte functions, including their metabolic activity, is poorly understood. We are going to address these questions in future studies to reveal the whole picture of adrenergic control of adaptive immune responses.

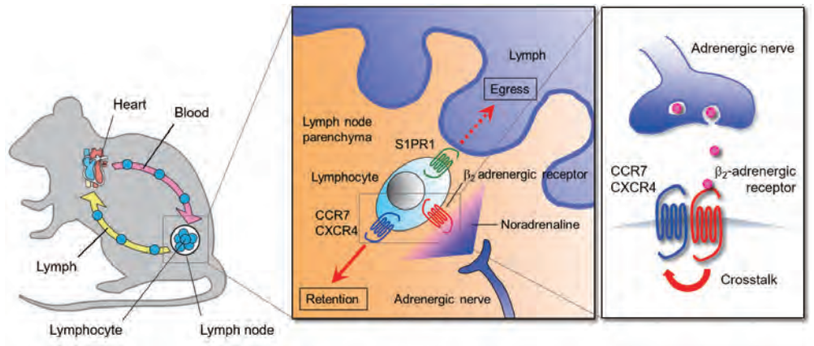


Figure 1. Control of lymphocyte egress from LNs through β_2ARs.
Activation of lymphocyte β_2ARs enhances signals through CCR7 and CXCR4, chemokine receptors that promote lymphocyte retention in LNs, and inhibits lymphocyte egress from LNs.

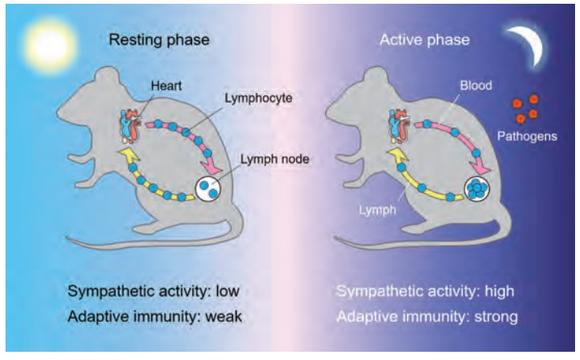


Figure 2. Diurnal control of adaptive immunity by adrenergic nerves.
During the period of high adrenergic nerve activity, lymphocyte egress from LNs is restricted, which leads to an increase of lymphocyte numbers in LNs. Immunization during the period of lymphocyte accumulation in LNs enhances adaptive immune responses.

Recent Publications

- Suzuki K and Nakai A. Immune modulation by neuronal electric shock waves. J. Allergy Clin. Immunol. (2018) in press.
- Suzuki K and Nakai A. Control of lymphocyte trafficking and adaptive immunity by adrenergic nerves. Clin. Exp. Neuroimmunol. 8, 15-22 (2017).
- 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).
- Suzuki K and Nakai A. Autonomic control of inflammation. Clin. Exp. Neuroimmunol. 7, 10-17 (2016).
- Nakai A, Hayano Y, Furuta F, Noda M. and Suzuki K. Control of lymphocyte egress from lymph nodes through β_2-adrenergic receptors. J. Exp. Med. 211, 2583-2598 (2014).

Systems Immunology

Daron M Standley, PhD

Professor	**Daron M Standley**
Associate Professor	**Kazutaka Katoh**
	Shunsuke Teraguchi
Assistant Professor	**Songling Li**
	Floris Van Eerden
Postdoctoral Fellow	**2**
Research Assistant	**2**
Support Staff	**2**

In 2017, our lab continued to pursue two major research themes: functional analysis of B cell receptor (BCR) and T cell receptor (TCR) repertoires and protein-nucleotide interactions. Both of these themes have the potential to make a strong contribution to basic and applied immunology research at IFReC. To this end, we develop bioinformatics methods in our lab, including methods for multiple sequence alignment, machine learning and 3D structural modeling. We also collaborate intensively with wet immunology groups in order to fine-tune our bioinformatics methods.

Functional analysis of BCR and TCR repertoires

Functional analysis of BCR and TCR repertoires can be defined as identification of the target antigen. In 2017, we focused on prediction of BCR or TCR structure from sequence. We developed a web-based tool (Repertoire Builder) for modeling BCR and TCR structure from sequence accurately in a high-throughput manner (Schritt, D. and Li, S. et al.). To our knowledge, Repertoire Builder is the fastest and most accurate tool available (Figure 1). In a typical run, 10,000 sequences can be modeled in under 30 minutes with an average error of under 2Å. We are currently pursuing the challenge of antigen binding. For BCRs, we have applied this approach to the successful modeling of anti-nuclear antibodies associated with acute systemic lupus erythematosus (Sakakibara, S. et al. 2017).

Protein-nucleotide interactions

In the second research direction, we have improved a web-based tool for predicting RNA binding sites on proteins (aaRNA; Li, S. et al. 2014) and have extended this tool to DNA and for docking the nucleotides onto the proteins. Such flexible nucleotide docking has been validated in the case of Regnase-1 (Yokogawa et al. 2016), Arid5a-OX40 (H Hanieh et al. 2017), AUF1-AU-rich repeat interactions (Nyati, K.K. et al. 2017), APOBEC3G-RNA (Izumi et al) and for the anti-nuclear antibody project mentioned above (Figure 2). We are currently applying aaRNA to a larger range of RNA-binding proteins, with emphasis on those proteins that interact with Regnase-1 in order to understand the basis for target specificity.

Multiple sequence alignment

We are continuously improving the performance and usability of the MAFFT multiple sequence alignment software. As one example, a MAFFT option for high-quality alignment has been extended to large-scale sequence data (Figure 3). The accuracy of this method was well demonstrated in previous benchmarks but was impractical for large-scale data sets. By using parallel programming techniques, the calculation speed was improved significantly (Nakamura et al, 2018). Other new features have been added to our online service in response to user demands (Katoh, K. et al. 2017). We also carried out sequence and structural analysis of the autophagy-related 8 (Atg8) protein family (Sasai, M. et al. 2017).

Structural modeling

We supported structural modeling for several wet labs in 2017. Using both 3D modeling and molecular docking we modeled SPP inhibitors of HCV (Hirano, J. et al 2017). We also helped to model the complex and novel structural arrangement of the Ragulator-Rag GTPase complex (R Yonehara, et al. 2017).

Error (Root-mean square deviation) of TCR and BCR models

	Repertoire Builder	Abody Builder	Pigs Pro	Lyra
BCR	**1.89**	1.99	2.06	2.06
BCR H3	**4.20**	4.67	4.76	4.94
TCR	**2.04**	NA	NA	2.32
TCR B3	**3.17**	NA	NA	3.75

Significantly high error (compared to Repertoire Builder): -log(p) > 5 | -log(p) > 10

Figure 1. Repertoire Builder BCR and TCR models significantly more accurate than other methods. The table shows root-mean square deviations of BCR models, BCR CDRH3 regions, TCR models, and TCR CDR alpha-3 regions for Repertoire Builder and three popular methods. The background color indicates statistical significance.

Flexible DNA Docking

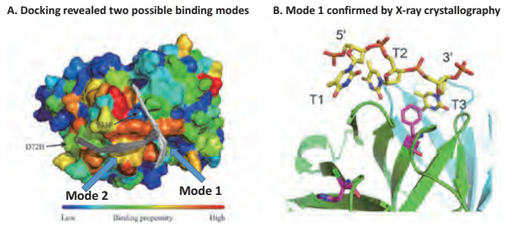


Figure 2. ssDNA docked to Anti-nuclear antibody model.
A, Docking before x-ray structure determined revealed two possible DNA bining models. B, X-ray crystallography confirmed model 1.

Alignment Accuracy

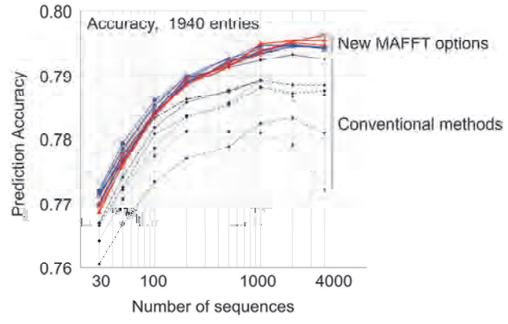
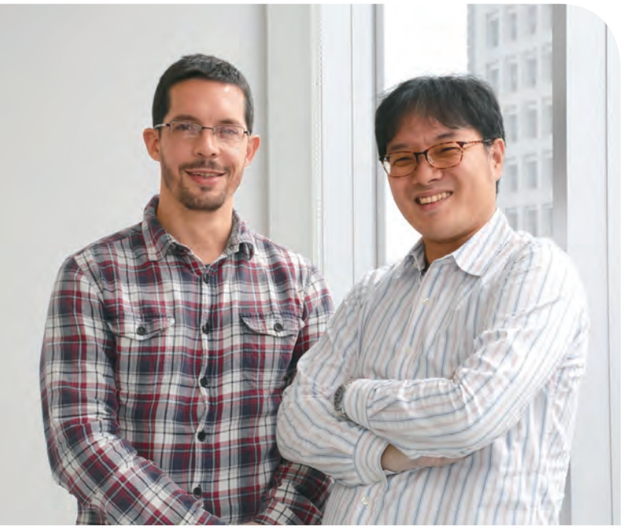


Figure 3. Improvements in MAFFT.
Over a wide-range of data sizes, new options of MAFFT consistently perform more accurately than alternative methods.

Recent Publications

- Yonehara R. et al. Structural basis for the assembly of the Ragulator-Rag GTPase complex. Nat. commun. 8, 1625 (2017).
- Sasai M. et al. Essential role for GABARAP autophagy proteins in interferon-inducible GTPase-mediated host defense. Nat. Immunol. 18, 899-910 (2017).
- Sakakibara S. et al. Clonal evolution and antigen recognition of anti-nuclear antibodies in acute systemic lupus erythematosus. Sci. Rep. 7, 16428 (2017).
- Nyati KK. et al. TLR4-induced NF-kappaB and MAPK signaling regulate the IL-6 mRNA stabilizing protein Arid5a. Nucl. Acids Res. 45, 2687-2703 (2017).
- Hirano J. et al. Characterization of SPP inhibitors suppressing propagation of HCV and protozoa. Proc. Natl. Acad. Sci. USA. 114, E10782-E10791 (2017).

Quantitative Immunology



Associate Professor	**Diego Diez**
Assistant Professor	**Yutaro Kumagai**
Research Assistant	**1**
Support Staff	**2**

The Quantitative Immunology Research Unit is a team of researchers with expertise in different scientific fields including immunology and bioinformatics. Our aim is to understand how the immune system works by combining three different but closely interconnected approaches; (1) quantitative measurement of molecular dynamics at single cell and single molecule level, (2) integration of "big data" from multiple sources into network models, and (3) development of mathematical frameworks to understand the immune system's dynamics through the analysis of these massive datasets. These approaches are combined in several projects that aim to get insight into specific questions related to the immune system. Some of these projects are described below to highlight specific topics.

Quantitative approaches

Accurate quantification of biological responses is critical for understanding the dynamics of complex systems. Previously, we have developed a fluorescent protein reporter system for the quantitative monitoring of IFN-a6 (Kumagai et al. 2007). Now we are trying to increase the "dimension" of the observation in two ways: time and perturbation. Time lapse imaging of type I interferon expression under microscope is combined with multiple fluorescent protein knock-in cells to monitor genes induced upon antiviral responses. We are also developing automated computational algorithms to extract important quantities to understand interferon regulation from such time lapse imaging data.

Receptor molecule dynamics such as dimerization and clustering with downstream molecules is important for immune system signaling. However, because of limitations in biochemical assay resolution, the details of this process are still poorly understood for most immune signaling pathways. To address this problem, we are applying, in collaboration with RIKEN QBiC and other laboratories in IFReC, Total Internal Reflection Fluorescent Microscopy (TIRFM) to monitor the dynamics of single immune molecules. We are using this technique to study TLR signaling, and successfully monitored TLRs and their adaptors at the single molecular level (Sato et al, 2017). We developed a novel algorithm to quantify the diffusion dynamics of single molecules without bias, even under high molecular density conditions (Teraguchi and Kumagai, in press). This highly quantitative technique can be used to describe the dynamics of the immune system's signaling pathways.

Data integration

High-throughput ("omics") technologies have brought biology into the big data era and the need for approaches that integrate, summarize and extract relevant information that reveals the relation between biological components.

We are developing methods that integrate measurements of transcription factor binding with transcriptome data from different experimental conditions, and with protein-protein interaction data from public databases, to obtain insight into signaling and gene regulatory immune networks. We apply these methods to study the mechanisms behind respiratory diseases, such as chronic obstructive pulmonary disease (COPD) and silicosis (Diez et al. 2015). A common feature of these diseases is that inflammation and disease progression are irreversible even after removing exposure to the harmful components (tobacco for COPD and silica for silicosis). Combining ATAC-seq and RNA-seq measurements of transcriptional activity in a mouse model of silicosis, we study the regulatory pathways associated with irreversible inflammation.

We are integrating a novel de novo RNA motif search algorithm with RNA degradation time course in mouse dendritic cells after LPS stimulation. Transcriptomic data is used to determine degradation kinetics by clustering the time course data into patterns with similar degradation kinetics. Then, de novo sequence and structure motif identification is applied to the 3' UTR sequences of each cluster. We identified known structural motifs, including the stem-loop structure containing motifs bound by the RNA-binding proteins Roquin and Regnase-1 (Kumagai et al. 2016). We also identified novel motifs for which their role in controlling RNA degradation during the immune response is being validated.

Mathematical modeling

The accurate quantification of immunological responses and the integration of massive data open the door to approach the immunology research from the theoretical perspective. We are developing novel mathematical frameworks for the quantitative description of the immune system.

A common obstacle for constructing dynamical models of cellular signaling is the biochemical determination of many parameters. To circumvent this problem, we have developed a mathematical framework called Stochastic Binary Modeling (SBM), which also allows us to represent the stochastic and heterogeneous nature of cell populations (Teraguchi et al. 2011). We have developed a system to automatically identify the structure and parameters of the network of regulatory pathways from multidimensional data. Now we are applying this system to infer the network dynamics of immune signaling.

Transcriptional regulatory networks are important determinants of cell identity and function. In this process thousands of regulators, including transcription factors, determine the expression level of their target genes. We model these processes using linear regression methods. We assume that the expression level of each gene depends on the activity of a small number of regulators. Furthermore, regulators contribute in an additive way to the expression level of their target genes. We validate the feasibility of these assumptions using simulated networks resembling different types of real networks. Using the expression level of the regulators as a proxy for their activities we apply these methods to several immune datasets.

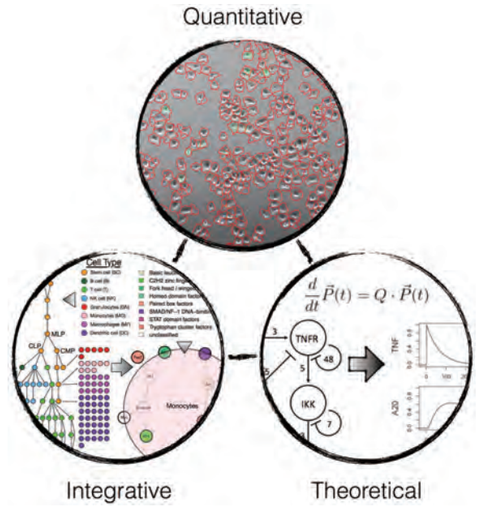


Figure.
The Quantitative Immunology Research Unit combines quantitative, integrative and theoretical approaches.

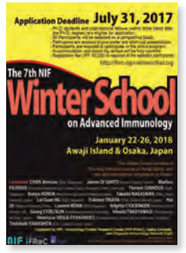
Recent Publications

- Teraguchi S & Kumagai Y. Estimation of diffusion constants from single molecular measurement without explicit tracking. BMC Syst. Biol. 12 (Suppl. 1), 15 (2018).
- Sato R, Kozuka J, Ueda M, Mishima R, Kumagai Y, Yoshimura A, Minoshima M, Mizukami S & Kikuchi K. Intracellular Protein-Labeling Probes for Multicolor Single-Molecule Imaging of Immune Receptor-Adaptor Molecular Dynamics. J. Am. Chem. Soc. 139(48), 17397-17404 (2017).
- Kumagai Y, Vandenbon A, Teraguchi S, Akira S & Suzuki Y. Genome-wide map of RNA degradation kinetics patterns in dendritic cells after LPS stimulation facilitates identification of primary sequence and secondary structure motifs in mRNAs. BMC Genomics 17, 1032, doi:10.1186/s12864-016-3325-7 (2016).
- Bahrini I, Song JH, Diez D & Hanayama R. Neuronal exosomes facilitate synaptic pruning by up-regulating complement factors in microglia. Scientific reports 5, 7989, doi:10.1038/srep07989 (2015).
- Diez D, Agusti A & Wheelock CE. Network analysis in the investigation of chronic respiratory diseases. From basics to application. Am. J. Respir. Crit. Care Med. 190, 981-988, doi:10.1164/rccm.201403-0421PP (2014).

Events & Outreach Activities

The 7th NIF Winter School on Advanced Immunology

The 7th NIF Winter School on Advanced Immunology was held with Singapore Immunology Network (SIgN) at The Awaji Yumebutai International Conference Center in Japan on 21-26 January 2018. The program comprised a four-day lecture course at Awaji Island and a one-day international symposium at Suita Campus of Osaka University. Fifty-four young researchers from around the world, selected from 213 applicants, learned cutting-edge immunology from 15 world-renowned senior researchers at the course and three up-and-coming researchers at the symposium. Three young researchers from companies collaborating with IFReC also attended and joined in the discussions. The Winter School was highly evaluated by the participants and the lecturers as an excellent opportunity for networking with young peers through active discussions and communications as well as its high scientific level.



- **Date：** **January 21- 26, 2018**
- **Venue：** **Awaji Yumebutai International Conference Center, Hyogo**
  Icho-Kaikan, Suita Campus, Osaka University, (The 9th International Symposium of IFReC)

Lecturer	Title
Jinmiao Chen (SIgN, Singapore)	Constructing Cell Lineages From Single-Cell Transcriptomes
James Di Santo (Institut Pasteur, France)	Developmental Options and Functional Plasticity of Human Innate Lymphoid Cells
Markus Feuerer (The University of Regensburg / German Cancer Research Center, Germany)	Specialization of regulatory T cells in tissues
Florent Ginhoux (SIgN, Singapore)	Human Dendritic Cells: From Development to Functions
Kenya HONDA (Keio University, Japan)	Modulation of the immune system by the gut microbiota
Takashi Nagasawa (IFReC, Osaka University, Japan)	Microenvironmental niches for hematopoietic stem cells and immune cells
Lai Guan Ng (SIgN, Singapore)	Watching The Great Leukocyte Migration
Yukinori Okada (IFReC, Osaka University, Japan)	Statistical genetics, disease biology, and drug discovery
Hai Qi (Tsinghua University, China)	T-B cell interactions and the germinal center reaction
Laurent Rénia (SIgN, Singapore)	Immune-mediated Pathologies During Malaria Infection
Brigitta Stockinger (The Francis Crick Institute, UK)	Environmental influences on intestinal homeostasis and inflammation
Georg Stoecklin (Heidelberg University, Germany)	Translation regulation during macrophage activation
Hiroshi Takayanagi (The University of Tokyo, Japan)	Osteoimmunology and autoimmunity
Henrique Veiga-Femandes (Champalimaud Center for the Unknown, Portugal)	Innate Lymphoid Cells and the Second Brain
Toshihide Yamashita (IFReC, Osaka University, Japan)	The spatiotemporal dynamics in the biological systems regulate the neuronal network in the central nervous system

The 9th International Symposium of IFReC - Immunology at the Forefront

IFReC invited many talented scientists who are future leaders in various research fields as speakers. It was an excellent opportunity to share ideas and expertise in order to contribute to further development in Immunology.

■ Date： January 26, 2018

■ Venue： Icho kaikan, Suita campus, Osaka University

Speaker	Title
Opening remarks by Director Shizuo Akira	
Takashi Shichita (Tokyo Metropolitan Institute of Medical Science, Japan)	The resolution of sterile inflammation after ischemic stroke
Daniel Mucida (Rockefeller University, USA)	Tissue adaptation: implications for gut tolerance and immunity
Etsushi Kuroda (Osaka University/ National Institutes of Biomedical Innovation, Health and Nutrition, Japan)	Mechanisms of allergic inflammation induced by inhaled fine particles
Michelle Linterman (University of Cambridge, UK)	CXCL13 recruits B cells to the lung and initiates tertiary germinal centre formation
Thomas Korn (Technische Universität München, Germany)	Modes and outcome of IL-6 signaling into T cells
Naganari Ohkura (Osaka University, Japan)	Significance of the regulatory T cell-specific epigenetics in autoimmune disease susceptibility
Takao Hashiguchi (Kyushu University, Japan)	Molecular dissection of virus entry and its inhibition by antibodies/inhibitors
Cevayir Coban (Osaka University, Japan)	Host-Plasmodium interactions at tissue level
Closing Remarks by Deputy Director Kiyoshi Takeda	

IFReC Seminars

IFReC holds seminars throughout the year with speakers from a variety of disciplines including immunology, imaging and informatics with the aim of promoting collaborative research, as well as to inspire and educate the next generation of scientists.

Since its establishment, IFReC has held more than a hundred seminars, which have served as a forum for effective interaction between researchers beyond national borders and academic disciplines. This program has certainly contributed to IFReC's mission of promoting internationalization and interdisciplinary research.

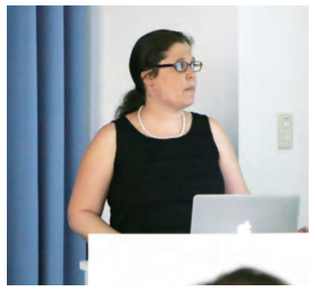
On July 20, 2017, a seminar by Mr. Osamu Nagayama (CEO and Chairman of Chugai Pharmaceutical Co.) was held in commemoration of the Comprehensive Collaboration Agreement between Osaka University and Chugai Pharmaceutical Co., Ltd.

2017

Special Lecture

Jul. 28	**Osamu Nagayama**	CEO, Chairman of Chugai Pharmaceutical Co., Ltd.	

Date	Speaker	Affiliation	Title
Sep. 4	**Christophe J. Desmet**	University of Liège / FRS-FNRS, Belgium	Lost in translation: of the importance of protein translation for normal function of hematopoietic stem and progenitor cells
Sep. 4	**Andrea Reboldi**	University of Massachusetts, USA	Mechanisms controlling intestinal IgA production
Sep. 4	**Meirav Pevsner -Fischer**	Weizmann Institute of Science, Israel	Host-microbiome interactions in health and disease
Oct. 3	**Claudia Mauri**	University College London, UK	Regulatory B cells in health: what goes wrong in patients with autoimmunity?
Oct. 20	**Axel Kallies**	University of Melbourne/Walter and Eliza Hall Institute of Medical Research, Australia	Molecular regulation of tissue-resident regulatory T cell development
Oct. 26	**Warren J. Leonard**	NHLBI, NIH, USA	Fine tuning IL-2: superenhancers, STAT5 tetramerization, and partial agonists
Nov. 7	**Hidehiro Yamane**	NIAID, NIH, USA	Mechanisms underlying the fine-tuning of CD4+ T cell positioning during T follicular helper cell differentiation



IFReC Colloquia

IFReC colloquia are a series of discussion meetings for IFReC members held once every other month since FY2011. At each colloquium, three speakers from IFReC laboratories give talks about their latest research progress followed by intensive discussion. After the colloquium, a small social gathering is held to further the discussions and encourage the exchange among IFReC members in an informal setting. These events serve as a platform to promote fusion researches and deepen understanding of researches conducted in IFReC.

- **Date :** 30th Colloquium : April 19, 2017
  31st Colloquium : June 21, 2017
  32nd Colloquium : August 30, 2017
  33rd Colloquium : October 11, 2017
  34th Colloquium : December 27, 2017
  35th Colloquium : February 21, 2018
- **Venue :** Taniguchi Memorial Hall, Osaka University

	Speaker	Title
30th	**Etsushi Kuroda** (Vaccine Science)	Cyclic GMP-AMP exacerbates asthma via IL-33
	Toshihide Yamashita (Molecular Neuroscience)	The spatiotemporal dynamics in the biological systems regulate the neuronal network in the central nervous system
	Shogo Tanabe (Molecular Neuroscience)	B-1 cells promote oligodendrogenesis during brain development
	Hiroshi Tsujioka (Molecular Neuroscience)	Interleukin-11 induces and maintains progenitor cells of various tissues during tail regeneration of Xenopus laevis tadpoles
	Yukinori Okada (Statistical Immunology)	Statistical genetics, disease biology, and drug discovery
	Sayuri Gyobu (Biochemistry and Immunology)	Mechanism and physiological role of phospholipid scrambling by TMEM16 family
31st	**Keiichi Namba** (Graduate School of Frontier Biosciences / Riken QBiC)	Recent progress of electron cryomicroscopy for structural analysis of protein assemblies at atomic level
	Sho Yamasaki (Molecular Immunology)	Immune recognition of pathogens and damaged-self via ITAM receptors
	Songling Li (Systems Immunology)	Quantifying structural and functional convergence in B cell receptor repertoires
	Takashi Nagasawa (Stem Cell Biology and Developmental Immunology)	Microenvironmental niches for hematopoietic stem cells and immune cells
32nd	**Takashi Saito** (Cell Signaling)	Regulation of T cell activation and function by innate signals-STING activation induces growth arrest and IFN production in T cells-
	Yuichiro Hori (Chemical Imaging Techniques)	Development of Chemical Tool for Imaging DNA Methylation
	Naoki Hosen (Immunopathology)	The activated conformation of integrin β7 as a target for multiple myeloma–specific chimeric antigen receptor T-cell therapy
	Wataru Ise (Lymphocyte Differentiation)	Selection of germinal center B cells into the plasma cell compartment
33rd	**Szandor Simmons** (Immunology and Cell Biology)	High-endothelial cell-derived sphingosine-1-phosphate regulates dendritic cell localization and vascular integrity in the lymph node
	Yoshiko Murakami (Immunoglycobiology)	Autoinflammation-Paroxysmal nocturnal hemoglobinuria (PNH) syndrome
	Eiji Umemoto (Mucosal Immunology)	Identification of bacteria-induced intestinal metabolites that influence immune responses
34th	**Hisashi Kanemaru** (Host Defense)	The antitumor effect of BATF2 through IL-12 p40 up-regulation in tumor-associated macrophages
	Patrick Michael Lelliott (Malaria Immunology)	A novel technique to quantify extracellular trap release
	Kazuki Kishida (Immunochemistry)	Antigen-specific immune regulation mediated by TCR-like antibodies
35th	**Nicolas Pavillon** (Biophotonics)	Label-free monitoring of activation and inhibition of macrophage responses
	Jisu Ma (Immunoparasitology)	A family member of phospholipase C is specifically involved in CD8 T cell-mediated immune responses
	Diego Diez (Quantitative Immunology Research Unit)	Inferring gene regulatory networks from omics data
	Akiko Nakai (Immune Response Dynamics)	The COMMD3/8 complex dictates the specificity of GRK recruitment to chemoattractant receptors

Science Café

The series of science Cafés is a long-term IFReC outreach activity to promote communication between researchers and the general public. It also enhances people's understanding of immunology researches and the researchers involved in them. In the two science cafés organized by IFReC in FY2017, a total of about 150 participants enjoyed novel topics in immunology in a relaxing atmosphere.

"Science Café on the Edge" at 2017 Icho Festival

- Speaker : Takashi Satoh (Assistant Professor, Host Defense, IFReC/RIMD)
- Date : April 30, 2017
- Venue : Techno Alliance Hall, Suita Campus, Osaka University

"Science Café on the Edge" as part of "Lab Café" by Osaka University

- Speaker : Jun Sakanoue (Associate Professor, RPMO, IFReC)
- Date : March 9, 2018
- Venue : Art Area B1 at Naniwabashi station (Keihan Electric Railway Co.)

Super Science High School Student Fair

Super Science High Schools (SSH) are selected high schools by MEXT in Japan, which promote advanced math/ science education as well as collaborative researches with universities, and activities to develop international perspectives.

In the SSH Student Fair 2017 held in Kobe, more than 200 schools, including several schools from overseas, held booths with posters to present their researches. WPI institutes held a collaborative booth and introduced the research activities of each institute using posters, booklets and demonstrations.

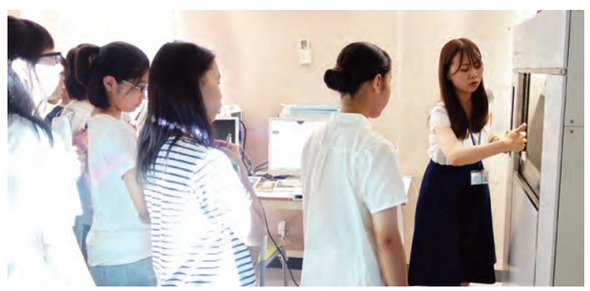
- Date : August 9-10, 2017
- Venue : Kobe International Exhibition Hall
- Host : MEXT, JST
- Support : Boards of Education (Kobe prefecture and Kobe city)

Students Visit and Facility tour

During the summer vacation, IFReC welcomed 30 SSH students from Nara Prefecture. They heard a lecture by Dr. Yutaro Kumagai (Quantitative Immunology Research Unit), and visited Akira Lab and the Core Instrumental Facility to try some experiments and to talk with researchers.

We also welcomed 55 junior high-school students and their parents. They also enjoyed the experiments and a tour of the RIMD History Museum.

- Date : August 3, 2017 (Junior High School)
  August 11, 2017 (High School)



WPI Science Symposium

IFReC co-organized the 6th WPI Science Symposium titled; Starting the Future!
At IFReC's booth, we introduced the images and movies acquired through IFReC researches, and provided leaflets of IFReC and WPI. Interaction with the visitors was quite stimulating for the WPI staff.

■ Date： February 11, 2018
■ Venue： National Museum of Emerging Science and Innovation (Miraikan), Tokyo





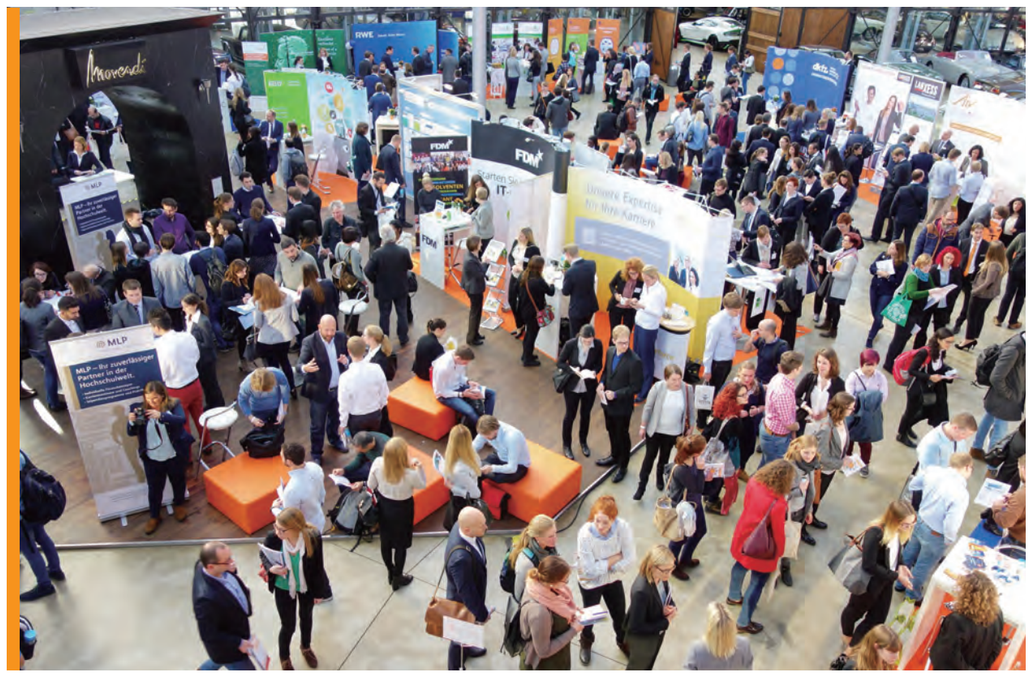
AAAS 2018 Annual Meeting


The American Association for the Advancement of Science (AAAS) is the biggest international scientific society in the world and its mission is to "advance science and serve society". The AAAS 2018 Annual Meeting was held in Austin, and it offered a broad range of activities including lectures, symposia, seminars and exhibits, with the theme of "Serving society through science policy ".

WPI institutes held a collaborative booth to introduce the WPI program and the institutes' activities using posters, booklets and a demonstration experiment. More than 400 participants visited the booth and gained interest in the WPI program and world leading researches in Japan.

■ Date： February 15-19, 2018
■ Venue： Austin Convention Center (Austin, Texas, USA)

Naturejobs Career Expo



Naturejobs is a worldwide career resource for scientists, providing a wide range of career advice and information across Nature Publishing Group journals as well as centrally on the website.

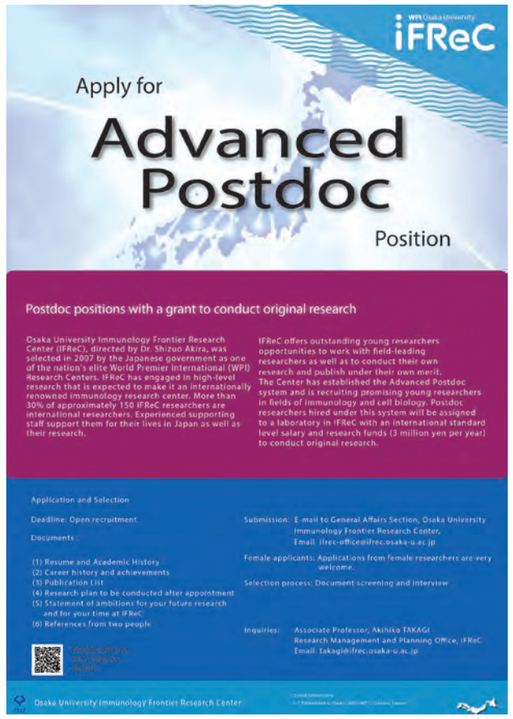
In 2017, Nature Publishing Group held their "Career Expo" in UK, USA and Germany. IFReC participated in "Naturejobs Career Expo in Dusseldorf". IFReC staff carried out recruiting activities for the Advanced Postdoc position starting in FY2017 by holding an exhibition booth and an oral session.

■ Date : November 17, 2017
■ Venue : Classic Remise, Dusseldorf, Germany

Advanced Postdoc System

For IFReC, fostering the education of young researchers is a responsibility as a global research institution. It is also vital for the institution to continually incorporate the original ideas of young researchers and to promote international brain circulation in order to further develop IFReC's research. Around the world, leading research institutions are in fierce competition to discover excellent young researchers. In order to employ young researchers who are expected to be internationally active at IFReC and to strengthen IFReC's function as a hub of international brain circulation, it is necessary to improve the conditions for researchers in Japan, to an international standard.

IFReC has, therefore, established the Advanced Postdoc system this fiscal year. It offers outstanding young researchers in the fields of immunology and cell biology opportunities to work with field-leading researchers in IFReC as well as to conduct their own research and publish under their own merit. IFReC has selected three excellent postdocs for three years under this system out of 78 applicants. They were assigned to laboratories in IFReC with an international standard level salary and research funds (3 million yen per year) to conduct original research.



Young Scientist Support Program for Research Abroad

To strengthen our international research network and our basis for international collaborative research, IFReC has provided financial support to young researchers who wish to participate in research activities at overseas institutions. The program aims to develop the practical skills and abilities of young researchers in international collaborative research and to develop their network with researchers overseas. 4 researchers used this support program in FY2017. Since the start of this program, IFReC has provided support for 53 visits overseas by young researchers.

Young Scientist Support Program for Research Abroad

Name	Country	Conference Attended
Rouaa Beshr	USA	Society of Nuclear Medicine and Molecular Imaging (SNMMI)
Romanov Victor	Australia	Annual congress of the european association of nuclear medicine (EANM'2017)
LIM Ee Lyn	Switzerland	World Immune Regulation Meeting XII
Taba Fargol	France	17th Annual Congress of the European Society for Photodynamic Therapy

Support provided for 36 overseas visits by young researchers in the past five years

IFReC has supported the active participation of young scientists in research activities overseas

Data

Common Facilities

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 large-capacity 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.

IFReC–RIMD Research Complex at Suita Campus of Osaka University

Photo : S. Higashiyama

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 DNA sequencing analysis
- Radio isotope facility

Kishimoto Foundation Fellowship

IFReC launched the Kishimoto Foundation Fellowship program for researchers in various fields of immunology in 2010. The program is supported by the Kishimoto Foundation and designed to support overseas researchers in order to promote and develop immunological research and international exchanges at IFReC. The fellowships are open to international postdoctoral researchers who seek to collaborate with IFReC researchers. The recipients are provided with a salary and an airfare to Japan.

The Kishimoto Foundation was established in 2008 in honor of Tadamitsu Kishimoto, who, during the 1980s and 90s, elucidated the function of interleukin-6 (IL-6), a key molecule for stimulating immune responses. He later developed the anti-IL6 receptor-based therapy, tocilizumab, to treat immune disorders such as Castleman's disease or rheumatoid arthritis.

FY2017 Kishimoto Fellowship Recipients

Position of Recipient	Name (initials)	Nationality	Host researcher	Period
Specially Appointed Researcher	D. H.	Australia	Sakaguchi	Oct. 16, 2015 - Oct 15, 2018
Specially Appointed Researcher	J. P.	Vietnam	Kishimoto	Apr. 1, 2016 - Mar. 31, 2019
Specially Appointed Researcher	N. T.	Slovenia	Quantitative Immunology Research Unit	Oct. 1, 2016 - Jul. 15, 2017
Specially Appointed Researcher	H. J.	China	Arase	May 1, 2017 - Apr. 30, 2018
Visiting Researcher	H. H.	Jordan	Kishimoto	Jun. 26, 2017 - Aug. 18, 2017
Visiting Researcher	P. K.	Turkey	Coban	Mar. 1, 2018 - May 28, 2018
Visiting Researcher	E. E.	Turkey	Coban	Mar. 1, 2018 - May 28, 2018
Visiting Researcher	N. N.	Vietnam	Kishimoto	Mar. 10, 2018 - Mar. 22, 2018
Visiting Researcher	D. L.	Vietnam	Kishimoto	Mar. 10, 2018 - May 8, 2018

Major Awards

Shimon Sakaguchi — Person of Cultural Merit of Japan

The Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan announced that Prof. Shimon Sakaguchi, Deputy Director of IFReC was named a Person of Cultural Merit. MEXT commented that Prof. Sakaguchi was awarded for his outstanding achievements in studies of Regulatory T cell (Treg); discovering and clarifying the roles of Treg in autoimmune diseases and allergies. This is the third time for the scientist of IFReC to be honored as a Person of Cultural Merit, others include Shizuo Akira (Director of IFReC) in 2009 and Toshio Yanagida in 2013.

Four IFReC researchers — Highly Cited Researchers 2017

Highly Cited Researchers are researchers with papers that have a large number of citations from all over the world as selected by ©Clarivate Analytics. They are thought to be leading the way in solving the world's biggest challenges. In 2017, some 3,300 researchers from 21 fields were selected as Highly Cited Researchers. Eight researchers in three fields were selected from Osaka University, and four of them are from IFReC.

- **Shizuo Akira**
  Director of IFReC

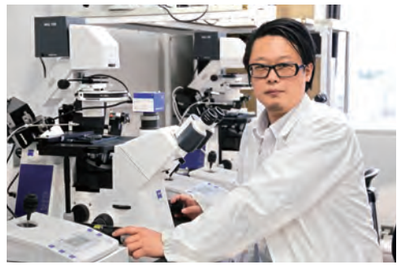
- **Shimon Sakaguchi**
  Deputy Director of IFReC

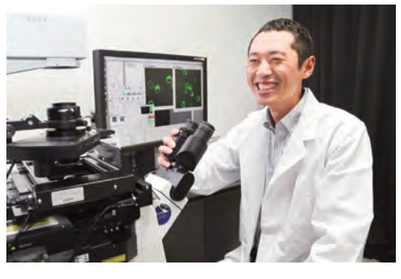
- **Kiyoshi Takeda**
  Deputy Director of IFReC

- **Masahiro Yamamoto**
  Immunoparasitology, IFReC

Satoh and Segawa — The Young Scientists' Prize by MEXT

Takashi Satoh (Host Defense) and Katsumori Segawa (Biochemistry & Immunology) were given the Young Scientists' Prize of Commendation for Science and Technology by the Minister of MEXT. The prize is given to young scientists who have been recognized to have a high level of research ability and development in the science and technology field in Japan.





Takashi Satoh (L) and Katsumori Segawa (R).

Cevayir Coban — JSI Women Immunologist Award

Cevayir Coban (PI, Malaria Immunology) was awarded the 4th Japanese Society for Immunology (JSI) Prize for Women Immunologists. The awarded title is "Revealing the mechanism of host-Plasmodium parasite interactions".

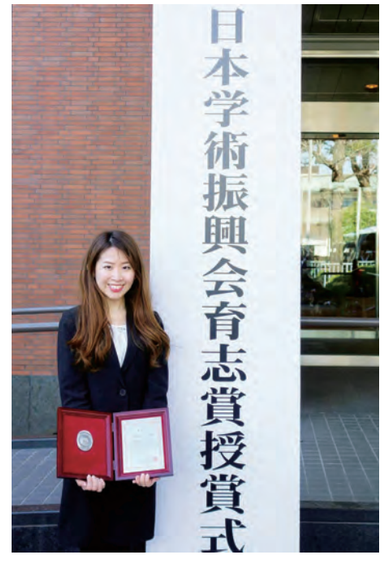
Taroh Kinoshita — Takeda Prize for Medical Science, JSI Human Immunology Research Award

This prize is given to scientists who have made remarkable contributions in the field of medical science. Kinoshita was honored for research on GPI-anchored proteins and mechanisms of its deficiencies.

Cevayir Coban (L) and Taroh Kinoshita (R) at the venue of the commemorative lectures

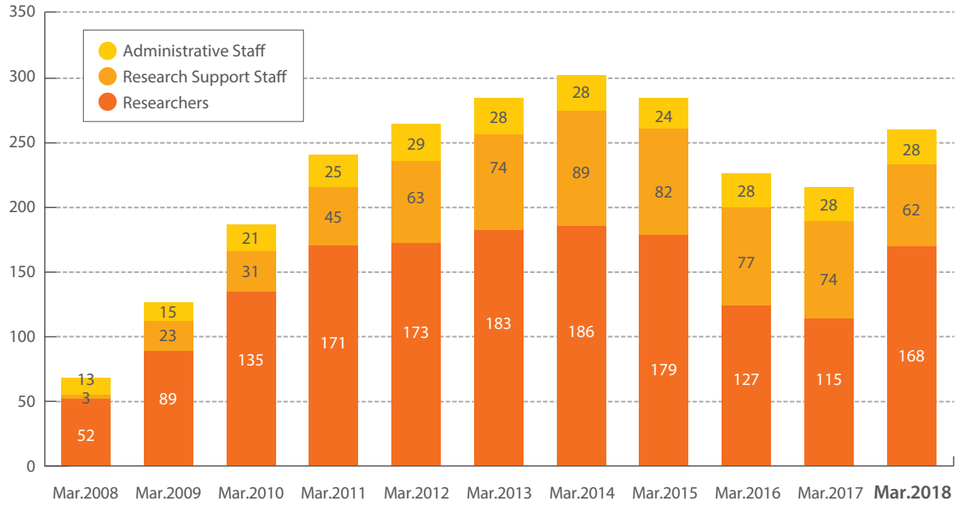
Michelle Sue Jann Lee — JSPS Ikushi Prize

IFReC Assistant Professor Michelle Sue Jann Lee was awarded the 8th JSPS Ikushi Prize. The 18 prize winners including Dr. Lee were unequivocally recognized for their excellent results in each research area. The award ceremony was held at the headquarters of Japan Academy (Gakushi-in) in the presence of their Imperial Highnesses Prince and Princess Akishino.

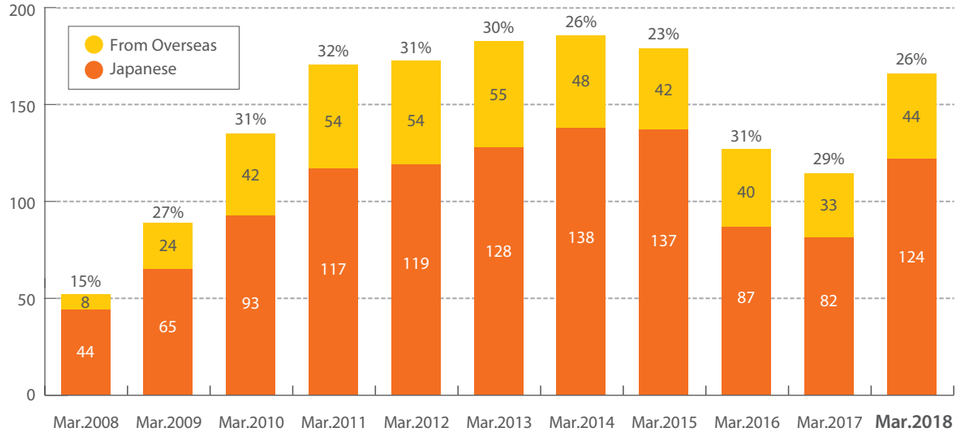


Composition

Number of IFReC Staff

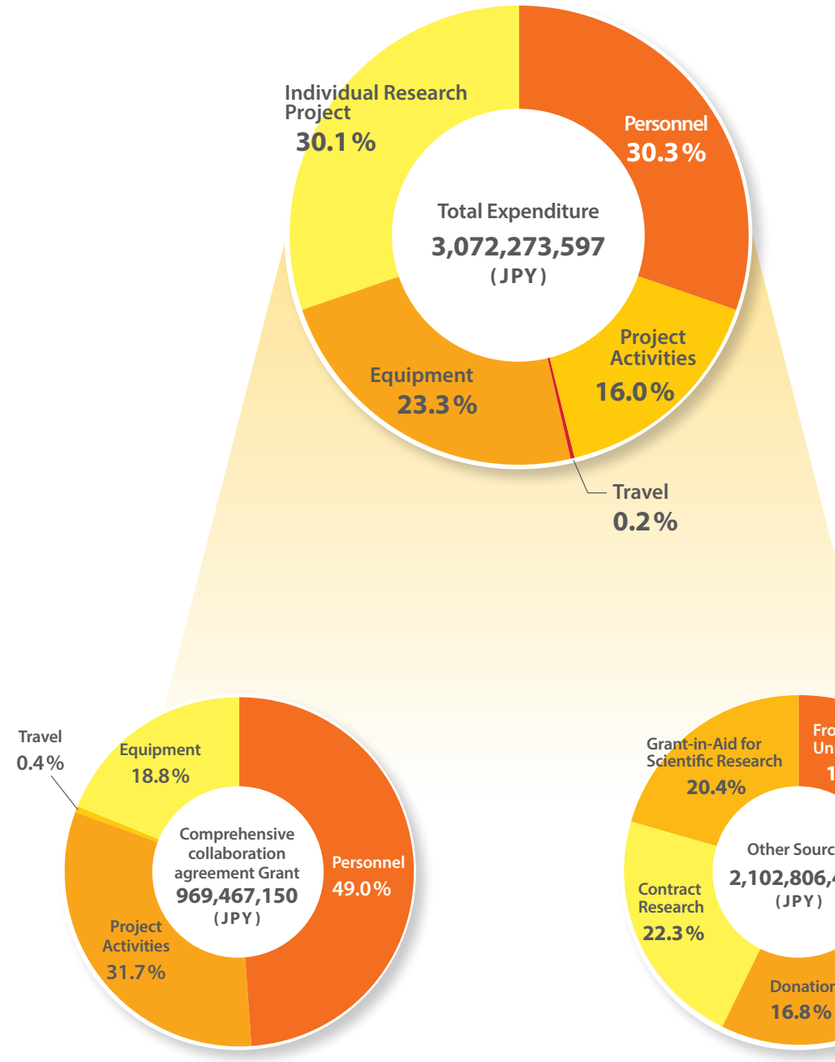


Number of Researchers



Finance

Breakdown of total expenditure at IFReC in FY2017



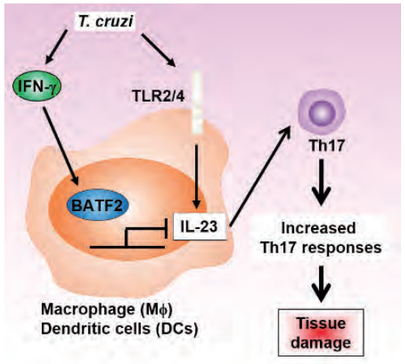
BATF2 inhibits immunopathological Th17 responses by suppressing Il23a expression during Trypanosoma cruzi infection.

J. Exp Med. 214(5):1313-1331, 2017. doi: 10.1084/jem.20161076.

Kitada S, Kayama H, Okuzaki D. et al.

Kiyoshi Takeda's group and others have revealed the regulation of immune response after Chagas disease causing Trypanosoma cruzi (T. cruzi) infection.

T. cruzi infection induces IL-23 production by host immune cells, and antigen-specific Th17 responses are then promoted. However, whether the IL-23-Th17 axis is controlled by IFN-γ-dependent mechanisms during T. cruzi infection remains unclear. In this study, they have demonstrated that IFN-γ-inducible BATF2 exerts its immunoregulatory function through the suppression of Il23a by interacting directly with c-JUN in innate immune cells, thereby preventing immunopathological Th17 responses during T. cruzi infection.



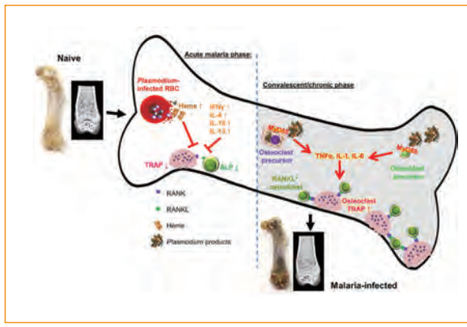
Plasmodium products persist in the bone marrow and promote chronic bone loss

Science Immunology. 2(12) eaam8093, 2017. doi: 10.1126/sciimmunol. aam8093.

Lee MSJ, Maruyama K, Fujita Y. et al.

Cevayir Coban's group revealed that malaria infection induces robust immune activation and invasion of parasite by-products into the bone marrow leading to harmful outcomes on bone homeostasis.

The Coban Team has used mouse malaria models to show that even after one time malaria infection, Plasmodium products gradually accumulate in the bone marrow niche and are "eaten-up" by bone resorbing cells osteoclasts. These accumulated malarial products in bone marrow niche induce MyD88-dependent inflammatory responses in osteoclast and osteoblast precursors, leading to increased RANKL expression and over-stimulation of osteoclastogenesis favoring bone resorption. Infection with a mutant parasite with impaired hemoglobin digestion that produces little hemozoin, a major Plasmodium by-product, did not cause bone loss. Importantly, malaria-mediated bone loss was found to be more severe in those of younger age, leading to growth retardation.



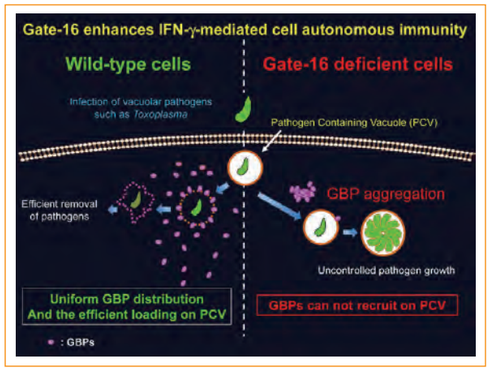
Essential role for GABARAP autophagy proteins in interferon-inducible GTPase-mediated host defense.

Nature Immunology. 18(8):899-910, 2017. doi: 10.1038/ni.3767.

Sasai M, Sakaguchi N, Ma JS. et al.

Masahiro Yamamoto's group and others revealed evealed a unique role of GABARAPs, in particular Gate-16, in interferon-γ (IFN-γ)-mediated antimicrobial response. The group showed that Gate-16 specifically associates with Arf1 to mediate uniform distribution of IFN-inducible GTPases, and that lack of Gate-16 reduces Arf1 activation, leading to formation of IFN-inducible GTPase-containing aggregates, hampering their function. Furthermore, mice lacking Gate-16 alone are susceptible to Toxoplasma. Thus, GABARAPs are uniquely required for antimicrobial host defense through cytosolic distribution of IFN-inducible GTPases.

These results will help Gate-16 targeted strategy for the treatment of toxoplasmosis and alimentary intoxication caused by Toxoplasma and Salmonella.

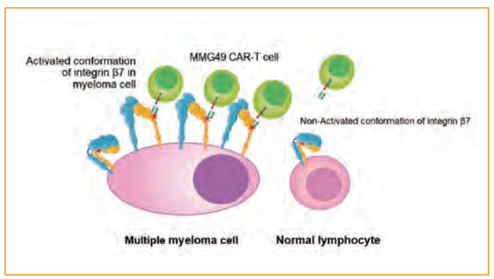


The activated conformation of integrin β7 is a novel multiple myeloma-specific target for CAR T cell therapy.

Nature Medicine. 23(12):1436-1443, 2017. doi: 10.1038/nm.4431.

Hosen N, Matsunaga Y, Hasegawa K. et al.

Naoki Hosen, Athushi Kumanogoh and their research group showed that the active conformer of an integrin can serve as a specific therapeutic target for multiple myeloma (MM). They screened >10,000 anti-MM mAb clones and identified MMG49 as an MM-specific mAb specifically recognizing a subset of integrin β7 molecules. The MMG49 epitope, in the N-terminal region of the β7 chain, is predicted to be inaccessible in the resting integrin conformer but exposed in the active conformation. Elevated expression and constitutive activation of integrin β7 conferred high MMG49 reactivity on MM cells, whereas MMG49 binding was scarcely detectable in other cell types including normal integrin β7+ lymphocytes. T cells transduced with MMG49-derived chimeric antigen receptor (CAR) exerted anti-MM effects without damaging normal hematopoietic cells. Thus, MMG49 CAR T cell therapy is promising for MM, and a receptor protein with a rare but physiologically relevant conformation can serve as a cancer immunotherapy target.



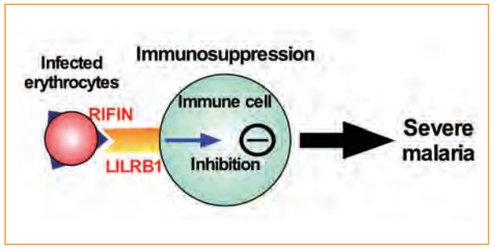
Immune evasion of Plasmodium falciparum by RIFIN via inhibitory receptors.

Nature. 552(7683):101-105, 2017. doi: 10.1038/nature24994.

Saito F, Hirayasu K, Satoh T. et al.

Malaria, one of the world's three major infectious diseases alongside tuberculosis and AIDS, has been reported to affect approximately 300 million people every year, accounting for about 500,000 deaths, and there is still no effective vaccine.

Hisashi Arase and others showed that P. falciparum uses immune inhibitory receptors to achieve immune evasion. RIFIN proteins are products of a polymorphic multigene family comprising approximately 150–200 genes per parasite genome3 that are expressed on the surface of infected erythrocytes. We found that a subset of RIFINs binds to either leucocyte immunoglobulin-like receptor B1 (LILRB1) or leucocyte-associated immunoglobulin-like receptor 1 (LAIR1). LILRB1-binding RIFINs inhibit activation of LILRB1-expressing B cells and natural killer (NK) cells. Furthermore, P. falciparum-infected erythrocytes isolated from patients with severe malaria were more likely to interact with LILRB1 than erythrocytes from patients with non-severe malaria, although an extended study with larger sample sizes is required to confirm this finding. Our results suggest that P. falciparum has acquired multiple RIFINs to evade the host immune system by targeting immune inhibitory receptors.

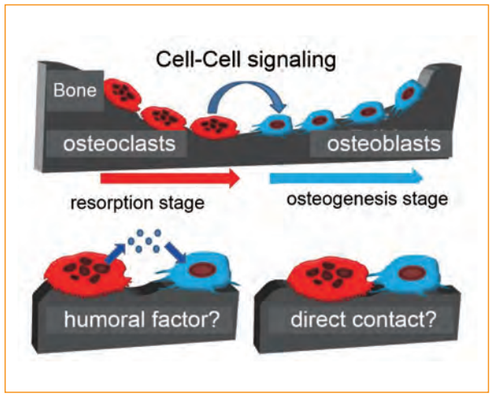


Direct cell–cell contact between mature osteoblasts and osteoclasts dynamically controls their functions in vivo.

Nat Commun. 9(1):300, 2018. doi: 10.1038/s41467-017-02541-w.

Furuya M, Kikuta J, Fujimori S. et al.

Masaru Ishii and his group showed, by using an intravital imaging technique, that mOB and mOC functions are regulated via direct cell–cell contact between these cell types. The mOBs and mOCs mainly occupy discrete territories in the steady state, although direct cell–cell contact is detected in spatiotemporally limited areas. In addition, a pH-sensing fluorescence probe reveals that mOCs secrete protons for bone resorption when they are not in contact with mOBs, whereas mOCs contacting mOBs are non-resorptive, suggesting that mOBs can inhibit bone resorption by direct contact. Intermittent administration of parathyroid hormone causes bone anabolic effects, which lead to a mixed distribution of mOBs and mOCs, and increase cell–cell contact. This study reveals spatiotemporal intercellular interactions between mOBs and mOCs affecting bone homeostasis in vivo.

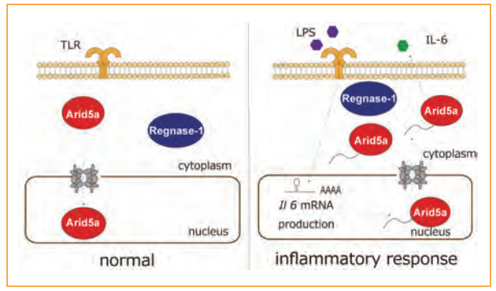


Regulation of inflammatory responses by dynamic subcellular localization of RNA-binding protein Arid5a.

Proc Natl Acad Sci USA. 115(6):E1214-E1220, 2018. doi: 10.1073/pnas.1719921115.

Higa M, Oka M, Fujihara Y. et al.

Tadamitsu Kishimoto and his research group revealed the regulatory mechanism of subcellular localization of Arid5a in response to inflammation. It has been known that an inflammatory accelerator, Arid5a, is localized in the nucleus, and an inflammatory brake, Regnase-1, is localized in the cytoplasm. In this study, they showed that 1) Arid5a translocates to the cytoplasm from the nucleus in response to inflammation, 2) bimax, which inhibit cNLS-dependent nuclear import via high-affinity interactions with NLS-binding sites of importin-α, inhibits the nuclear import of Arid5a, 3) CRM1 inhibitor, Leptomycin B, inhibits the nuclear export of Arid5a after LPS stimulation.

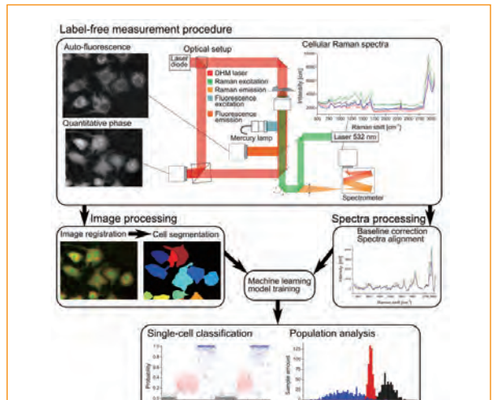


Combination of multiple optical measurements can assess macrophage activation at single-cell level without any contrast agent.

Proc Natl Acad Sci USA. 115(12):E2676-E2685, 2018. doi: 10.1073/pnas.1711872115.

Pavillon N, Hobro AJ, Akira S, and Smith N.

Nicholas I. Smith and the collaborators developed a label-free multimodal microscopy platform that allows the non-invasive study of cellular preparations without the need of any additional chemicals or contrast agent. The parameters extracted from these measurements, coupled with machine algorithms, enable the study of fine cellular processes such as macrophage cells activation upon exposure to lipopolysaccharide (LPS). The authors demonstrate that activation, as well as partial activation inhibition, can be observed at single-cell level through phenotypic and molecular characterization purely through non-invasive optical means.



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The articles were published between April, 2017 and March, 2018. The data were acquired using Web of Science Core Collection™ on May 25, 2018, and sorted by alphabetical order of the first authors.

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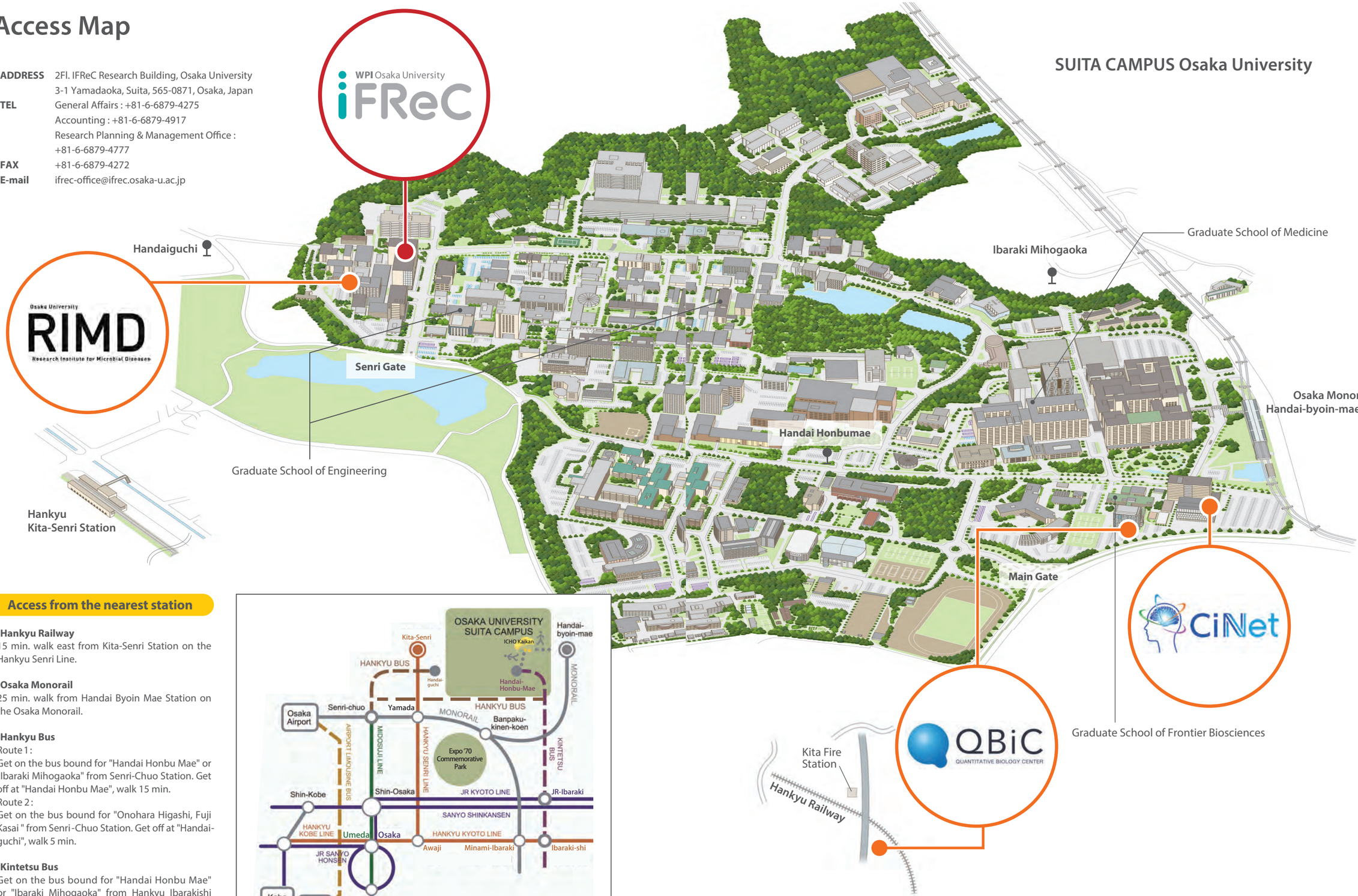

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By Hankyu Bus
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