Edit : Research Planning & Management Office, IFReC
Published in June, 2017

WPI Immunology Frontier Research Center FY2016

Osaka University

Annual Report
of IFReC
FY 2016

World Premier International
Research Center

Osaka University

Immunology Frontier Research Center

wpi
World Premier International
Research Center Initiative

WPI Osaka University
iFReC

Contents

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 2016.

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.

IFReC was created as part of the WPI program, a national project led by the Japanese Ministry of Education, Culture, Sports, Science and Technology. However, from FY2017, IFReC will mark a new stage in its history with a novel agreement for academic-industry partnership. This governance system is an ambitious program without precedent.

Although the governing structure will change, 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.

In FY2016, IFReC organized the "International Symposium on Advanced Immunology" to celebrate IFReC's first decade and Professor Tadamitsu Kishimoto's 77th birthday. In the symposium, the researchers of IFReC recognized past achievements, and shared the challenges in diverse research fields for the future.

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

Shizuo Akira

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

Looking back on IFReC's activities over the years

Jun Sakanoue (Research Planning and Management Office, IFReC)

IFReC's Research Achievements over the past decade

Since the establishment of IFReC in 2007, there have been 1270 scientific articles published by IFReC researchers. Over 10% of these articles have appeared in high impact journals such as Science, Nature, Cell and their affiliates.

The productivity of scientific articles by IFReC researchers is shown in Figure 1. In 2015, the number of articles dipped which is thought to be due to the end of the WPI grant by MEXT from FY2017. However, the conclusion of contracts between Osaka University and several pharmaceutical companies offered a bright outlook and people began to look toward the future again for the next generation of IFReC. For this reason, the number of researchers at IFReC increased again in 2016, and the number of papers recovered.

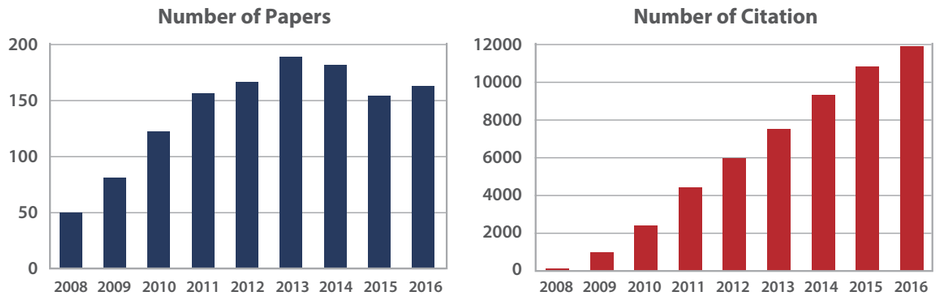

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Figure 1. Research Output in 2008-2016

Table 1 shows the extracted parameters for institution evaluation in all the research fields of IFReC.

Table 1. Number of Published Papers and their Citations in All the Research Fields

Number of Papers	1,270
Number of Citations	54,840
Citation Impact	43.2
h-index	96

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Table 2 shows the performance comparison between IFReC and two world top research institutions in the life science field, La Jolla Institute for Allergy and Immunology (LIAI), and Walter and Eliza Hall Institute of Medical Research (WEHI), Australia.

Since each of the three institutes has a different composition of research fields, to be fair, the parameters for comparison were calculated only in the three research areas of Immunology, Biochemistry & Molecular Biology, and Cell Biology.

Table 2. Performance Comparison between IFReC and Other Institutions

	WPI Osaka University iFReC	La Jolla Institute for Allergy and Immunology	Walter+Eliza Hall Institute of Medical Research
Staff (approx.)	200	250	1,000
Papers in Immunology, Biochemistry & Molecular Biology, Cell Biology	619	715	1,247
Citation Number	36,325	22,720	40,447
Citation Impact	58.7	31.8	32.4
h-index	77	71	94
Documents in Top 1%	6.6%	4.7%	5.4%
Documents in Top 10%	29.8%	23.4%	27.3%

Web of Science™ © 2017 Clarivate Analytics/Citation number and h-index as of March 10, 2017

Since IFReC has a lower number of researchers than LIAI or WEHI, the total citation number or h-index of IFReC did not match these institutes. However, IFReC's figures for Citation Impact, and Documents in Top 1% and Top 10% compared favorably with the other two institutes, allowing us to conclude that IFReC researchers have produced high-quality papers for the past ten years.

Internationalization and Interdisciplinary Researches

Promotion of internationalization and interdisciplinary research is an important mission of the WPI program and WPI institutions including IFReC.

The ratio of international co-authored papers is considered to be one of the indexes for internationalization of the institute. The ratio for IFReC remained around 41 to 43% after 2010 (Figure 2), whereas the average values by all Osaka University researchers in the immunology field are around 25% (data not shown). IFReC had been internationalized to some extent at an early stage of the WPI program, and generally continued to be flat thereafter. The ratio of international collaborative papers of LIAI and WEHI were 52% and 48%, respectively (2014-2016).

The ratio of papers in interdisciplinary researches areas in IFReC was 21% (2014-2016), and is still increasing.

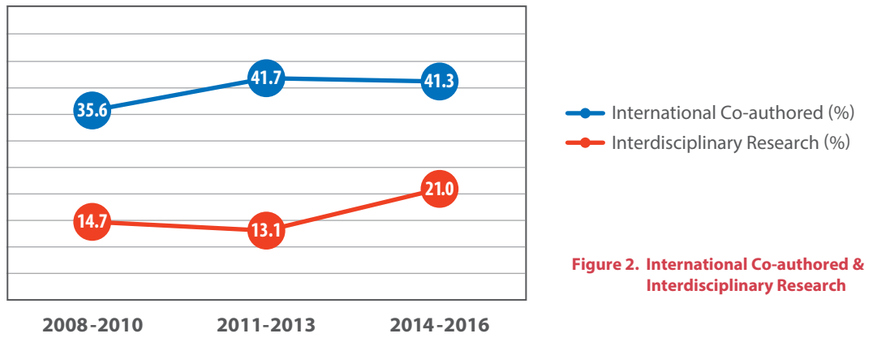

Figure 2. International Co-authored & Interdisciplinary Research

IFReC, which has created a large number of quality science papers, and promoted internationalization and interdisciplinary research, is surely commensurable with institutes honored with World Premier Status.

References

- Annual Reports of IFReC FY2010-2015
- Web of Science™ © 2017 CLARIVATE ANALYTICS
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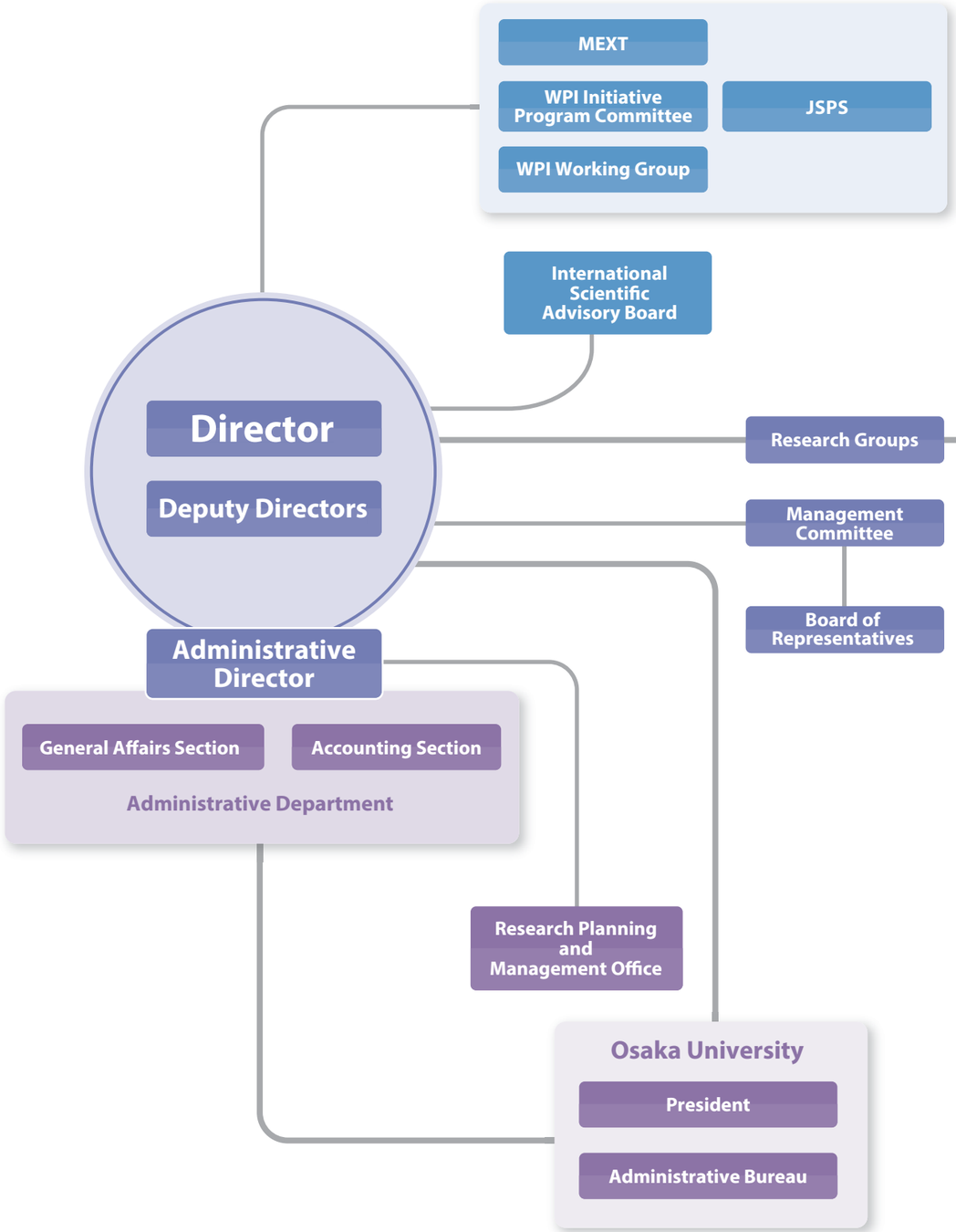
Organization

As of March, 2017

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

Imaging Group

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

Informatics Group

Information Systems	Yutaka Hata
Systems Immunology	Daron M Standley

Units for Combined Research Fields

Quantitative Immunology	Yutaro Kumagai Diego Diez
Immuno-Genomics	Alexis Vandenbon Hiromasa Morikawa

Common Facilities

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

Cooperative Institutions

Domestic

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

Overseas

- Convergent Research Consortium for Immunologic Disease, Seoul, St Mary's Hospital, Catholic University of Korea
- Indian Institute of Science Education and Research, India

Committee and Advisory Board for IFReC

As one of the nine centers selected for the World Premier International Research Center Initiative (WPI), IFReC has been the subject of evaluations including site visits and follow-ups by the WPI Program Committee.

In close cooperation with the Program Directors, the Program Officer and working group members, the WPI Program Committee conducts follow-up activities on progress being made by the WPI institutes including IFReC, with an eye to developing them into "highly visible research centers."

WPI Program Committee

Program Director

as of FY2016

Toshio Kuroki	Senior Advisor, Research Center for Science Systems, Japan Society for the Promotion of Science (JSPS), Japan

Deputy Program Director

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

Program Committee Members

Toshiaki Ikoma	Special Adviser, Canon Inc., Japan
Hiroto Ishida	President Emeritus, Kanazawa Gakuin University, Japan
Shinichiro Ohgaki	President, Japan Water Research Center, Japan
Maki Kawai	Director General, Institute for Molecular Science, National Institutes of Natural Sciences, Japan
Kiyoshi Kurokawa	Adjunct Professor, National Graduate Institute for Policy Studies, Japan
Makoto Kobayashi	Honorary Professor Emeritus, High Energy Accelerator Research Organization, Japan Nobel Laureate in Physics (2008)
Norihiko Suzuki	Chair of the Board/President, Akita International University
Ryozo Nagai	President, Jichi Medical University, Japan
Michiharu Nakamura	Counselor to the President, Japan Science and Technology Agency (JST), Japan
[Chairperson] Ryoji Noyori	Director-General, Center for Research and Development Strategy, JST, Japan Nobel Laureate in Chemistry (2001)
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

Working Group Leader and Assigned Members

as of FY2016

[Program Officer] Takehiko Sasazuki	University Professor, Institute for Advanced Study, Kyushu University, Japan
Hiroshi Kiyono	Professor, Institute of Medical Science, The University of Tokyo, Japan
Nagahiro Minato	Executive Vice-President for Research, Planning, and Hospital Administration, Kyoto University, Japan
Kazuhiko Yamamoto	Professor and Chairman, Department of Allergy and Reumatology, Graduate school of Medicine, The University of Tokyo, Japan
Günter J. Hämmerling	Professor, Tumorimmunology Program, German Cancer Research Center DKFZ, Germany
Hisataka Kobayashi	Associate (chief) scientist, Molecular Imaging Program, National Cancer Institute, National Institutes of Health, USA
Philippe Kourilsky	Professor, Collège de France/Honorary Director-General, The Institute of Pasteur, France

International Scientific Advisory Board

The International Scientific Advisory Board conducts evaluations on scientific achievements of IFReC PIs by reviewing their reports or direct interviews.

as of FY2016

Jeffrey Ravetch	The Rockefeller University, USA	**Immunology**
Christopher Goodnow	Australian National University	**Immunology**
Richard Locksley	University of California, San Francisco, USA	**Immunology**
Lewis L. Lanier	University of California, San Francisco, USA	**Immunology**
Anne O'Garra	The Francis Crick Institute, UK	**Immunology**
Kiyoshi Takatsu	Toyama Prefectural Institute for Pharmaceutical Research, Japan	**Immunology**
Kazuo Sugamura	Miyagi Cancer Center Research Institute, Japan	**Immunology**
Yale Goldman	University of Pennsylvania, USA	**Imaging**
Yasuyoshi Watanabe	Center for Life Science Technologies, RIKEN, Japan	**Imaging**
Masamitsu Iino	Nihon University School of Medicine, Japan	**Imaging**
Akinori Kidera	Yokohama City University, Japan	**Informatics**

All researches performed at IFReC are required to be reviewed by external scientists. Therefore an in-depth evaluation of research activities at IFReC was performed in a peer-review style by the International Scientific Advisory Board (ISAB), which consists of ten highly qualified scientists. Since this is the final year of WPI program, the entire research activities since joining IFReC of each PI were evaluated.

The evaluation was conducted through both document and interview. For each PI, one board member was assigned for document evaluation and two board members were assigned for the interview evaluation. Ten ISAB members gathered at IFReC on February 2nd and 3rd, 2017 for the interviews.

The document evaluation was based on a submitted research report including future prospectus, publication list, obtained patents, awards, invited lectures and list of external funding. In addition, the PIs attached copies of the five most important publications as an appendix to their progress report.

The board members evaluated the following items and provided the score and the comments.

Evaluation Items
1. Level of achievement
2. Scientific/technical merit
3. Research output and outcomes (research achievements, patents, invited lectures, etc.)
4. Promoting interdisciplinary research
5. Future prospects

Score 5 : Excellent, 4 : Good, 3 : Average, 2 : Fair, 1 : Poor

After summarizing the evaluation results, anonymous comments and scores made by board members were conveyed individually to PIs. The evaluations and advice received are invaluable to each PI and will surely contribute to their research in the future. Also, IFReC will make use of the comments and suggestions to open up new paths over the next ten years.

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 & Management Office

- Research Promotion & Support
  (Consultation for grants & patents, Fusion research program, etc.)
- Establishing Research Environments
  (Facility & Safety management, Research agreement, etc.)
- Fostering Young Scientists
  (Winter School etc.)
- Organizing Scientific Events
  (Symposia, Colloquia, Seminars, etc.)
- Public Relations
  (Publishing, Website, Outreach to citizens, etc.)
- WPI evaluation issues
  (Progress report, Advisory Board meeting, etc.)

As of March, 2017

Laboratories

Host Defense

Shizuo Akira, MD/PhD

Professor	**Shizuo Akira**
Associate Professor	**Kazuhiko Maeda**
Assistant Professor	**Takashi Satoh**
	Kenta Maruyama
	Hiroki Tanaka
Postdoctoral Fellow	**2**
Research Assistant	**9**
Visiting Scientist	**4**
Support Staff	**7**

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

Macrophages consist of at least two subgroups, M1 and M2. M1 macrophages are pro-inflammatory and have a central role in host defense. Whereas, M2 macrophages are associated with responses to anti-inflammatory reactions, and tissue remodeling. Previously we reported that Trib1 is critical for the differentiation of tissue-resident macrophages—that share characteristics with M2 macrophages (which we term M2-like macrophages). Trib1 deficiency results in a severe reduction of M2-like macrophages in various organs, including bone marrow, spleen and adipose tissues. Trib1 is critical for adipose tissue maintenance and suppression of metabolic disorders by controlling the differentiation of tissue-resident M2-like macrophages.

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. However, the specific subtypes of monocytes and macrophages that are involved in fibrosis have not yet been identified. We showed 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. Cebpb-deficiency results in a complete lack of SatM. Furthermore, the development of bleomycin-induced fibrosis, but not inflammation, was prevented in chimeric mice with Cebpb-/- haematopoietic cells. Adoptive transfer of SatM into Cebpb-/- mice resulted in fibrosis. Notably, SatM are derived from Ly6C-FcεRI+ granulocyte/macrophage progenitors, and a newly identified SatM progenitor downstream of Ly6C-FcεRI+ granulocyte/macrophage progenitors, but not from macrophage/dendritic-cell progenitors. Our results show that SatM are critical for fibrosis and that C/EBPβ licenses differentiation of SatM from their committed progenitor.

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 the CCCH-type zinc finger domain containing proteins. Regnase-1 knockout mice developed spontaneous autoimmune diseases accompanied by splenomegaly and lymphadenopathy. Regnase-1 is an endogenous RNase that destabilizes a set of mRNAs through cleavage of their 3' UTRs such as IL-6 and IL-12p40 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 (TCR) 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 understanding of the precious role of Regnase-1 in immune cells by using Regnase-1 mutant mice 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.

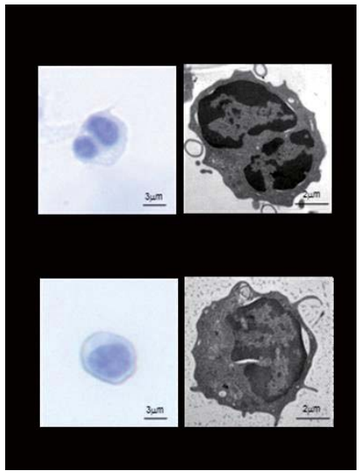


Figure 1.
The morphology of SatM

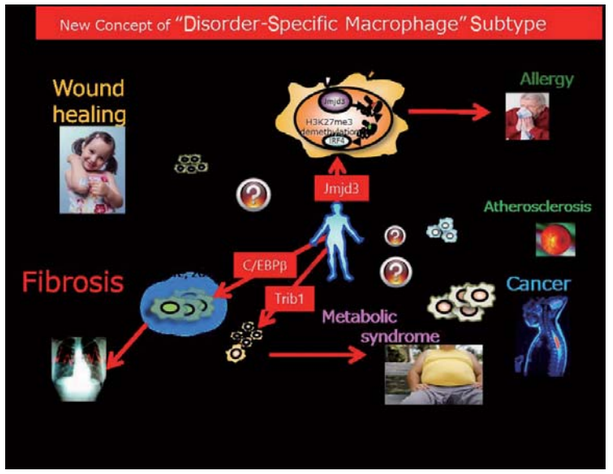


Figure 2.
Concept of disorder specific macrophages

Recent Publications

- Satoh T, et al. Identification of an atypical monocyte and committed progenitor involved in fibrosis. Nature 541, 96-101 (2017).
- Maeda K & Akira S. Regulation of mRNA stability by CCCH-type zinc-finger proteins in immune cells. Int. Immunol. (2017) in press.
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- Maeda K & Akira S. TLR7 Structure: Cut in Z-Loop. Immunity 45, 705-707 (2016).

Immunoglycobiology

Taroh Kinoshita, PhD

Professor	Taroh Kinoshita
Associate Professor	Yusuke Maeda
	Yoshiko Murakami
Assistant Professor	Yuko Tashima
	Noriyuki Kanzawa
Postdoctoral Fellow	2
Research Assistant	1
Visiting Scientist	3
Support Staff	3

More than 150 different proteins with various functions, such as receptors, adhesion molecules, enzymes, enzyme inhibitors and complement regulatory proteins, are anchored to the cell surface via glycosylphosphatidylinositol (GPI) that is amide-bonded to the carboxyl-terminus. Our laboratory has been working to clarify the mechanism of biogenesis, biological functions and medical significance of GPI anchors and GPI-anchored proteins (GPI-APs). In 2016 we made several discoveries.

Characterization of new pathogenic mutations responsible for inherited GPI deficiencies (IGDs):We reported the first cases of inherited GPI deficiency caused by mutations in *PIGC* and *PIGP* genes in collaborations with research groups from Canada and Israel (Edvardson, S. et al., *J. Med. Genet*., 54:196-201, 2016; Johnstone, D. L. et al, *Hum. Mol. Genet*., 2017). Three affected individuals from two families having global developmental delay, severe intellectual disability and seizures had homozygous or compound heterozygous loss-of-function mutations in the *PIGC* gene. Two siblings with biallelic loss-of-function *PIGP* mutations showed early-onset seizures, hypotonia and global developmental delay. Both PIGC and PIGP are components of the enzyme that mediates the initial step in GPI biosynthesis, transfer of N-acetylglucosamine to phosphatidylinositol (PI) (Figure). To date, pathogenic mutations that cause IGDs were identified in 16 of 27 genes involved in biosynthesis, transfer to proteins and maturation of GPI anchors.

Demonstration of new function of PIGN: PIGN is an enzyme that transfers ethanolaminephosphate side-chain to the first mannose in step 8 in GPI biosynthesis (Figure). In collaboration with Dr. Ihara's group in the National Institute of Genetics, we show PIGN is required for quality control in mammalian cells (Ihara, S. et al, *J Cell Sci*., 130:602-613, 2017). In *C. elegans pign-1* mutants, several proteins fail to be secreted and instead form abnormal aggregation in the endoplasmic reticulum (ER). *PIGN*-knockout HEK293 cells also showed similar protein aggregation. Certain mutation in *C. elegans pign-1* caused protein aggregation in the ER without affecting GPI-anchor biosynthesis. These results show that PIGN-1/ PIGN has a conserved and non-canonical function to prevent deleterious protein aggregation in the ER independently of the GPI-anchor biosynthesis. A defect in the non-canonical function of PIGN may contribute to clinical symptoms of individuals affected by IGD caused by *PIGN* mutations.

In collaboration with Dr. Yamaguchi's group in RIKEN, we have been characterizing p24γ2 complex, a cargo receptor for efficient transport of GPI-anchored proteins from the ER (Figure). In 2016, we reported crystal structures of GOLD domains of p24β1, p24γ2 and p24δ1 component of the cargo receptor (Nagae, M. et al, *J. Mol. Biol*., 428:4087-4099, 2016; *Proteins*, in press). The results support the idea that the p24 cargo receptor is an octamer consisting of two each of four components.

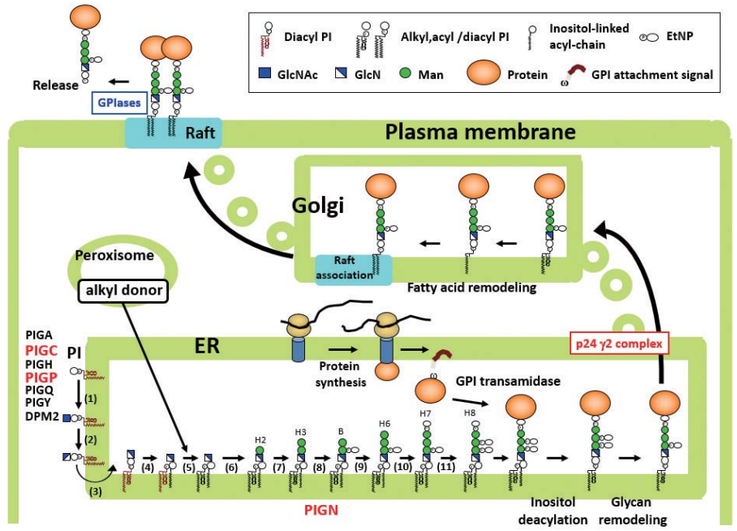

Figure.
Biosynthesis and transport of GPI-anchored proteins. PIGC and PIGP are components of N-acetylglucosaminine transferase that mediates the initial step in GPI biosynthetic pathway. We reported individuals with inherited GPI deficiencies caused by pathogenic mutations in PIGC and PIGP. PIGN is involved in step 8 in GPI biosynthesis. We reported that PIGN is required for protection of protein aggregation in addition to GPI biosynthesis. GPI-anchored proteins are packaged into transport vesicles by a cargo receptor consisting of four p24 proteins, α2, β1, γ2 and δ1. We report evidence for an octameric complex including two each of four components.

Recent Publications

- Lee GH, Fujita M, Takaoka K, Murakami Y, Fujihara Y, Kanzawa N, Murakami K, Kajikawa E, Takada Y, Saito K, Ikawa M, Hamada H, Maeda Y and 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 and Murakami Y. Pathogenic variants in PIGG cause intellectual disability with seizures and hypotonia. Am. J. Hum. Genet. 98, 615-626 (2016).
- Nagae M, Hirata T, Morita-Matsumoto K, Theiler R, Fujita M, Kinoshita T and Yamaguchi Y. 3D structure and interaction of p24β and p24δ Golgi dynamics domains: implication for p24 complex formation and cargo transport. J. Mol. Biol. 428, 4087-4099 (2016).
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- Theiler R, Fujita M, Nagae M, Yamaguchi Y, Maeda Y and Kinoshita T. The alpha helical region in p24γ2 subunit of p24 cargo receptor is pivotal for the recognition and transport of glycosylphosphatidylinositol-anchored proteins. J. Biol. Chem. 289, 16835-16843 (2014).

Immunopathology

Atsushi Kumanogoh, MD/PhD

Professor	**Atsushi Kumanogoh**
Assistant Professor	**Shohei Koyama**
Support Staff	**5**

Our research team is involved in two approaches; 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 diagnosis/therapy for human immunological disorders, such as autoimmunity, allergy, immune deficiency, cancer/metastasis, and neurodegenerative diseases. We focus on the functions of Semaphorin 4D (SEMA4D) as an immune check point for neutrophils and its pathological involvement in anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV).

We found that serum levels of soluble SEMA4D were elevated in AAV patients and were correlated with clinical disease scores. In neutrophils from AAV patients, cell surface expression of SEMA4D was significantly down-regulated, a consequence of ADAM17-mediated neutrophils-specific proteolytic shedding of SEMA4D. Soluble SEMA4D exerted pro-inflammatory effects on endothelial cells. Moreover, we found that neutrophil membrane-bound SEMA4D interacted with plexin B2 on endothelial cells, and this binding was required for the suppression of reactive oxygen species (ROS) production and resultant neutrophil extracellular trap (NET) formation. Treating neutrophils with recombinant plexin B2 led to the suppression of Rac1 activation through SEMA4D's intracellular domain, and resulted in the inhibition of the pathogen or ANCA IgG-induced neutrophil oxidative burst. Collectively, neutrophil surface SEMA4D functions as a negative regulator of neutrophil activation. Proteolytic cleavage of SEMA4D as observed in AAV patients may amplify neutrophil-mediated inflammatory responses. SEMA4D is a promising biomarker and potential therapeutic target for AAV.

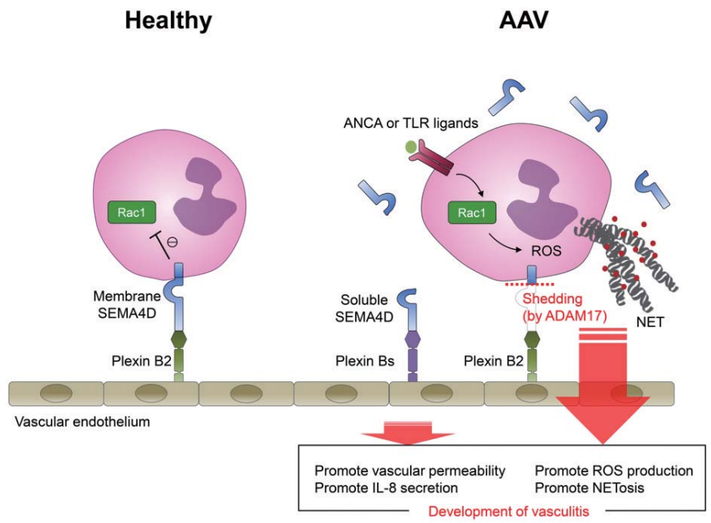

Figure. Graphical scheme of this study.
Under healthy conditions, the interaction between endothelial plexin B2 ligand and neutrophil cells surface SEMA4D receptor inhibits Rac1 activation and negatively regulates the generation of ROS and NET formation. In contrast, in AAV patients, neutrophil surface SEMA4D is shed by ADAM17. Soluble SEMA4D had pro-inflammatory functions on endothelial cells. In addition, alteration of the SEMA4D–plexin B2 interaction results in aberrant activation of neutrophils, and this dichotomous effect is involved in the pathogenesis of AAV.

Recent Publications

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Immunochemistry

Hisashi Arase, MD/PhD

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	Kouyuki Hirayasu
Postdoctoral Fellow	**4**
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We have been working 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.

Interaction between immune receptors and herpesviruses

PILRα is one of the immune inhibitory receptors that are expressed on various immune cells. We have previously found that both PILRα and PILRβ recognize CD99 as a host ligand (Shiratori et al. J. Exp. Med. 2004). In addition, we have identified PANP as a new ligand for PILR (Kogure et al. Biochem. Biophys. Res. Commun. 2011). PILRα is a unique receptor that that has binding sites for both sugar chains and protein structures (Wang et al. J. Immunol. 2008; Kuroki et al. Proc. Natl. Acad. Sci. USA. 2014). We 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). We further analyzed host cell molecules that associate with HSV-1 gB and found that non-muscle myosin heavy chain (NMHC-IIA) associates with gB and is involved in HSV-1 infection (Arii et al. *Nature* 2010). We also found that Siglec-4 (MAG, myelin associated glycoprotein) associates with VZV gB and mediates VZV infection. Because Siglec-4 is specifically expressed in neural tissues, Siglec-4 seemed to be involved in neurotropic characteristic of VZV (Suenaga et al. Proc. Natl. Acad. Sci. USA. 2010; Suenaga et al. J. Biol. Chem. 2015).

PILRα plays an important role in regulation of inflammation

We analyzed function of PILRα in immune response using PILRα-knockout mice. PILRα-deficient mice were susceptible to LPS-induced endotoxin shock. Further analyses revealed that infiltration of neutrophils in liver and lung was significantly increased in PILRα-deficient mice. (Wang et al. Nat. Immunol. 2012). Furthermore, PILRα-deficient mice showed severe DSS-induced colitis (Kishida et al. Int. Immunol. 2015) as well as increased tissue fibrilization (Kohyama et al. Eur. J. Immunol. 2016). These findings indicated that PILRα plays an important role in the regulation of inflammation by regulating integrin function.

Identification of new sensing system for immune abnormalities

We found that LILRA2 (also called ILT1, LIR-7 and CD85H), an orphan activating receptor expressed on human myeloid cells, recognizes abnormal immunoglobulins cleaved by microbial proteases but not normal immunoglobulins. Because immunoglobulins are quite important molecules in host defense, degradation of immunoglobulins is a very dangerous situation for immunity. Therefore, LILRA2 is a sensor to detect immunoglobulin abnormalities in microbial infection (Figure 1, Hirayasu et al. Nat. Microbiol. 2016).

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). 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).

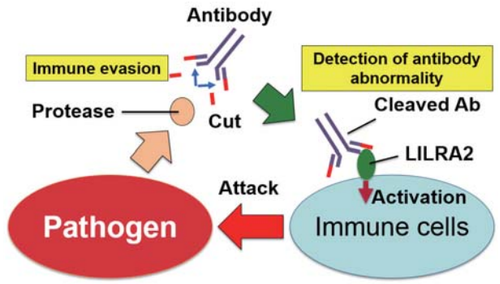

Figure 1. Microbially cleaved immunoglobulins are sensed by the innate immune receptor LILRA2
Microbial pathogens produce various proteases and degrade immunoglobulins to evade immune system. However, immune system has acquired a novel receptor, LILRA2, which specifically recognizes degraded immunoglobulins to detect immune abnormalities caused by microbial pathogens. The recognition of degraded immunoglobulins by LILRA2 plays an important role in host defense against pathogens (Hirayasu et al. Nat. Microbiol. 2016).

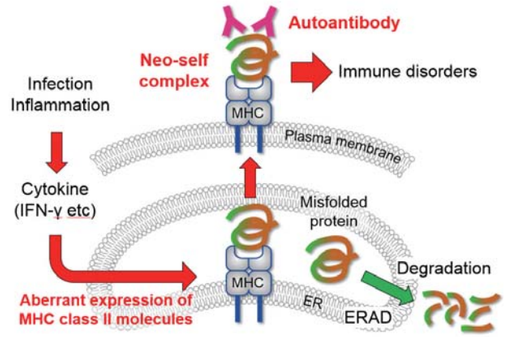

Figure 2. Misfolded proteins transported to the cell surface by MHC class II molecules are targets for autoantibodies
Cellular misfolded proteins are generally degraded in the cells and are not transported to outside the cells. Therefore, misfolded proteins transported to the cell surface by MHC class II molecules may be recognized as 'neo-self' antigens by 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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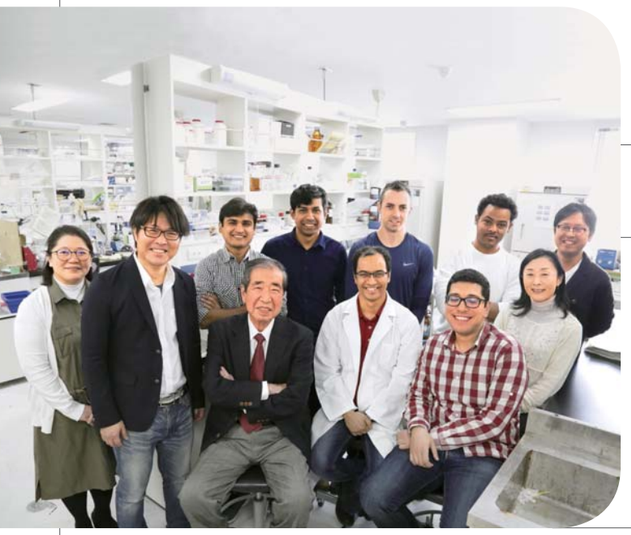
Immune Regulation



Tadamitsu Kishimoto, MD/PhD

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Introduction

Interleukin-6 (IL-6) is in important cytokine in early phase of acute immune responses, by which lymphocytes, hematopoietic cells and vascular endothelial cells are activated to protect the body against invasion of pathogens. In contrast, persistent production of IL-6 leads to development of various chronic diseases such as rheumatoid arthritis (RA) and juvenile idiopathic arthritis (JIA). Regarding the significance of IL-6 in the pathogenesis of chronic diseases, tocilizumab, a humanized anti-IL-6 receptor antibody successfully proved its outstanding efficacy against those chronic immune disorders. However, high levels of IL-6 expression have also been revealed in the sera of patients from 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, in which dysfunction led to sustain 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 has been shown to stabilize IL-6 mRNA (Figure 1). In fact, Arid5a deficient mice are resistant to development of endotoxin-shock and EAE mice model. In addition, we found a T cell-intrinsic role of Arid5a through stabilization of STAT3 mRNA. Reduction of STAT3 level in Arid5a deficient T cells led to the imbalance of STATs (STAT1, 3, 5) activation under Th17 polarizing conditions, which contributed to the alteration of the character of inflammatory T cells into that of anti-inflammatory T cells. Importantly, we revealed that Arid5a regulates naïve T cells-fate through selective stabilization of STAT3 mRNA, suggesting that Arid5a is required for RNA stabilization for IL-6 and STAT3 mRNAs.

Arid5a exacerbates IFN-γ-mediated septic shock by stabilizing T-bet mRNA. (Published)

Arid5a deficient mice are shown to be completely resistant to lipopolysaccharide (LPS) mediated shock. Histological analysis by H&E staining of lung, liver and spleen tissue indicated that Arid5a deficient mice are protected from tissue injury after LPS induced shock mice model. Recently, our studies have shown that Arid5a regulates IFNγ production through Tbx21 mRNA stabilization, which encodes T-bet, known as master regulator of Th1 cell differentiation. Consistent with the data, serum level of IFNγ was significantly reduced in LPS-induced Arid5a deficient mice. Consequently, reduction of IFNγ production contributed to the recovery of mortality in endotoxin shock. Additionally, Arid5a-deficient mice in *Propionibacterium acnes*-primed endotoxin shock model, which elicits Th1 responses, showed lower levels of IFNγ, IL-6 and TNFα, with a higher survival rate. These results strongly suggest that Arid5a controls the stabilization of many inflammatory mRNAs, which in turn, contributes to the pathogenesis of inflammatory and autoimmune disease (Figure 1).

Arid5a-deficient mice are highly resistant to bleomycin-induced lung injury (Published)

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are clinical conditions involving acute respiratory dysfunction with high mortality. We examined whether Arid5a is involved in the pathogenesis of acute lung inflammatory diseases. Interestingly, we observed that Arid5a deficient mice are resistant to bleomycin-induced lung injury-mediated mortality. Arid5a deficiency suppressed not only for IL-6 production, but also reactive oxygen species (ROS) expression in lung. Moreover, immunohistological data of lung tissue suggests that Arid5a deficiency could protect mice from bleomycin-induced lung fibrosis which indicates the important role of Arid5a in lung tissue fibrosis. Therefore, the control of Arid5a expression should be a potential therapeutic target for treatment of acute lung inflammatory disorders.

Rabex-5 is a lenalidomide target molecule that negatively regulates TLR-induced type 1 IFN production. (Published)

A recent study identified Cereblon as the receptor for immunomodulatory drugs (IMiDs) compounds responsible for thalidomide teratogenicity. To understand the mechanism underlying the anti-inflammatory properties of IMiDs we generated Cereblon deficient mice using the CRISPR/Cas9 system. Importantly, thalidomide was effective in inhibiting TLR4 induced TNFα production even in the absence of Cereblon. Thus, we concluded that the inhibitory effect of thalidomide on TLR induced cytokine production occurs independently of Cereblon. The significance of this study is that certain therapeutic properties of IMiDs can be separated from pathways leading to teratogenicity. However, the anti-inflammatory properties of IMiDs are unlikely to be entirely Cereblon-independent. To identify IMiD target molecules that might contribute to the anti-inflammatory properties of IMiDs we searched for Cereblon interacting proteins affected by IMiD binding. We subsequently identified Rabex-5 as a Cereblon interacting protein. Signficantly, IMiD binding prevented the association of Cereblon with Rabex-5. Knockdown of Rabex-5 in a human macrophage cell line upregulated TLR induced type-1 interferon production *via* a MYD88 dependent STAT1 activating pathway. We have proposed that disruption of the Cereblon-Rabex-5 complex releases Rabex-5 to suppress TLR induced STAT1 activation, contributing to the efficacy of these compounds in autoimmune disorders (Figure 2).

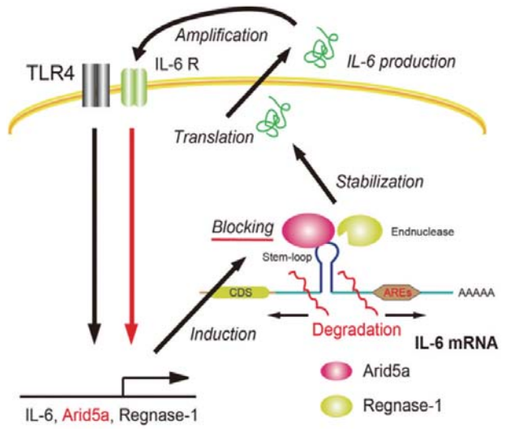

Figure 1.
Amplification of IL-6 level by Arid5a.

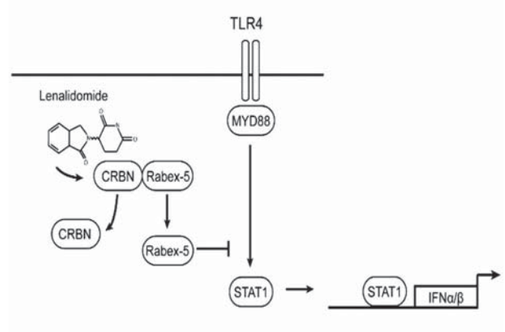

Figure 2.
Lenalidomide disrupts a Celeblon-Rabex-5 complex to suppress TLR induced type-1 interferon production.

Recent Publications

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- Masuda K, et al. Arid5a regulates naive CD4+ T cell fate through selective stabilization of Stat3 mRNA. J. Exp. Med. 213(4), 605-19 (2016).

Mucosal Immunology

Kiyoshi Takeda, MD/PhD

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Assistant Professor	**Hisako Kayama**
	Ryu Okumura
	Mari Murkami
Research Assistant	**6**
Support Staff	**3**

Our laboratory is analyzing the mechanisms how intestinal homeostasis is maintained to reveal the pathogenesis of inflammatory bowel diseases (IBD). Several recent evidences have indicated that both intestinal environmental factors including microbiota and intestinal immunity (genetic predisposition) contribute to the development of IBD. However, there exists a single layer of intestinal epithelial cells between intestinal environmental factors and intestinal immunity. We thought that intestinal epithelial cells possess some barrier functions that separate intestinal environmental factors and intestinal immunity, and analyzed the barrier function of intestinal epithelial cells.

Barrier function of intestinal epithelial layers

Genome-wide association studies have identified several susceptible loci for IBD including ulcerative colitis (UC). Among genes that are located within these loci, we tried to find a gene that is highly expressed in colonic epithelial cells. A gene encoding RING finger protein *186* (*RNF186*), which was located within UC-susceptible loci, was found to be highly expressed in epithelial cells in the colon. Therefore, we analyzed the role of *RNF186* in the regulation of intestinal homeostasis. RNF186, which harbored RING finger domain, acted as an E3 ligase mediating polyubiquitination of its substrates. Comparison of protein expression levels using liquid chromatography-tandem mass spectrometry showed that expression of several proteins was increased in colonic epithelial cells of *Rnf186*$^{-/-}$ mice. These included occludin. RNF186 was found to associate with these proteins and induce polyubiquitination. The polyubiquitination induced by RNF186 was K48-mediated, which leads to protein degradation. These findings indicate that RNF186 regulates protein expression the colonic epithelial cells by inducing polyubiquitinaton. The disturbed protein homeostasis in *Rnf186*$^{-/-}$ mice correlated with enhanced endoplasmic reticulum (ER) stress in colonic epithelia and increased sensitivity to intestinal inflammation after dextran sulfate sodium (DSS) treatment. A previous deep sequencing study of the UC-susceptible loci had found a single nucleotide polymorphism (SNP) in a coding region of the *RNF186* gene. Introduction of an UC-associated *Rnf186* SNP led to impaired E3 ligase activity and increased sensitivity to DSS-induced intestinal inflammation in mice. Taken together, these findings demonstrate that *RNF186* is responsible for the maintenance of gut homeostasis by controlling ER stress in colonic epithelia (Figure 1).

Dysbiosis triggers arthritis development

We also analyzed the involvement of microbiota in several disorders other than IBD. Several previous studies using mouse models of arthritis indicated that the intestinal microbiota is involved in arthritis pathogenesis. Therefore, we analyzed whether microbiota is involved in the pathogenesis of rheumatoid arthritis (RA). We first compared the composition of fecal microbiota between healthy volunteers and RA patients. Altered microbiota composition, called dysbiosis, was found in some RA patients. These RA patients harbored intestinal microbiota dominated by *Prevotella copri*. We then analyzed how dysbiosis contributes to arthritis development using arthritis-prone SKG mice harboring human microbiota. SKG mice reared in a specific pathogen-free facility developed arthritis upon fungal component injection; however, elimination of intestinal microbiota by oral administration of antibiotics protected these mice from joint inflammation. Then, germ-free SKG mice were generated and these mice were colonized with human fecal microbiota from healthy volunteers and *Prevotella*-dominated RA patients. SKG mice harboring both the microbiota of healthy controls and the *Prevotella*-dominated microbiota from RA patients (RA-SKG) were successfully produced. RA-SKG mice developed severe arthritis after fungal component injection. Lymphocytes in regional lymph nodes and colon, but not spleen, of RA-SKG mice showed enhanced IL-17 responses to the arthritis-related autoantigen, RPL23A. The RPL23A-induced T cell response was attenuated in germ-free SKG mice. Naïve SKG T cells, co-cultured with *P. copri*-stimulated dendritic cells, responded to RPL23A and rapidly induced arthritis. SKG mice monocolonized with *P. copri* developed severe arthritis. Thus, SKG T cells are activated to vigorously react to autoantigens by dysbiotic microbiota in the intestine, causing joint inflammation (Figure 2). These findings indicate that dysbiosis is an environmental factor that triggers arthritis development in genetically susceptible mice.

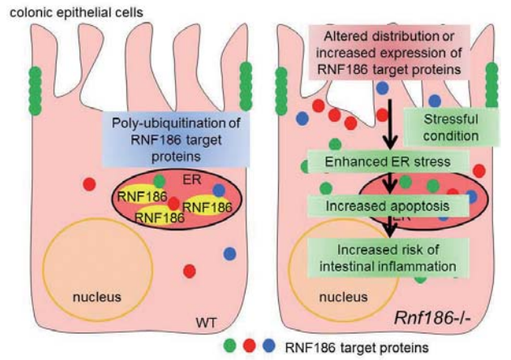

Figure 1.
RNF186 is responsible for the maintenance of protein homeostasis in the colonic epithelial cell. In the absence of RNF186, expression of RNF186-targeted protein was increased, causing high susceptibility to stress-induced cell death, which leads to the high sensitivity to intestinal inflammation.

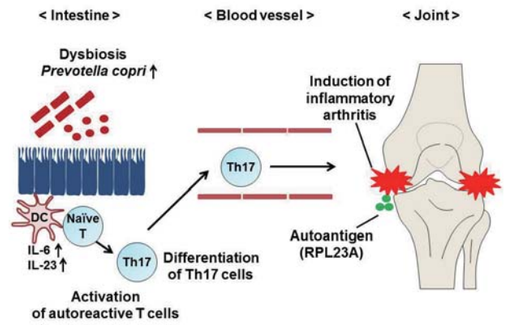

Figure 2.
A subpopulation of rheumatoid arthritis (RA) patients showed dysbiotic microbiota dominated by *Prevotella copri*. SKG mice harboring *Prevotella copri* -dominated microbiota from RA patients (RA-SKG mice) showed severe inflammatory arthritis as well as increased number of Th17 cells in the regional lymph nodes and large intestine. Moreover, lymphocytes in regional lymph nodes and large intestine of these mice showed enhanced IL-17 responses to arthritis-related autoantigen (RPL23A). Thus, dysbiosis triggers arthritis development via activation of autoreactive T cells in the intestine.

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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. 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 K_D 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 (Figure 1). 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. Our result provides a new structural insight into antigen recognition by anti-DNA antibodies in SLE.

Tracking of 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 in sinonasal cavity, such as *Streptococcus pyogenes*. Next, to comprehend the development pathway of such antigen-specific IgE, deep sequencing of NP-associated BCR repertoires was performed (Figure 2). 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.

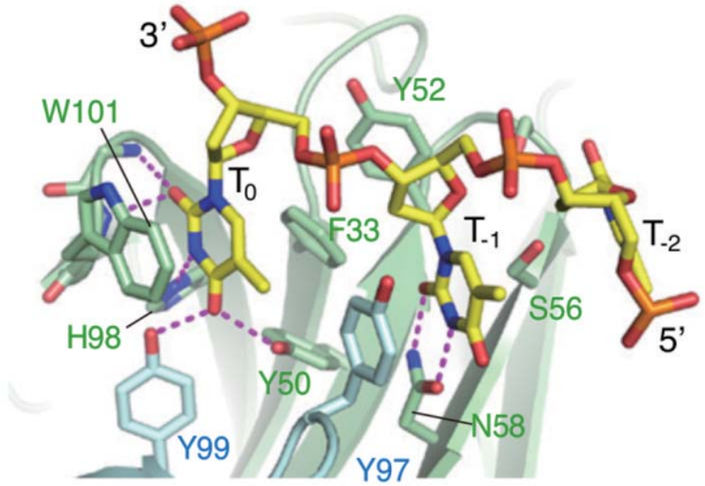

Figure 1.
X-ray crystallography of anti-DNA antibody 71F12.
The Fab binds to thymines through a stacking interaction (W101 of the heavy chain) and several hydrogen bonds. It should be noted that mutated F33 of the heavy chain locates at the antigen recognition site.

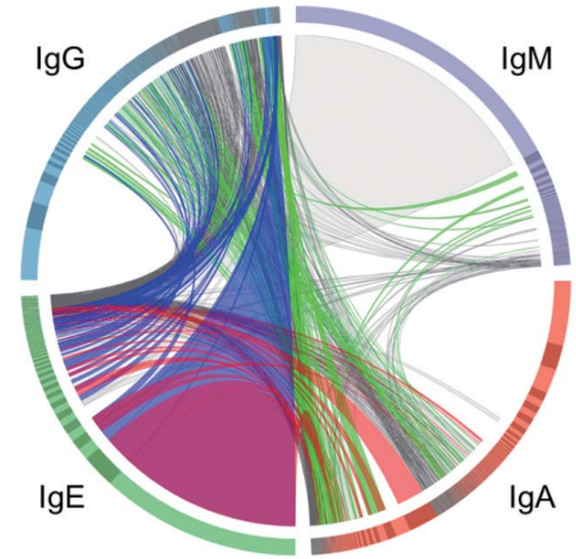

Figure 2.
Clonal connectivity between isotypes in NP-associated immunoglobulins.
Deep sequencing analysis of expressed BCR repertoires unraveled the clone distribution across immunoglobulin isotypes. Large IgE clones were strongly connected with IgG and IgA lineages. This analysis was performed by Dr. Shota Nakamura, "Dr. Kazuo Yamashita" and Prof. Daron M. Standley in RIMD, Osaka Univ.

Experimental Immunology

Shimon Sakaguchi, MD/PhD

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Postdoctoral Fellow	**3**
Research Assistant	**4**
Support Staff	**6**

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. The majority of them develop in the thymus as a functionally distinct and mature T-cell subpopulation, with their stable Foxp3 expression chiefly maintained by Treg-specific DNA demethylation (Ohkura et al., Immunity 2012, 2013; Morikawa et al., PNAS 2014). It is poorly understood, however, how Treg-specific transcriptional and epigenetic changes are initiated and coordinated to determine the Treg cell lineage in the thymus. This year, with recently demonstrated associations of super-enhancers with cell type-specific gene regulation and lineage determination in various cell types, we identified Treg cell-specific super-enhancers (Treg-SEs), many of which were associated with the Treg signature genes, such as *Foxp3*, *Ctla4* and *Il2ra* (Kitagawa et al., Nat. Immunol. 2017). The establishment of Treg-SEs developmentally began in Treg progenitor cells before *Foxp3* transcription and Treg-specific DNA demethylation, facilitating early induction of the associated genes. It required the genome organizer Satb1, which bound to Treg-SEs before their activation and extended its binding sites within the SEs along Treg cell differentiation. T cell-specific deletion of Satb1 impaired Treg-SE formation in Treg precursor cells, hindering both Treg-specific DNA demethylation and the transcription of Treg-SE-associated genes including *Foxp3*. The consequent arrest of Treg cell differentiation at the precursor stage resulted in spontaneous development of severe autoimmunity and IgE hyperproduction. Our results thus demonstrate how Satb1-dependent Treg-SE establishment and subsequent transcriptional and epigenetic changes control Treg cell linage specification in the thymus, and how molecular anomaly in this process causes autoimmune and other immunological diseases via affecting Treg cell development.

Based on the findings described above with mice, we are now studying how Treg-specific transcriptional and epigenetic alterations control the function, development, and functional stability of Treg cells in humans, and how genetic variations of Treg-specific functional genes contribute to the development of autoimmune and other immunological diseases.

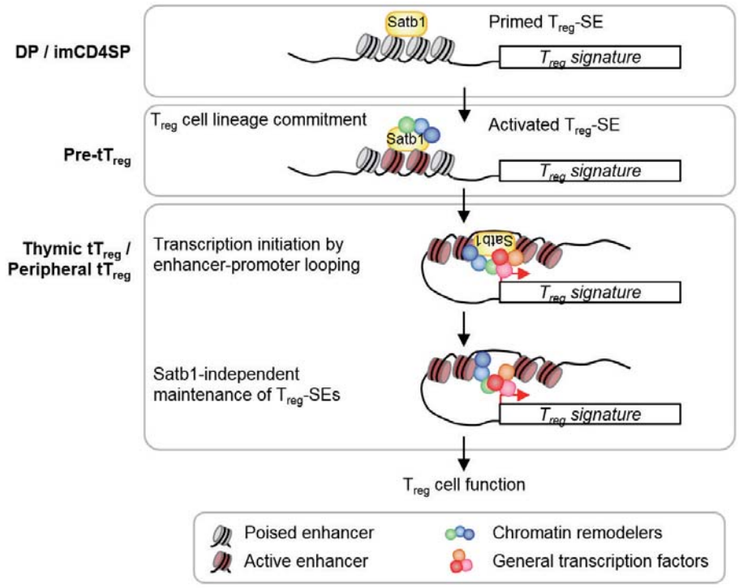

Figure. Establishment of Treg-specific super-enhancers for Treg cell development
A model of super-enhancer (SE) establishment and subsequent transcriptional regulation during thymic Treg cell development. Treg-SE regions are poised at least from the CD4/CD8 double-positive (DP) stage and bound by Satb1. Upon receiving the signal to direct Treg cell differentiation via the immature CD4 single-positive (SP) stage, Treg-SE regions undergo Satb1-dependent activation, likely through the recruitment of epigenetic modifying enzymes and become Treg lineage-committed precursor cells. As Treg cell development proceeds, enhancer-promoter looping facilitates the expression of associated Treg cell signature genes, including *Foxp3*, as well as other epigenetic modifications such as T_{reg}-specific DNA demethylation, histone modification of promoter regions and chromatin loosening of enhancer and promoter regions. Once Treg cell development is complete, Foxp3 amplifies pre-established molecular features and Satb1 transcription is repressed by Foxp3; however, mediator, cohesin, and various transcription factors, including Foxp3, occupy the sites where Satb1 initially bound, maintaining the local chromatin structure.

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- Kitagawa Y, Ohkura N, Kidani Y, Vandenbon A, Hirota K, Kawakami R, Yasuda K, Motooka D, Nakamura S, Kondo M, Taniuchi I, Kohwi-Shigematsu T, Sakaguchi S. Guidance of regulatory T cell development by Satb1-dependent super-enhancer establishment. Nature Immunol. 18, 173-183, (2017).
- Saito T, Nishikawa H, Wada H, Nagano Y, Sugiyama D, Atarashi K, Maeda Y, Hamaguchi M, Ohkura N, Sato E, Nagase H, Nishimura J, Yamamoto H, Takiguchi S, Tanoue T, Suda W, Morita H, Hattori M, Honda K, Mori M, Doki Y and Sakaguchi S. Two $FOXP3^+CD4^+$ T-cell subpopulations distinctly control the prognosis of colorectal cancers. Nature Med. 22, 679-684 (2016).
- 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).
- Ito Y, Hashimoto M, Hirota K, Ohkura N, Morikawa H, Nishikawa H, Tanaka A, Furu M, Ito H, Fujii T, Nomura T, Yamazaki S, Morita A, Vignali DA, Kappler JW, Matsuda S, Mimori T, Sakaguchi N, and Sakaguchi S. Detection of T-cell responses to a ubiquitous cellular protein in autoimmune disease. Science. 346, 363-368 (2014).
- Wing JB, Ise W, Kurosaki T, and Sakaguchi S. Regulatory T-cells control antigen-specific expansion of Tfh cell number and humoral immune responses via the coreceptor CTLA-4. Immunity. 41, 1013-1025 (2014).

Cell Signaling

Takashi Saito, PhD

Professor **Takashi Saito**

T cells play central roles in initiating and regulating immune responses. They start immune response, induce activation, and generate various effector T cells, which function for protection against infection and tumorigenesis. Aberrancy in T cell function results in infectious and autoimmune diseases. Our group aims to determine the molecular mechanism of T cell activation, differentiation and homeostasis, particularly from the perspective of signaling regulation.

T cells are activated upon antigen recognition by association with antigen-presenting cells such as dendritic cells (DC). Association between T cells and DC generates immune synapse at the interface. We have found that activation is induced through TCR-microclusters (MC), the signaling clusters to induce T cell activation, prior to immune synapse formation. TCR-MCs are generated by recruiting TCR and proximal signaling molecules such as kinases, adaptors, and effector molecules. We found that each TCR-MC is surrounded by an adhesion-ring composed of integrin and focal adhesion molecules, such as LFA-1, paxillin, Pyk2 and vinculin, at the initial stage of activation. The structure resembles immune synapse in micro-scale, thus termed *microsynapse* (Figure). TCR-MCs, ZAP70, SLP76 and F-actin were detected at the center, and LFA1, Paxillin, Pyk2 are in the outside ring of microsynapse. Microsynapse supports integrin outside-in signal and cell adhesion, which help the formation of MCs and cSMAC, and consequently T cell activation. Microsynapse is particularly important in activation of T cells under weak stimulation conditions because weak stimulation induces long-lasting microsynapse whereas strong stimulation merely induces transiently. Thus, microsynapse and MCs seem to be the major structures to induce activation signal in T cells. Under weak stimulation, T cells do not induce the formation of cSMAC, however, TCR-MCs function to induce activation with the support of microsynapse for cell adhesion.

We continued to analyze spatio- and temporal-regulation of T cell activation, particularly by analyzing individual critical activation pathways. Since activation of the Ras-MAPK pathway is spatially regulated, dynamics of two Ras GEFs, Ras-GRP1 and Sos, were investigated. Whereas Sos is initially co-localized with TCR-MC, Ras-GRP1 is accumulated in a unique mesh-like cytoskeletal structure upon stimulation. Since RasGRP1 dominantly functions as GEF in T cells, we concentrated on analyzing the dynamics of RasGRP1. The membrane localization was mediated through C-terminus region of RasGRP1. Mass analysis identified Plectin, a known cytoskeletal linker, as a binding molecule to this region of RasGRP1. Plectin-deficient T cells failed to generate the mesh structure of RasGRP1 and consequently reduced IL-2 production upon TCR stimulation. Thus, Ras activation is mediated by spatial regulation of RasGRP1 through linking cytoskeleton.

We have also investigated the regulation of T cell activation and function by innate signals. We have already analyzed the functions and signaling of TLRs, and nucleic acid recognition in T cells. STING is now a major intracellular DNA sensor in innate cells. Since we found that STING is highly expressed in T cells, the function of STING activation in T cells is analyzed. Surprisingly, STING stimulation resulted in inhibition of T cell proliferation and induces anti-viral and anti-tumor responses including type I-IFN secretion. Therefore, STING ligands possibly serve as specific modulators of activated effector T cells.

The ultimate aim of our approaches to analyze T cell signaling and function is to elucidate the mechanism of inducing autoimmune diseases through aberrant T cell signaling and function and to be able to modulate signaling and function in order to inhibit/prevent autoimmunity and allergic inflammation. We are analyzing function and regulation by phosphatase (PTPN22, PTPN2) whose mutations and deficiency are related to induction of autoimmune diseases. Then the onset of autoimmunity will be analyzed in mouse models.

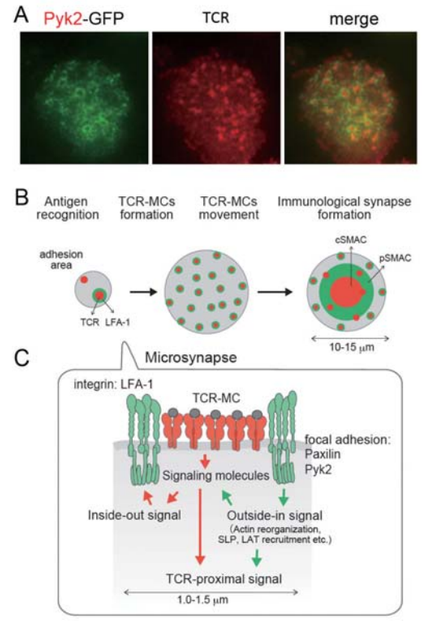
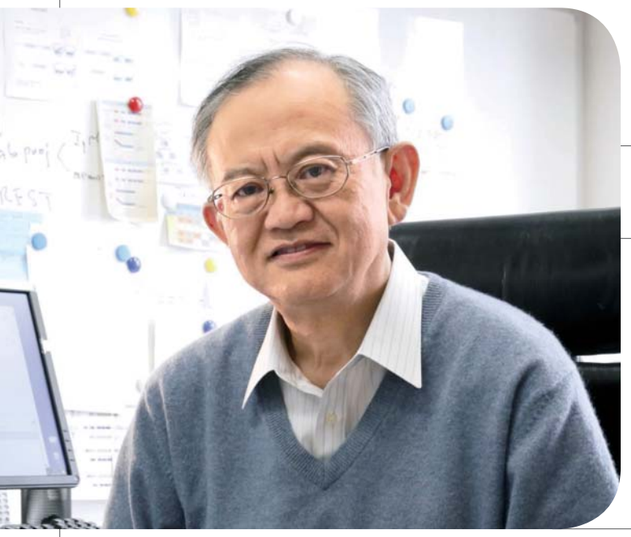


Figure. Microsynapse composed of micro-adhesion ring surrounding TCR-microcluster is essential for T cell activation
(A) Focal adhesion molecule Pyk2 (green) forms micro-adhesion ring around TCR-microclusters (red) upon T cell stimulation.
(B) Microsynapse composed of micro-adhesion ring around TCR-microcluster is formed in early activation and help formation of microcluster and cSMAC and T cell activation, particularly under weak stimulation.
(C) Adhesion-ring in microsynapse serves inducing outside-in signals through LFA1/focal adhesion molecules helps inducing activation signals through TCR-microclusters.

Recent Publications

- Takeuchi A and Saito T. CD4$^+$ CTL, a Cytotoxic Subset of CD4$^+$ T cells, Their Differentiation and Function. Front. Immunol. 8, 194 (2017).
- Ishikawa E, et al. Protein kinase D regulates positive selection of CD4$^+$ thymocytes through phosphorylation of SHP-1. Nat. Commun. 7, 12756 (2016).
- Hashimoto-Tane A, et al. The Micro adhesion-ring surrounding each TCR microclusters forms synapse-like structure 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).
- Matsumoto T, et al. Overexpression of CTLA-4 prevents atherosclerosis in mice. Arterioscl. Throm. Vas. 36, 1141-1151 (2016).

Lymphocyte Differentiation



Tomohiro Kurosaki, MD/PhD

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Assistant Professor	**Takeshi Inoue**
	Ryo Shinnakasu
	Shinya Tanaka
Research Assistant	**2**
Support Staff	**4**

The immune system acts in a trade-off manner between enhanced immune response and an increased risk of autoimmunity. For example, the PTPN22^{R620W} polymorphism provides increased protection against tuberculosis and is associated with increased susceptibility to several autoimmune diseases.

Involvement of DNA demethylases, Tet2 and Tet3 in B cell tolerance

Genome-wide association studies (GWAS) have identified hundreds of gene polymorphisms that are associated with an increased risk of developing autoimmunity. Among them, Tet3 was identified to be associated with SLE; Tet3 is one of the recently identified Ten-eleven translocation (Tet) dioxygenase family proteins (Tet1, Tet2, and Tet3). They act as DNA demethylase by catalyzing the conversion of 5-methylcytosine (5mC) in DNA to 5-hydroxymethylcytosine (5hmC) and other intermediate products in the DNA demethylation pathway. We showed that deficiency of Tet DNA demethylases, Tet2 and Tet3, in B cells led to accumulation of effector T cells, autoantibody formation, and nephritis. By using this mouse model, we found that Tet2- and Tet3-deficient B cells manifested hyper-responsive to B cell receptor, thereby inducing co-stimulatory molecules CD80/86 at the initiating phase in lupus. At the promoting phase, treatment of anti-CD4 antibodies ameliorated aberrant activation of B cells and autoantibody titers. Thus, our results suggest that dysregulation of CD80/86 on B cells is one of the key drivers for initiating the positive loop between autoreactive B and T cells, resulting in full-blown autoimmunity.

Selection of high-affinity antibodies during germinal center reactions

The hallmark of antibody-mediated adaptive immunity is the progressive increase in the ability of serum antibodies to bind antigen and efficiently provide immune protection. This phenomenon, known as affinity maturation, takes place majorly in the germinal center (GC), where antigen-specific GC B cells diversify their BCRs by somatic hyper-mutation. We found that the IRF4^{+}Bcl6^{lo} light zone (LZ) GC subset with high affinity BCRs favored the plasma cell fate over GC cycling. Cells in this plasma-prone fraction was increased or decreased by stronger T cell help or haplo-insufficiency of CD40, respectively. ICAM and SAP on GC B cells were upregulated by strong CD40 signals, thereby affording stronger interaction between IRF4^{+}Bcl6^{lo} cells and T_{FH} cells. Together, our data suggest that high CD40 signal induces stronger T-B interaction, thereby conferring differentiation potential towards plasma cells concomitantly with losing GC potential (Fig.1).

Regulation of germinal center B cell proliferation by Foxo1

Foxo1, forkhead box protein O1, is a transcriptional regulator that localizes to the nucleus and controls gene expression. As GC-derived lymphomas frequently carry mutations in Foxo1, Foxo1 might play a critical role in controlling gene expression within GC B cells. To address Foxo1 function in GC biology, we conducted an experimental system in which Foxo1 is inducibly deleted specifically in GC B cells. We found that ablation of Foxo1 after GC development led to the loss of the GC DZ B cells, suggesting that Foxo1 exerts a proliferative role, rather than promoting quiescence as previously thought. Mechanistically, upon provision of adequate T cell help, Foxo1$^{-/-}$ GC LZ B cells could not induce transcription factors, c-Myc ant BATF, both of which are essential for GC B cell proliferation. We confirmed that BATF was transiently induced in LZ B cells in a Foxo1-dependent manner and that depletion of BATF similarly led to GC disruption. Thus, our results support a model in which the switch from the LZ to DZ is triggered after receipt of T cell help, and suggest that Foxo1-mediated BATF up-regulation plays an important role in this process (Fig.2).

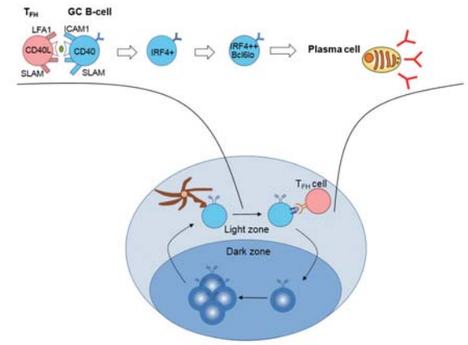

Figure 1. Strong T cell help confers the differentiation potential toward plasma cells
GC-B cells with high affinity BCR receive strong T cell help via CD40, which leads to up-regulation of adhesion molecules such as ICAM-1 or SLAM. The consequence of strong interaction with TFH is the induction of IRF4+ Bcl6lo GC B cells which favor plasma cell differentiation rather than GC re-cycling.

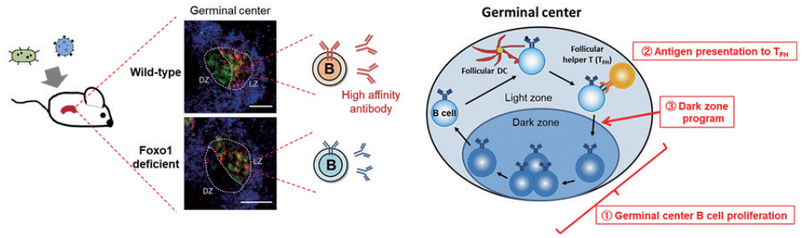

Figure 2. Regulation of GC B cell proliferation by Foxo1
(Left) Without Foxo1, germinal center structure is disorganized and mice could not produce high affinity antibodies against foreign antigens. (Right) DZ is lost and the GC is collapsed upon Foxo1 ablation. Foxo1 has also important roles in the LZ B cells, including antigen presentation to TFH cells and expression of DZ program genes, including BATF.

Recent Publications

- Shinnakasu R, Kurosaki T. Regulation of memory B and plasma cell differentiation. Curr. Opin. Immunol. 45, 126-131 (2017) Epub ahead of print.
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- 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), 861-9 (2016).
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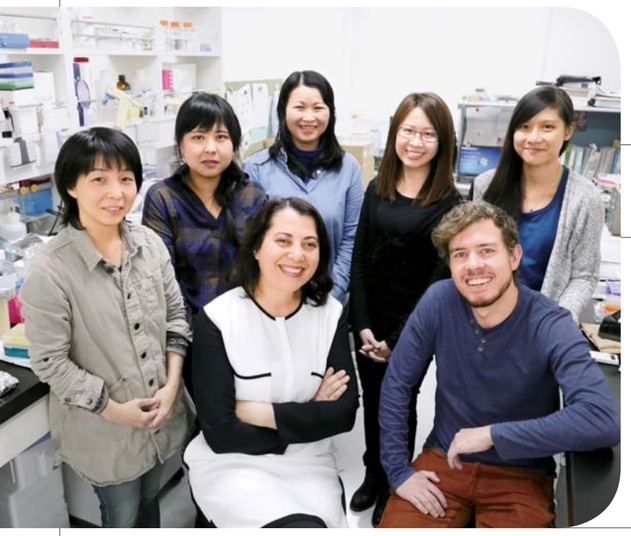
Malaria Immunology



Cevayir Coban, MD

Professor	**Cevayir Coban**
Postdoctoral Fellow	**2**
Research Assistant	**2**
Support Staff	**2**

Malaria continues to be a global burden to the world that kills millions every year due to a lack of a fully successful vaccine or potent drugs for the complete elimination of *Plasmodium* parasites. Therefore, research in our laboratory focuses on the elucidation of host-pathogen interactions in the context of malaria to understand better how these parasites interact with the host. Our broad and final aim is to investigate host-parasite interactions both in animal models and humans, and translate our understanding into safe intervention such as vaccines and drugs.

During blood-stage life cycle of *Plasmodium* parasites, infection can cause disease manifestations such as fever, vomiting, muscle pain and anemia and severe complications including cerebral malaria and, respiratory distress, suggesting that the interaction between infected erythrocyte and tissue environment could be different. Therefore, our studies have mainly focused on the understanding of tissue-specific immunopathology caused by malaria in different tissues/organs. Our final goal is to create new interventions from this understanding as adjunct therapy to anti-malarials (Figure 1).

We have taken advantage of using imaging technologies such as MRI, multiphoton live imaging and micro-computed tomography (µCT) readily available at IFReC facilities in accordance with IFreC/WPI program's aims.

One of the topics we have investigated is the pathology of cerebral malaria, an acute and deadly outcome caused by *Plasmodium falciparum* infections in humans and *P. berghei*ANKA parasites in mice. We have recently reported a new understanding of cerebral malaria pathogenesis using ultra-high field MRI during experimental cerebral malaria. 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 have provided evidence that olfactory functional impairment (loss of smell) could be a valuable early diagnosis marker for cerebral malaria (*Cell Host Microbe, 2014*).

On the other hand, despite severe complications, the majority of patients recover from malaria. However, there is evidence that malaria survivors experience long-term 'hidden' pathologies that are as yet poorly defined. For instance, chronic exposure to *Plasmodium* parasites promotes prolonged immune activation and enhances the risk of lymphoma formation. Growing evidences suggest that physical growth retardation in young children in Africa is associated with infectious diseases, with a high prevalence among malaria-infected children regardless of nutritional status. An increased incidence of porous bone lesions has also been reported in malaria endemic regions, suggesting infection may compromise bone integrity. 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 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, supplementation of alfacalcidol, a vitamin D3 analog, could prevent 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 (*Lee et al., Science Immunology, 2017, in press*).

The final goal of our research is the translation of our understanding of host-pathogen interactions into vaccines or drugs to treat disease(s). Given that there is still more to do for the development of potent vaccines against malaria, we 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 (Lee et al., *Vaccine, 2016*). These studies were performed according to GLP procedures in rats and concluded that synthetic hemozoin is a safe adjuvant that displays very low autoimmune properties.

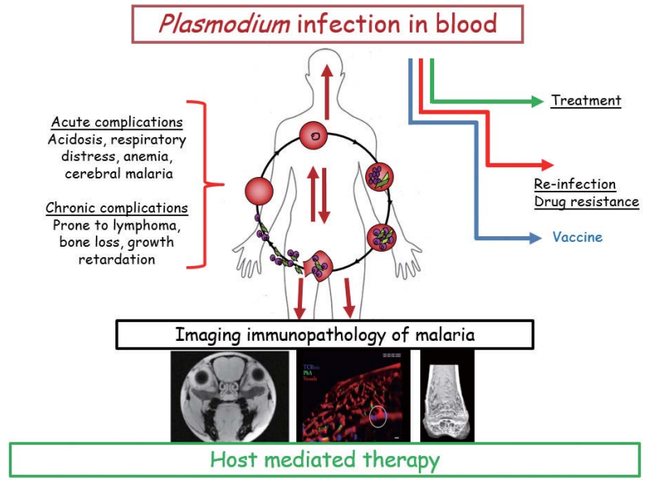


Figure.
Blood stage *Plasmodium* infection causes deadly complications. However, most people develop partial immunity and suffer from mild symptoms. Recent evidences suggest that the incomplete recovery from infection causes chronic illnesses such as bone loss or growth retardation. High prevalence of drug resistance and look of successful vaccines are drawbacks in the efforts to eliminate malaria. Therefore additional treatment modalities should be investigated to prevent and treat acute and chronic complications caused by malaria. Our approach to this problem is to use imaging technologies to understand immunopathology caused by *Plasmodium* parasites.

Recent Publications

- 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. (2017) in press.
- Lee MSJ, Igari Y, Tsukui T, Ishii KJ, Coban C. Current status of synthetic hemozoin adjuvant: A preliminary safety evaluation. Vaccine 34(18), 2055-61 (2016).
- Zhao H et al. Olfactory Plays a Key Role in Spatiotemporal Pathogenesis of Cerebral Malaria. Cell Host Microbe 15(5), 551-63 (2014).
- Zhao H et al. Lipocalin 2 bolsters innate and adaptive immune responses to blood-stage malaria infection by reinforcing host iron metabolism. Cell Host Microbe 12(5), 705-16 (2012).
- Coban C et al. Immunogenicity of Whole Parasite Vaccines Against Plasmodium falciparum Involves Malarial Hemozoin and Host TLR9. Cell Host and Microbe 7(1), 50-61 (2010).

Vaccine Science

Ken J. Ishii, MD/PhD

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Assistant Professor	**Natsuko Kishishita**
Postdoctoral Fellow	**4**
Research Assistant	**4**
Visiting Scientist	**6**
Support Staff	**1**

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.

Our recent study revealed another face of aluminum nano- to micro-particle as a part of PM2.5 (Kuroda E et al, *Immunity 2016 in press*).

<Basic and translational vaccine science>

Old, but newly evolving adjuvant research

It is known that particle pollutions such as PM2.5 are involved in allergic inflammation. Many papers have shown that these particulates have a strong adjuvant activity and induce Type-2 immune responses. However, the mechanisms by which particulates trigger allergic responses are unclear. In FY2016-2017, we have shown that particulates induce alveolar macrophage death and release IL-1α as a DAMP (damage-associated molecular pattern). Released IL-1α contributes to IgE production and inducible bronchus-associated lymphoid tissue (iBALT) formation in the lungs. We suggest that iBALT formation plays an important role for local IgE responses induced by particulate in the lungs. In this study, we examined the detailed mechanisms of iBALT formation in the lungs (Kuroda E et al *Immunity* 2016).

We used alum as inflammatory particulate. Alum was administered by intratracheal (i.t.) instillation and then mice were exposed to OVA aerosol. Previously, we found that iBALT formation and IgE responses were regulated by IL-1R signaling. In addition, we observed the migration of DCs into the lungs are also controlled by IL-1R signaling. Using CD11c-DTR mice, we examined the role of migrated DCs for iBALT formation and found that depletion of CD11c+ cells displayed reduced levels of IgE responses and iBALT formation. Next, we examined the role of Tfh cells in the lungs using Cd4-cre Bcl6flox/flox mice, as a model of Tfh-deficient mouse. The same as requirement of DCs, Tfh cells were also required for iBALT formation, in addition to IgE responses.

These results indicate that DCs and Tfh cells play a pivotal role for particulate-induced lung inflammation, and might be a good therapeutic target for treatment of this type of inflammation (Figure 1).

<Human immunology, clinical development of novel adjuvants and their biomarkers>

Clinical studies on seeking bio-marker(s) for safety as well as efficacy of adjuvanted vaccines

In 2012 we launched a national project called Adjuvant Data Base project supported by Ministry of Health, Labor and Welfare (Figure 2). We conducted a whole organ transcriptome as well as miRNA analysis after the most representative 20-25 adjuvants, some of which are approved in the market or are being developed, injected in mice and rats. Our datasets provided a detailed biological feature of each adjuvant in vivo, unbiasedly classified those adjuvants into 6 groups with associated mechanisms of the actions (Manuscript submitted). Adding another data of AS04 adjuvant demonstrated that current data work as a reliable and standardized core for new adjuvant characterization. We believe this approach will be an important platform for adjuvant biology and immunology, and contribute new adjuvant development in the future.

Cohort as well as retrospective analysis of human samples obtained from volunteers of vaccine clinical trials and patients of relevant immunological disorders are being conducted by four groups including our lab in IFReC and those in National Institute of Biomedical Innovation, Health and Nutrition (NIBIOHN). Preliminary results suggest serum miRNA may provide useful biomarkers to predict safety and immunogenicity of adjuvanted vaccines (manuscript in preparation).

Future prospect

The Forefront Vaccine Development: Development of next-generation vaccines is verging on practical possibility based on molecular designing. Toward such an end, outcomes from studies in my lab at IFReC are also expected to make considerable contributions. These include searching of modality of antigen delivery system and adjuvants. 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 lifestyle-related diseases. We will continue innovative research and development vaccines against these diseases together with National Institute of Biomedical Innovation, Health and Nutrition (NIBIOHN) accompanying active exchanges of researchers.

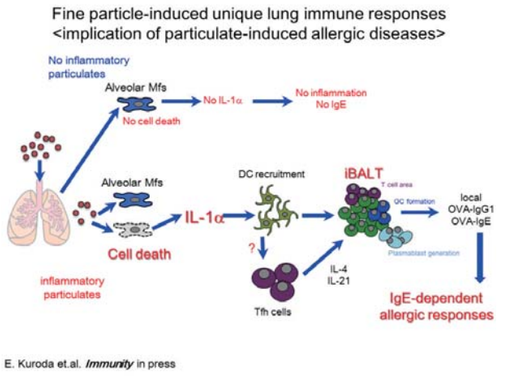

Figure 1.
Lung-specific allergic inflammation by PM2.5 and other allergen via alveolar macrophage and IL-1.

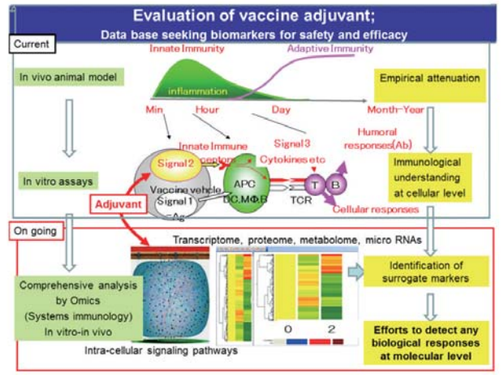

Figure 2.
Adjuvant data base project to evaluate adjuvant efficacy as well as safety by comprehensive transcriptome analysis.

Recent Publications

- Kanuma T, et al. CD63-mediated antigen delivery into extracellular vesicles via DNA vaccination results in robust CD8+ T cell responses J. Immunol. (2017) in press.
- Kuroda E, et al. Inhaled Fine Particles Induce Alveolar Macrophage Death and Interleukin-1α Release to Promote Inducible Bronchus-Associated Lymphoid Tissue Formation. Immunity. 45(6), 1299-1310 (2016).
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- Kobiyama K, et al. Species-dependent role of type I IFNs and IL-12 in the CTL response induced by humanized CpG complexed with β-glucan. Eur. J. Immunol. 46(5), 1142-51 (2016).
- Temizoz B, et al. Vaccine adjuvants as potential cancer immunotherapeutics. Int. Immunol. 28(7), 329-38 (2016).

Immunoparasitology

Masahiro Yamamoto, PhD

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Assistant Professor	**Miwa Sasai** **Jisu Ma** **Youngae Lee**
Support Staff	**1**

The host immune system produces a proinflammatory cytokine, interferon γ (IFN-γ), against infection of the obligatory intracellular protozoan pathogen *Toxoplasma gondii*. IFN-γ stimulates the cell-autonomous innate immune response, causing the disruption of the membranes of parasitophorous vacuole (PV), which *T. gondii* forms inside infected cells, by IFN-γ-inducible GTPases, such as immunity-related GTPases (IRGs) and guanylate-binding proteins (GBPs). GBPs and effector IRGs (called GKS-IRGs), such as Irga6 and Irgb6, are recruited to the *T. gondii* PV membranes and are thought to cooperate in PV disruption. The regulatory IRGs (called GMS-IRGs), such as Irgm1 and Irgm3 (Irgm1/m3), and some essential autophagy components, such as Atg3, Atg5, Atg7, and Atg16L1, positively control the recruitment of IRGs and GBPs to the *T. gondii* PVs.

When a healthy host is infected with *T. gondii*, it develops acquired immunity involving a parasite-specific $CD8^+$ T cell response, which is important for both acute and chronic resistance to *T. gondii* and protective immunity against infection. Endogenous parasite-derived proteins, including GRA6, or a model protein such as ovalbumin (OVA) that is secreted into the PVs, has been shown to efficiently elicit strong parasite-specific $CD8^+$ T cells. Professional hematopoietic antigen-presenting cells (APCs), such as dendritic cells (DCs) and macrophages, as well as nonprofessional nonhematopoietic cells, such as fibroblasts and astrocytes, process the vacuolar proteins and present the antigens on major histocompatibility complex class I (MHCI) molecules in a TAP1-dependent manner. Furthermore, the direct interaction between the PV and the host endoplasmic reticulum (ER) provides a route of entry for the MHCI-restricted antigen presented on DCs. The priming of macrophages by the IFN-γ and tumor necrosis factor alpha (TNF-α) further enhances the activation of antigen-specific $CD8^+$ T cells in an Irgm3-dependent manner, suggesting a potential role for IFN-γ-inducible GTPases in antigen presentation. However, the molecular link between IFN-γ-induced cell-autonomous innate immunity and vacuolar-antigen-specific $CD8^+$ T cell-dependent acquired immunity remains unclear.

Here, we show that p62/Sqstm1, a protein with a ubiquitin-binding domain and that is involved in the selective autophagic elimination of intracellular pathogens, is recruited together with ubiquitin to the *T. gondii* PVs, following IFN-γ stimulation, and plays a specific role in vacuolar-antigen-specific $CD8^+$ T cell activation after PV disruption by IFN-γ-inducible GTPases.

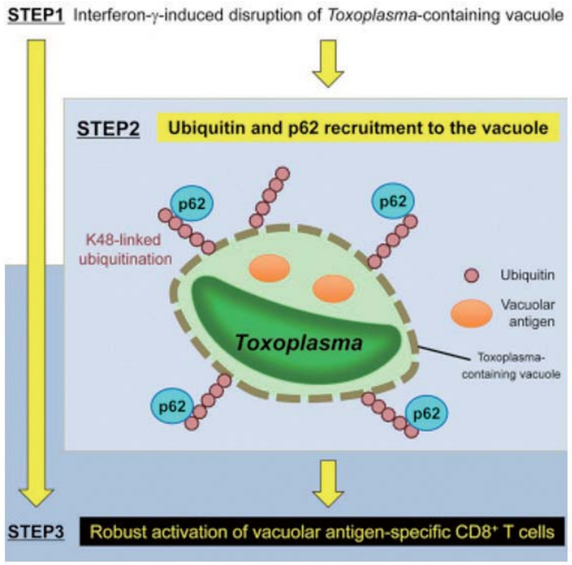

Figure.
p62 Plays a Specific Role in Interferon-γ-Induced Presentation of a Toxoplasma Vacuolar Antigen

Recent Publications

- Man SM, Karki R, Sasai M, Place DE, Kesavardhana S, Temirov J, Frase S, Zhu Q, Malireddi RK, Kuriakose T, Peters JL, Neale G, Brown SA, Yamamoto M, Kanneganti TD. IRGB10 Liberates Bacterial Ligands for Sensing by the AIM2 and Caspase-11-NLRP3 Inflammasomes. Cell. 167, 382-396 (2016).
- Lee Y, Sasai M, Ma J, Sakaguchi N, Ohshima J, Bando H, Saitoh T, Akira S, Yamamoto M (Corresponding author). p62 plays a specific role in interferon-γ-induced presentation of a Toxoplasma vacuolar antigen. Cell Reports 13, 223-233 (2015).

Biochemistry and Immunology

Shigekazu Nagata, PhD

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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 flippase, 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 scrambles 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 of apoptotic cells, and as scaffolds 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 recently 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. A high concentration of Ca^{2+} inhibits their flippase activity, too.

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 by forming a dimer. Thus, when cells are activated, the high intracellular concentration of Ca^{2+} reversibly inactivates the flippases, and reversibly activates TMEM16F scramblase to expose PtdSer to the cell surface (Figure 1).

When the intracellular Ca^{2+} level returns to normal, the flippase re-establishes the asymmetrical distribution of phospholipids. Among these five members that function as scramblases, TMEM16F is ubiquitously expressed in various cells, while others are tissue-specific; they 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, thus 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. When Xkr8$^{-/-}$ mouse embryonal fibroblasts (MEF) and fetal thymocytes undergo apoptosis, they do not expose PtdSer and can not be engulfed by macrophage. The unengulfed dead cells are believed to undergo secondary necrosis, and intracellular materials are released from dead cells. This may activate the immune system, leading to systemic lupus erythematosus-type autoimmune disease.

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.

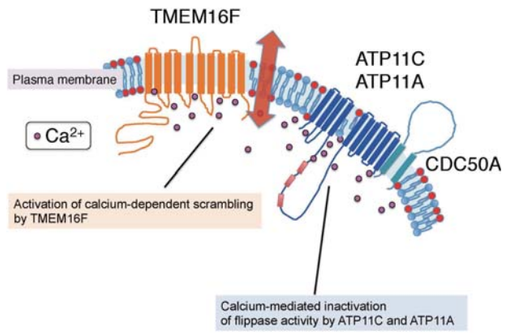

Figure 1. The molecular mechanism for PtdSer exposure in cells with high Ca^{2+}-concentration.
The flippase comprised of P4-ATPase (ATP11A or ATP11C) and CDC50A, and a Ca^{2+}-dependent scramblase (TMEM16F) are schematically shown. In activated platelets, the intracellular Ca^{2+} concentration increases 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.

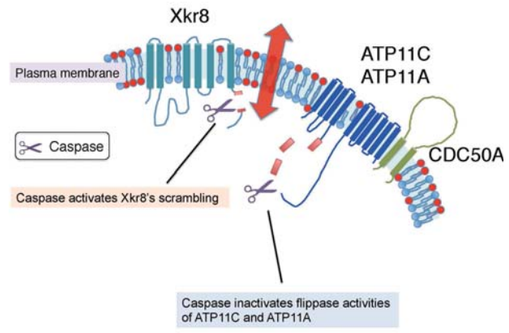

Figure 2. The PtdSer-exposure in apoptotic cells.
A caspase-dependent phospholipid scramblase of Xkr8, and flippase (ATP11A/ATP11C associated with CDC50A) are schematically shown. When cells undergo apoptosis, caspase 3 or caspase 7 in the downstream of the caspase-cascade cleaves Xkr8 to activate its 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, Tanaka M. Programmed cell death and the immune system. Nat. Rev. Immunol. 17, 333-340 (2017).
- Suzuki J, Imanishi E, Nagata S. The Xkr8 phospholipid scrambling complex in apoptotic phosphatidylserine exposure. Proc. Natl. Acad. Sci. USA 113, 9509-9514 (2016).
- Gyobu S, et al. A role of TMEM16E carrying a scrambling domain in sperm motility. Mol. Cell Biol. 36, 645-659 (2016).
- Fujii T, Sakata A, Nishimura S, Eto K, Nagata S. TMEM16F is required for phosphatidylserine exposure and microparticle release in activated mouse platelets. Proc. Natl. Acad. Sci. USA 112, 12800-12805 (2015).
- Segawa K, et al. Caspase-mediated cleavage of phospholipid flippase for apoptotic phosphatidylserine exposure. Science 344, 1164-1168 (2014).

Single Molecule Imaging

Toshio Yanagida, PhD

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	Yutaka Komai
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Use of Morin hydrate for manganese mapping

Recently, manganese (Mn) became an important paramagnetic contrast agent in magnetic resonance imaging. Mn^{2+} can enter neurons through voltage dependent Ca^{2+} channels, and thus acts as an indirect marker of calcium channel activity. Morin is a chemical isolated from Maclura pomifera, which when it binds divalent metals shifts from a red to green emission peak, and thus it allows visualization of the distribution of Mn^{2+} at a cellular level under fluorescence microscopy. To demonstrate the detectability of Mn in tissue, mice were exposed to Mn^{2+} for 5 weeks by way of drinking water containing 50mM $MnCl_2$. The brains of these mice were harvested and cryo-sectioned using the standard procedures. Tissue sections were stained with 20mM Morin hydrate in 60% ethanol solution, embedded in a mixture of glycerol, ethanol and water (5:3:2), and imaged using two-photon microscopy. The histogram indicates that Mn accumulation (pixels with high green signal) appeared most prominently in amygdala. The procedure we developed successfully delineated the spatial distribution of Mn and evaluated the accumulation of Mn with drinking water exposure.

Immune responses modelling

We showed that regulatory T cells (Treg) cells enhance cell-interactions which stabilize the tolerant state with both theoretical and experimental studies. Modeling showed that T cell interaction with APC is crucial for maintaining stable unresponsiveness in addition to inhibition of activation-signaling when ligands for stimulation are limited and there is competition among T cells (Fig. 1). To robustly inhibit T cell proliferation in the presence of specific Treg cells even where individual T cells behave stochastically, Treg cells were required to inhibit two processes; dissociation and activation. Supporting this theory, in vitro experiments showed antigen-specific Treg cells actually enhance the expression of adhesion molecules and inhibit the expression of costimulatory ligands on APC. Notably, the model optimized to discriminate reactivity under self and non-self conditions well reproduced the proliferation patterns of T cells under various conditions examined in in vitro experiments. Based on the predictions of the model, we also found a way to manipulate immune response in vivo. Transient reduction of T cell number enhanced the proliferation of antigen specific T cells in draining lymph nodes. Thus, the combined studies of theoretical modeling and immunological experiments revealed a novel ability of Treg cells to inhibit cell-dissociation, which gives insight into immune tolerance and into manipulation of immune responses in various clinical settings.

Observation of single T cell activation

To answer how many T cells are activated by contact with APC, we manipulated single T cell to interact with APC and activation was monitored. T cell activation can be monitored using fluorescent Ca^{2+}-reporter. By counting the number of activated T cells attached to APC, we found that only 22% became activated when antigen was presented by APC. On the other hand, 11% of T cells showed activation even when antigen was absent, indicating that activation of T cells is not deterministic. To understand how the immune system distinguishes between self and non-self despite the non-deterministic manner of T cells, we further investigated the effect of Treg cells (Fig. 2), and found that in the presence of Treg cells, activation of T cells was suppressed in the absence of antigen peptide, whereas activation probability did not change in the presence of antigen peptide. This observation show that Treg cells act as a faucet for a leaky immune system.

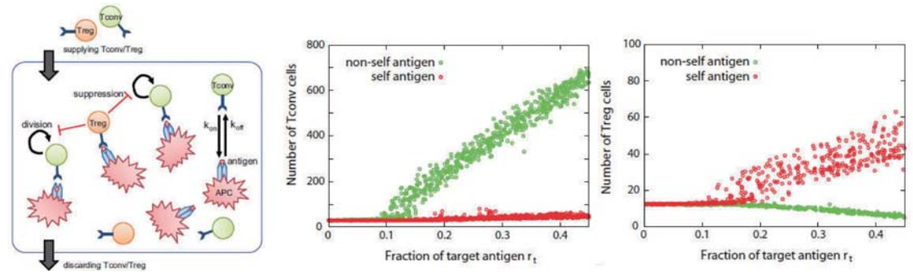

Figure 1.
Multicellular model of T cell response. Schematic illustration of the model (left), response of T cell population with respect to antigen presentation (middle), and average number of Treg cells as a function of the fraction of the target antigen (right).

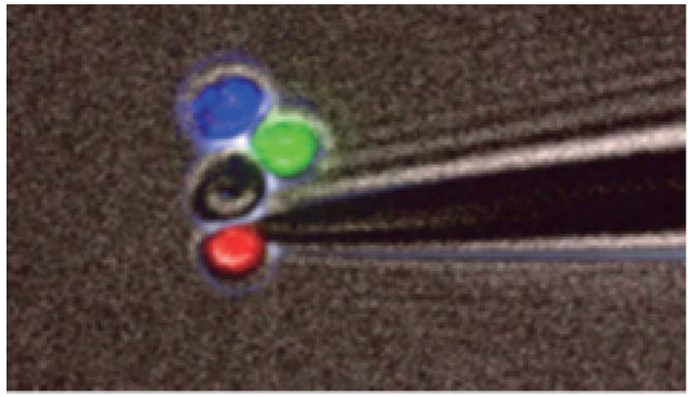

Figure 2.
Counting T cell activation upon engagement with APC in the presence of regulatory T cell. Rhod2 stained T cells (red) were held with a glass micro-needle and placed on APC and fluorescence was monitored. Regulatory T cell (blue) and conventional T cell (green) are also present on APC.

Recent Publications

- Chiu LD, Ichimura T, Sekiya T, Machiyama H, Watanabe T, Fujita H, Ozawa T, and Fujita K. Protein expression guided chemical profiling of living cells by the simultaneous observation of Raman scattering and anti-Stokes fluorescence emission. Sci. Rep. 7, 43569 (2017).
- Furusawa C, Yamaguchi T. Robust and Accurate Discrimination of Self/Non-Self Antigen Presentations by Regulatory T Cell Suppression. PLoS One. 11(9), e0163134 (2016).
- Ichimura T, Chiu LD, Fujita K, Machiyama H, Yamaguchi T, Watanabe TM and Fujita H. Non-label immune cell state prediction using Raman spectroscopy. Sci. Rep. 6, 37562 (2016).
- Iwaki M, Wickham S, Ikezaki K, Yanagida T, Shih W. A programmable DNA origami nanospring that reveals force-induced adjacent binding of myosin VI heads. Nature Comm. 7, 13715 (2016).
- Fujita K, Iwaki M, Yanagida T. Transcriptional bursting is intrinsically caused by interplay between RNA polymerases on DNA. Nature Comm. 7, 13788 (2016).

Immunology and Cell Biology

Masaru Ishii, MD/PhD

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	Takao Sudo
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Visiting Scientist	**1**
Support Staff	**3**

The immune system is highly dynamic and a variety of cell types are migrating throughout the body and mutually communicating at defined sites. The 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 our advanced imaging techniques, we have investigated the dynamic nature of different cell types in a time-dependent manner, in addition to the spatial and structural information. We are now elucidating the dynamic systems in bone biology and immunology as well as biological science such as hematopoiesis and cell lineage commitment and other 'niches', cell dynamics during inflammation in various kinds of tissues and organs (see the Figure).

Intravital bone imaging revealing osteoclast and osteoblast dynamics in vivo

Our lab has originally elaborated the novel imaging system for visualizing inside the bones. We have first elucidated the in vivo behaviors of bone-resorbing macrophage, osteoclast, i.e., the migration and positioning of their precursor macrophages, their mode of bone-resorbing function in vivo and the functional and physical coupling with bone-reforming osteoblasts. By utilizing this methodology, we showed sphingosine-1-phosphate (S1P) as a key factor in controlling the migratory behavior of osteoclast precursors in concert with various chemokines (Nature 2009; J Exp Med 2010). We also showed the substantial contribution of S1P-mediated migratory control of bone cells by S1P by generating knockout mice deficient for endogenous S1P transporter (J. Clin. Invest. 2012). Moreover, we demonstrated that vitamin D significantly blocks bone destruction by modulating S1P-mediated migration control of osteoclast precursor monocytes (Proc. Natl. Acad. Sci. USA 2013).

Through the improvement of bone imaging system, we succeeded in visualizing the function of mature osteoclasts 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. Th17 cells, a bone destruction-prone T cell subset, express RANKL on their surface, although its functional role remains elusive. This novel imaging system showed that RANKL-bearing Th17 could stimulate osteoclastic bone destruction by contacting N state osteoclasts directly to convert them to the R state, a critical mechanism underlying bone erosion in arthritic joints. Furthermore, 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). By using this probe, we could grasp the real time course of osteoclastic bone resorption which was finely regulated by cell-cell contact with bone-forming osteoblasts and other bone cell types.

Immunometabolism of bone homeostasis

Osteoclasts dramatically alter their metabolic activity during cell differentiation. This change in the metabolic status is termed 'metabolic reprogramming', but its role in osteoclast is not fully understood. Using metabolomics approach, we found that metabolic reprogramming during osteoclast differentiation increased intracellular S-adenosyle methionine (SAM), a metabolite of the methionine cycle. SAM is the universal methyl donor for methylation reactions, including histone and DNA methylation. Furthermore, we identified the *de novo* DNA methyltransferase 3a (Dnmt3a) as a transcription factor that couples these metabolic changes to osteoclast differentiation. We found that SAM-mediated DNA methylation by Dnmt3a regulates osteoclastogenesis via epigenetic repression of anti-osteoclastogenic genes. Dnmt3a-deficient osteoclast precursor cells do not differentiate efficiently into osteoclasts and that mice with an osteoclast-specific deficiency in Dnmt3a have elevated bone mass due to a smaller number of osteoclasts. Furthermore, inhibition of DNA methylation by theaflavin-3,3′-digallate abrogated bone loss in models of osteoporosis. Thus, we revealed the role of epigenetic processes in the regulation of cellular metabolism and differentiation, which may provide the molecular basis for a new therapeutic strategy for a variety of bone disorders (Nat Med 2015).

Application of intravital imaging techniques for dissecting human immunology

Intravital imaging with multi-photon microscopy is an undoubtedly powerful tool for dissecting living 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 applicable for analyzing human samples. By collaborating with companies (supported by AMED), we are developing a new microscopy system for application to human tissues and organs in vivo. To date, we have succeeded in visualizing non-labelled human normal and cancer tissues, and we will be able to dissect human immunology in the future.

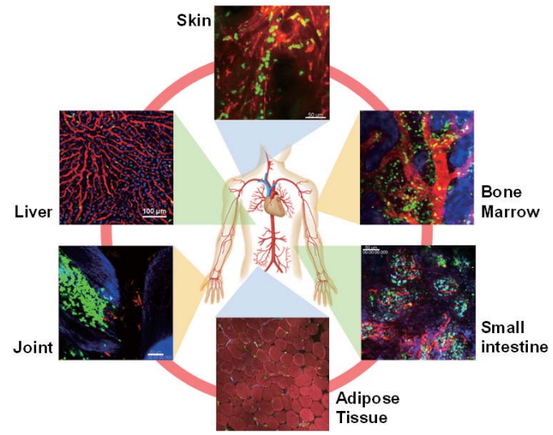

Figure. Intravital imaging for various immune systems.
Immune cells are high dynamic and interconnecting various tissues and organs, by forming a 'soft-wired' network. We are elucidating the basic principle controlling the dynamic nature of immune cells by visualizing in vivo behaviors with advanced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

Recent Publications

- Kon S, et al. Cell competition with normal epithelial cells promotes apical extrusion of transformed cells through metabolic changes. Nat. Cell Biol. 19(5), 530-541. doi: 10.1038/ncb3509 (2017).
- Maeda H, et al. Real-time intravital imaging of pH variation associated with cell osteoclast activity and motility using designed small molecular probe. Nature Chem. Biol. 12, 579-85 (2016).
- Sekimoto R, et al. Visualized macrophage dynamics and significance of S100A8 in obese fat. Proc. Natl. Acad. Sci. USA 112, 2058-66 (2015).
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- Kikuta J, et al. Dynamic visualization of RANKL and Th17-mediated osteoclast function. J. Clin. Invest. 123, 866-873 (2013).
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Nuclear Medicine

Jun Hatazawa, MD/PhD

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Research Assistant	**2**
Support Staff	**1**

Astrocyte activation is representative pathology in central nervous system of multiple sclerosis (MS) patients. Acetate has been revealed to be selectively uptaken and metabolized by astrocytes in the CNS. We aimed to visualize and investigate activation of astrocyte in the CNS and to clarify the activity MS in vivo by means of PET with C-11 acetate.

Patients with relapsing-remitting MS (6 females, 46 +/- 5.9 year old (yo)) and healthy volunteers (HV) (6 females, 58 +/- 5.8 yo) were enrolled in the present study. In all the subjects, MRI and PET scanning was performed. A static 20-min PET acquisition was started 20 min after intra-venous injection of C-11 acetate. The images were reconstructed using a filtered-back projection method. Parametric images of standardized uptake value (SUV) and the ratio of regional SUV to mean SUV in thalamus (SUVt) were produced for analysis. Volume-of-interest (VOI) analysis for gray matter (GM) and/or white matter (WM) based on segmentation technique for co-registered MRI. Voxel-based statistical parametric analysis were performed by using Statistical Parametric Mapping 8 (SPM8) to evaluate pathological change of regional C-11 acetate uptake. Correlation between C-11 acetate uptake and the lesion number in T1- and T2- weighted MR images were also assessed.

As a result, standardized uptake value (SUV) of 11C acetate relative to SUV in bilateral thalami (SUVt) was significantly increased in both white and gray matter in MS compared with HV. The ratio of SUV in white matter to SUV in gray matter was significantly higher in MS than HV. Voxel-based statistical analysis elucidated significant increase in uptake in broad area of white matter especially in subcortical white matter in MS patients. The numbers of T2 lesions and T1 black holes recognized as a marker of tissue destruction were significantly correlated with C-11 acetate uptake in white and gray matter. It seems that the C-11 acetate uptake reflecting reactive astrocytes correlates with both axonal damage and cortical astrogliosis. These results imply the widespread involvement of cortical astrocytes in MS pathology.

Inflammatory demyelination in WM is a cardinal feature in MS pathology. In addition to the demyelinated lesions detected by ordinary MRI, pathological changes exist even in normal appearing WM and GM. Astrocyte pathology precedes demyelination in an animal model; astrocyte hypertrophy occurs at the leading edge of acute MS lesions, followed later by astrocytic scarring. Thus, the altered astrocyte activation is presumably involved in MS pathophysiology.

Our present study suggests that pathologic white matter changes in patients with MS can be detected with non-invasive static C-11 acetate PET, which may be an effective MS diagnostic tool.

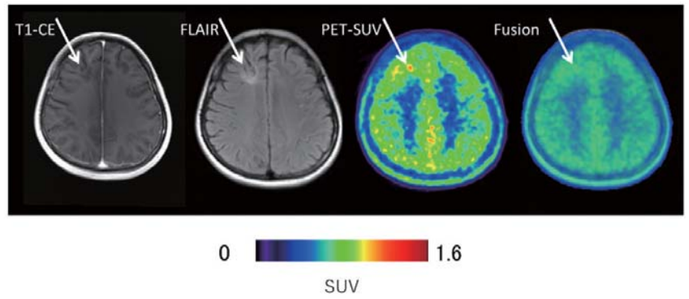


Figure 1.
A representative case of MS. (SUV: standardized uptake value,)

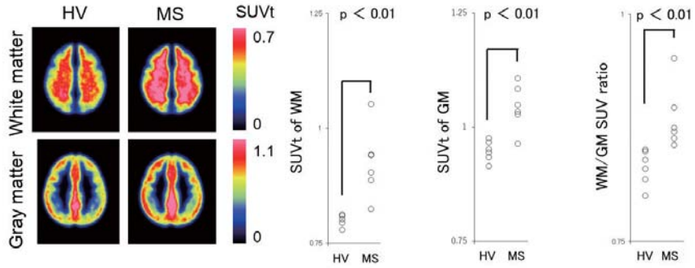


Figure 2.
Standardize uptake value of C-11 acetate in the MS patients and the healthy volunteers. (SUVt: ratio of regional SUV to mean thalamic SUV.)

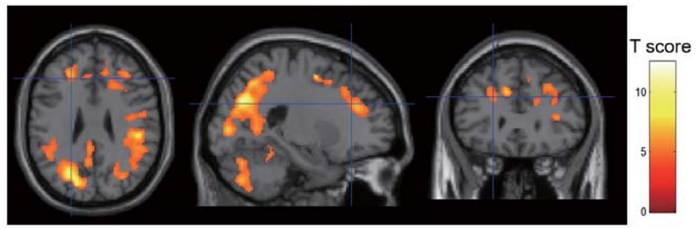
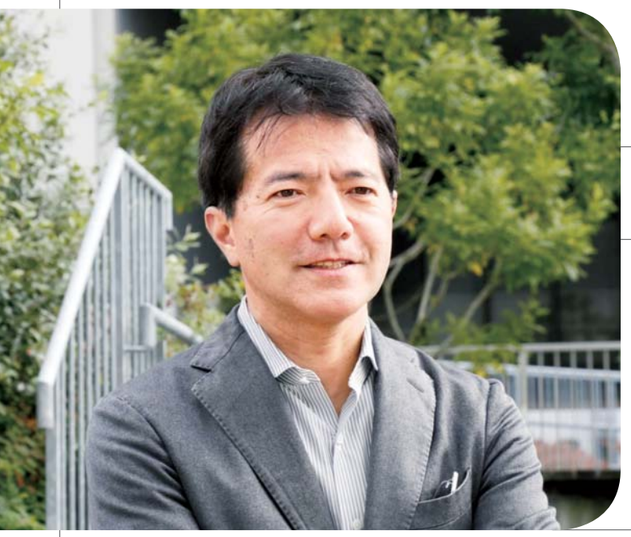


Figure 3.
Voxel-based statistical comparison C-11 acetate uptake between MS patients and healthy volunteers. Colored voxels indicate T-scores representing significantly increased C-11 acetate uptake (SUVt) in patients with MS compared to HV patients.

Recent Publications

- Watabe T, et al. Quantitative evaluation of oxygen metabolism in the intratumoral hypoxia: 18F-fluoromisonidazole and 15O-labelled gases inhalation PET. EJNMMI Res 7, 16. doi:10.1186/s13550-017-0263-6 (2017).
- Kato H, Shimosegawa E, Fujino K & Hatazawa J. CT-Based Attenuation Correction in Brain SPECT/CT Can Improve the Lesion Detectability of Voxel-Based Statistical Analyses. PloS one 11, e0159505, doi:10.1371/journal.pone.0159505 (2016).
- Watanabe S, Kato H, Shimosegawa E & Hatazawa J. Genetic and Environmental Influences on Regional Brain Uptake of 18F-FDG: A PET Study on Monozygotic and Dizygotic Twins. J. Nucl. Med. 57, 392-397. doi:10.2967/jnumed.115.164004 (2016).

Chemical Imaging Techniques



Kazuya Kikuchi, PhD

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Support Staff	**1**

Multicolor imaging techniques are useful for investigating the localization and movement of proteins in living cells. By combining these techniques with pulse-chase imaging of proteins, precise spatio-temporal information on proteins in living cells is obtained. In this type of imaging analyses, protein labeling using a protein tag and its specific probes is an attractive approach, because target proteins are conditionally labeled by the temporal addition of probes with different fluorophores that allow the multicolor pulse-chase imaging. Previously, we developed a protein labeling/imaging system based on photoactive yellow protein (PYP) tag and its specific probe, FCANB. PYP-tag is a novel protein tag, which is a small protein (14 kDa) derived from purple bacteria. The noticeable feature of this labeling method is that the fluorescence of FCANB is quenched and is increased upon the labeling reaction with PYP-tag. FCANB consists of a 4-hydroxycinnamic acid ligand, a fluorophore, a linker, and a nitrobenzene (NB) quencher. This modular scaffold is attractive, since various fluorophores emitting fluorescence at different wavelengths can be introduced into the probe. In addition, the NB quencher decreases fluorescence intensity of the fluorophore based on a contact quenching mechanism. This fluorogenic property allows the imaging of cell-surface proteins with low background signals, even when free probes are not washed out from cells. However, the current probes available for the no-wash imaging were created using fluorophores emitting short-wavelength fluorescence or have limitation in the availability of fluorophore, and thus reduce the benefit in multicolor imaging analyses.

In this research, to achieve multicolor protein imaging with high contrast, we synthesized new PYP-tag probes with two different long-wavelength fluorophores, which increase fluorescence intensity upon the labeling reactions. As the long-wavelength fluorophores, we chose sulfonated cyanine dyes, Cy3 and Cy5, which emit fluorescence with the maximum wavelengths of 570 nm and 670 nm, respectively. These fluorophores are bright and have been applied to a number of biological studies. As a fluorescence quencher, 3,5-(dinitrobenzyl)acetamide (DNB) was employed as a quencher that reduces fluorescence intensity based on contact quenching. We introduced DNB into the scaffold of the probe to develop Cy3DNB2 and Cy5DNB2 (Fig 1). SDS-PAGE analyses revealed that both the probes bind to PYP-tag covalently. As we expected, fluorescence intensity of the probes enhanced upon labeling reactions of PYP-tag (Fig 2). The probes labeled PYP-tag more rapidly than the previously reported probe, FCANB. It turned out that DNB increased the labeling reactions as well as fluorescence intensity. Both the probes specifically imaged EGFR in living cells (Fig 3). Particularly, Cy5DNB2 showed the good fluorogenic and kinetic properties and allowed no-wash imaging of the cell-surface proteins with high contrast. The strategy based on DNB quenching would offer a promising approach for design of fluorogenic probes with long-wavelength fluorophores. These probes thus will be attractive tools for in vivo or in situ imaging of various proteins.

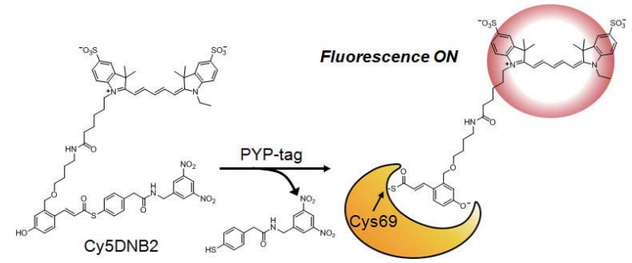

Figure 1.
Principle of fluorogenic PYP-tag labeling system using Cy5DNB2.

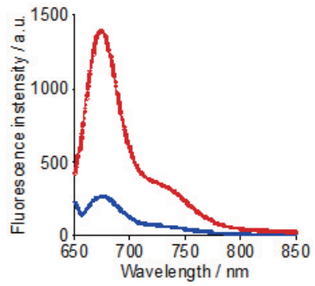

Figure 2.
Fluorescence enhancement of Cy5DNB2 upon labeling reactions with PYP-tag. Red and blue lines represent fluorescence spectra of Cy5DNB2 in the presence and absence of PYP-tag, respectively.

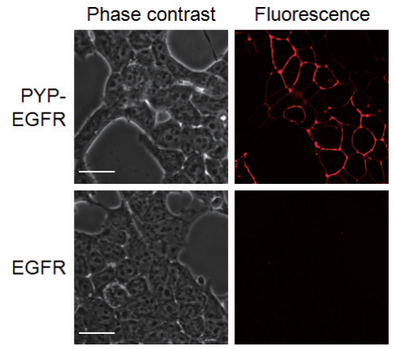

Figure 3.
Live-cell imaging of PYP-EGFR using Cy5DNB2.

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- Hirayama S, Hori Y, Benedek Z, Suzuki T, Kikuchi K. Fluorogenic Probes Reveal a Role of GLUT4 N-glycosylation in Intracellular Trafficking, Nat. Chem. Biol. 12, 853-859 (2016).
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Biophotonics

Nicholas Isaac Smith, PhD

Associate Professor	**Nicholas Isaac Smith**
Assistant Professor	**Alison Hobro**
	Nicolas Pavillon
Support Staff	**1**

The Biophotonics lab research theme is to develop optical techniques and apply them to study biological and immunological processes. Most of our research involves using optical measurements, without fluorescent labels, to provide images, spectral data, or both. Several different physical interactions of light can be exploited to measure samples and extract cellular information. We rely heavily on Raman scattering based measurements, using a high intensity laser beam, and measuring the shift in the wavelength of the scattered light. With different molecules expressed at different locations in the cell, we can produce an image where the image contrast is defined by the spatial and chemical distributions of endogenous molecules in a living cell or tissue sample. We also investigate how other light-based methods can be used in a complementary manner to the Raman-based measurements. With several years of development already underway, we are now moving more towards applying the tools that we have developed to study processes in immune cells, such as analysis of cell phenotype, and to discriminate cell activation levels, usually in lymphocytes and macrophages.

For T and B cells, the usual features for discrimination of immunological function are the presence or absence of surface markers. We recently completed a study using Raman scattering to discriminate between different lymphocyte cell lines (Hobro et al., 2016). Of note is the finding that the Raman signature of each cell type was distinct enough that it could be used to predict the cell type in a mixed group of cells. The Raman analysis is based on a measurement of the endogenous molecules, but depending on the nature of the measurements, most crucially the laser focus and optical sectioning, it should not detect contributions from surface markers. Therefore the Raman signature should instead be more related to the genome or transcriptome-based components of the cell. This gives a complementary approach to the more usual wet-lab methods of cell phenotyping. While the implications of this approach go beyond a simple discrimination method, using it as a method of discriminating T vs B cells and/or individual cell types from one another, resulted in high sensitivity and specificity (Hobro et al., 2016). Figure 1 shows a PLS-DA separation model of individual cell lines.

We also used Raman analysis in a non-imaging mode, where we can measure purified samples of interest (e.g. mRNA and small molecule interactions). The interactions between small molecules such as mRNA, and proteins can be measured by Raman with some advantages over other methods. We built a portable Raman detection system which can be used for analysis of cuvette-based samples, or other samples where imaging is not required. This includes detection of pathogens in blood or serum samples (e.g. Hobro et al., 2013), evaluation of tissue, or analysis of drugs in solution.

For the ultimate sensitivity (approaching single-molecule level) in the cell, however, Raman scattering alone can not generate sufficient signal, but by using plasmonic Raman enhancement, the scattering signal is amplified at a metallic surface and/or by chemical bonds that form between target molecules and the surface. The enhancement factor can then be in the order of 1 million times. These particular experiments require the introduction of a metal surface in the cell, and are not strictly label-free but the tradeoff is often worthwhile, depending on the target. Conceptually similar to the more well-known SPR sensors, our approach uses only the interface enhancement to detect endogenous molecules without any conjugated targeting. One difficulty for these enhanced Raman experiments is the need to introduce the metal surface inside the cell, usually an inert metal nanoparticle. This can be difficult since some cells do not readily uptake nanoparticles, and even for those that do, the particles do not typically pass the nuclear membrane, restricting the measurements to cytosolic regions. A novel method to solve this is to introduce gold salt solution, which can pass through the entire cell, and to form the nanoparticles in-situ by photoreduction (Smith et al., 2014), thereby creating the Raman enhancement directly inside the cell.

Due to the challenges of Raman imaging, we considered how best to optimize the measurements, and completed our first project on compressed sensing, using sub-sampling and optimized reconstruction models to reduce the amount of data points needed to form an image (Pavillon et al., 2016). One of the main problems in Raman microscopy is the signal-to-noise ratio. Higher laser power is usually not possible due to the desire to avoid sample damage. Therefore, relatively long imaging times are required. However, due to the inherent blur in the microscope imaging system, there is some amount of redundancy between measurements in typical imaging conditions. By using a reduced set of measurements, in combination with a model of the imaging properties, we can significantly speed up the Raman imaging process. As shown in figure 2, using up to 5 times less sampling, the image can still be reconstructed with minimal information loss. Although our lab is primarily focused on label-free imaging, we showed that the compressed sensing approach can be applied to laser scanning microscopy in general, such as confocal or multiphoton fluorescence imaging. This can then lead to a significant improvement in imaging speed and/or reduction in photobleaching which is very useful for collaborations with other groups that use fluorescent probes.

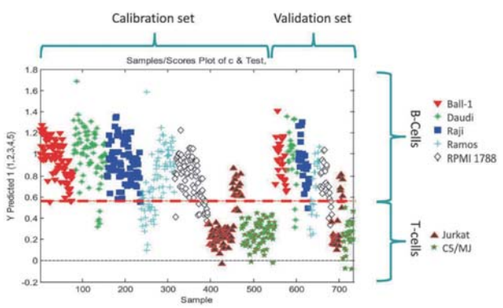

Figure 1.
B- and T-cell line discrimination by label-free Raman measurement. Each sample represents one Raman spectra from an individual cell, where each cell line is denoted by different symbols described in the figure key. The separation model is built from part of the data, with a portion of the data set aside and used to validate the model. The red dashed line represents a dividing line between the B- and T-cell spectra. Figure from Hobro et al. 2016.

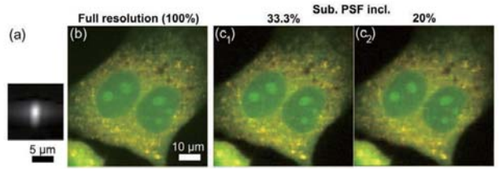

Figure 2.
Raman measurements on MEF cells, showing how compressed sensing can significantly improve Raman imaging speed by reducing the required measurements. Image (a) shows the point spread function of the Raman microscope, measured with 100 nm fluorescent beads. The Raman images are then measured at full resolution (b), and then reconstructed using only 1/3 (c1) or 1/5 (c2) of the original data, showing that up to five times reduction in sampling still produces usable images when combined with the compressed sensing method. The color contrast is generated from a C-H stretching band (2925 cm−1, green) and lipids (2853 cm−1, red). Figure from Pavillon et al 2016.

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- Hobro AJ, Pavillon N, Fujita K, Ozkan M, Coban C and Smith NI. Label-free Raman imaging of the macrophage response to the malaria pigment hemozoin. Analyst 140, 2350-2359 (2015).
- Smith NI, Mochizuki K, Niioka H, Ichikawa S, Pavillon N, Hobro AJ, Ando J, Fujita K and Kumagai Y. Laser-targeted photofabrication of gold nanoparticles inside cells. Nat. Commun. 5(5144), 1-9 (2014).
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Immune Response Dynamics

Kazuhiro Suzuki, MD/PhD

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

Control of lymphocyte trafficking through β_2-adrenergic receptors

It has long been proposed that various aspects of immune responses are influenced by nervous system activity. However, the cellular and molecular basis for neural regulation of immunity is largely unclear. 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 was 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 recently reported that inputs from adrenergic nerves control lymphocyte egress from LNs through β_2ARs (Nakai et al., 2014). 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). However, the physiological role of this mechanism in adaptive immune responses was unclear.

Physiological significance of adrenergic control of lymphocyte trafficking

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 accompanied by an increase of lymphocyte numbers in LNs and their concomitant decrease in lymph and blood. Consistent with these observations, we found that lymphocyte egress from LNs was restricted during the night time in mice. The diurnal variation of LN egress was dependent on neural inputs to lymphocyte β_2ARs (Suzuki et al., 2016).

We hypothesized that the accumulation of lymphocytes in LNs during the period of high adrenergic nerve activity may increase the chance of antigen encounter and potentiate adaptive immune responses. We found in mice that immunization in the night time, when lymphocyte numbers in LNs were high, induced more robust humoral immune responses than immunization 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 fluctuation 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).

Future perspectives

Our findings have added a novel layer of immune regulation by the nervous system. However, a few 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. In the short term, we are going to address these questions to reveal the whole picture of adrenergic control of lymphocyte trafficking. The activity of the sympathetic nervous system and thereby adrenergic nerves is influenced by psychological conditions. In the longer term, we plan to clarify cellular and molecular mechanisms by which brain functions are reflected in immune responses through adrenergic nerves. Currently, the neuro-immune interactions attract particular attention in biomedical science. We are going to carve a niche for ourselves in this rapidly growing field.

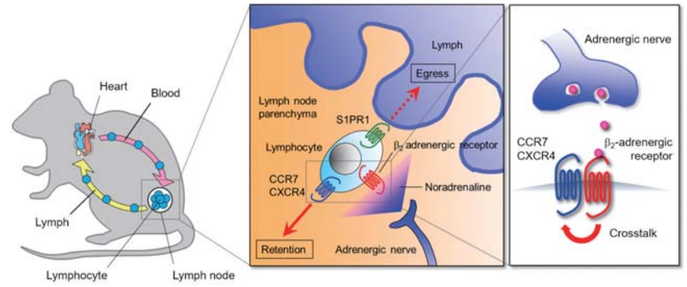

Figure 1. Control of lymphocyte egress from LNs through β_2ARs.
Inputs from adrenergic nerves stimulate β_2ARs expressed on lymphocytes. Activation of lymphocyte β_2ARs enhances signals through CCR7 and CXCR4, chemokine receptors that promote lymphocyte retention in LNs, and inhibits lymphocyte egress from LNs.

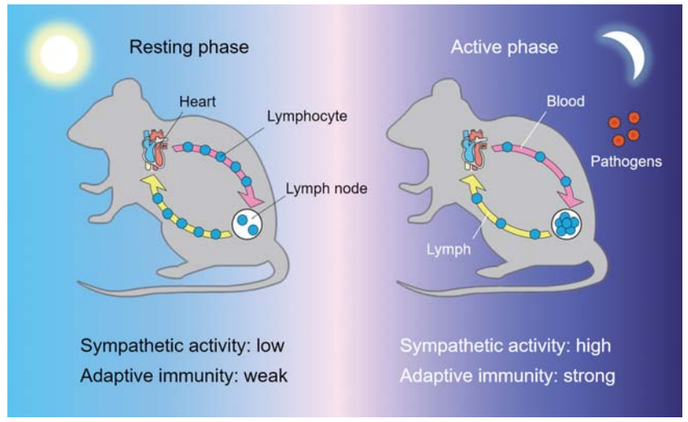

Figure 2. Diurnal control of adaptive immunity by adrenergic nerves.
During the period of high adrenergic nerve activity, lymphocyte egress from LNs is restricted, which leads to an increase of lymphocyte numbers in LNs. Immunization during the period of lymphocyte accumulation in LNs promotes adaptive immune responses. This diurnal variation of lymphocyte trafficking may have evolved to maximize the efficiency of host defense against pathogens during the active phase.

Recent Publications

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Brain-Immune Interaction

Ben Seymour, MD/PhD

Professor	**Ben Seymour**
Associate Professor	**Aya Nakae**
Assistant Professor	**Masaki Maruyama**
Postdoctoral Fellow	**1**

My lab studies interactions between the immune system and the brain, and combines immunology, neuroscience and information science. The research focuses on two main questions

1. How does the brain respond to peripheral neurogenic inflammation?
2. Can we isolate brain-based biomarkers for fatigue and pain in inflammatory arthritis (humans and rodent models)?

Brain responses during neurogenic inflammation.

The nervous system plays a critical role in the local tissue inflammatory response, by sensing bacterial antigens (immunoception), modulating the immune response (locally and systemically), and regulating autoimmunity (e.g. as in psoriasis). One way to probe the role of the nervous system in tissue inflammation is to study neurogenic inflammation, which can be done in humans by topical application the compound capsaicin. This sensitizes peripheral and spinal cord circuits that transmit information about injury to the brain, and modulate the local tissue inflammatory response through descending brain-to-spinal cord pathways.

We have been studying capsaicin inflammation using (Magnetoencephalography (MEG) and functional Magnetic Resonance Imaging (fMRI)) in humans. By combining the two imaging methodologies, we have been able to chart the dynamic signature of the brain response that uses information about peripheral inflammation to control behaviour. We have identified a brain region that seems critical for sensing inflammation and using this information to guide protective responses orientated to minimise further injury. This is the first demontsration that the brain can specifically plan behaviour based on inflammation or injury.

Biomarkers for fatigue and pain in inflammatory arthritis.

Patients with inflammatory arthritis report that fatigue and pain are the most severe symptoms they experience as part of their disease. Critically, these symptoms often respond poorly to immunologic therapies such as anti TNF, despite the fact the inflammatory markers often respond well. This highlights how little we understand about the nature of fatigue in inflammatory disease, and how it relates to immune activation. Part of the problem is that we have no objective biomarkers for these symptoms, which is a major problem not just for human and clinical studies, but also for translation with animal studies.

Using bioinformatic approaches, we have developed tools that can predict both pain and depression with reasonable accuracy by 'decoding' patterns of brain activity for patients with osteoarthritis, and we are now applying these to patients with rheumatoid arthritis. Furthermore, our cohort involves patients about to start anti-TNF therapy, and longitudinally following up these patients, we aim to prospectively predict their treatment response in terms of symtpoms of fatigue and pain. In parallel, we have been studying brain activity following joint inflammation (CFA model) in rodents (in collaboration with Shionogi). This has identified a brain region (the anterior cingulate cortex) that is active during joint inflammation, and is associated with the level of fatigue (indexed by the animals burrowing behavior) induced by inflammatory signals.

In other work, Aya Nakae has been developing a novel rodent model of post-operative pain, and has begun to characterize the immunological, endocrinological and neurophysiological basis of the persistent pain state that results. A vision for this research is to understand how immunological factors may contribute the chronic pain state that results from numerous common surgical procedures.

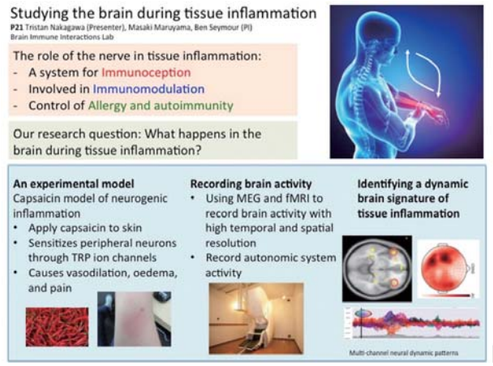

Figure 1.
Overview of our study program identifying the brain response to neurogenic inflammation.

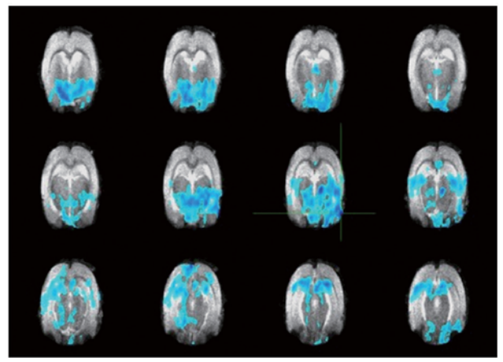

Figure 2.
Sequential images through the rat brain showing functional connectivity of the anterior cingulate cortex associated with fatigue in the CFA inflammatory arthritis model.

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Information Systems

Yutaka Hata, PhD

Professor	**Yutaka Hata**
Associate Professor	**Syoji Kobashi**
	Shugo Yasuda
	Shinichiro Shima
Assistant Professor	**Manabu Nii**
Support Staff	**1**

Three dimensional analysis of macrophage learning-tracking algorithm

It is effective to analyze 3-D dynamics of macrophages. Last year, we proposed an automated method based on Hungarian method in 11.7 time-lapse 2-D MR images. In this year, we extend the method to 3-D processing and evaluated the performance numerically. The method is based on background subtraction and the Hungarian algorithm. It was validated using mouse brain MR images and artificially synthesized images. The results with artificially synthesized images showed that the method tracked the macrophages well. The method successfully tracks the macrophages in the real mouse brain and visualizes the 3-D trajectory. A limitation of the proposed method is that it might suspend due to the noise influence.

A Monte Carlo simulation method of chemotactic motile cells

A Monte Carlo simulation method for chemotactic motile cells is developed based on a kinetic chemotaxis equation for motile cells coupled with reaction-diffusion equations for chemical cues. The Monte Carlo method is applied to the traveling pulse and pattern formation problems. It is demonstrated that the Monte Carlo method can reproduce the experimental results of the traveling pulse of chemotactic bacteria. Comparison of the Monte Carlo simulations and theoretical analysis on the pattern formation problem of chemotactic bacteria proves a solid mathematical foundation to the Monte Carlo method.

Region Extraction Algorithm

Chronic thromboembolic pulmonary hypertension (CTEPH) is one of the lung diseases caused by thrombi occurring in pulmonary arteries. By measuring the size of a region dominated by an arterial subtree which has thrombi, physicians find a higher treatment effect point. This research proposes an automated method to extract the lung region dominated by an arterial subtree from MDCT images as the basis of a region extraction algorithm. The method extracts an arterial subtree associated with a seed point and a region dominated by the extracted arterial subtree and visualizes them. The results show a clinical ability of visualization and extraction of dominant region from MDCT Images.

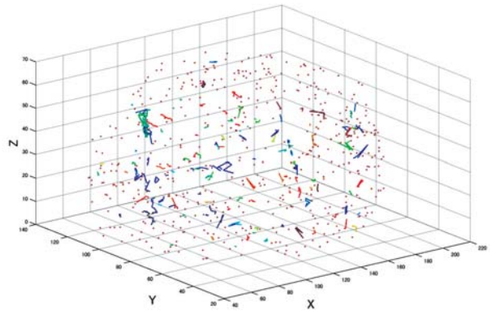

Figure 1.
3-D tracking result in the time-lapse MR images. (Dots are brain surface of the mouse. Lines are trajectories of macrophages. Different colors are different macrophages).

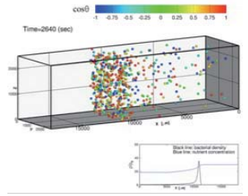

Figure 2.
The snapshot of Monte Carlo simulation for the traveling pulse of chemotactic bacteria in micro channel. The particles represent each chemotactic bacterium and their colors represent the moving direction. The inset shows the snapshot of the population density of bacteria and concentration of chemical cue along the channel.

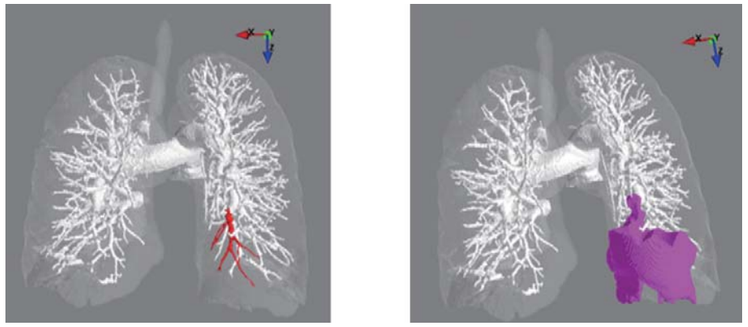

Figure 3.
Extraction result of dominant region (red region denotes pointed pulmonary artery and purple region is the extracted region corresponding to the pulmonary artery).

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Systems Immunology

Daron M Standley, PhD

Professor	**Daron M Standley**
Associate Professor	**Kazutaka Katoh**
Assistant Professor	**Songling Li**
Postdoctoral Fellow	**1**
Research Assistant	**2**
Support Staff	**1**

In 2016, the systems immunology lab began to shift from general bioinformatics to several specific topics in computational immunology. Although we still develop general bioinformatics methods, and collaborate with a wide range of experimental groups in other areas, our priority is to deliver state-of-the art software targeting three main areas: (1) structural modeling of protein-RNA complexes involved in post-translational regulation of immune responses; (2) Functional analysis of large-scale immune receptor sequence data; and (3) multiple sequence alignment methods, both as publicly available software (MAFFT) and as curated databases for sequence and structures of interest (e.g., RBPs, BCRs, TCRs, pathogens, etc.).

RNA-binding proteins (RBPs) are essential for proper immune responses, as they effect the strength and duration of activation (e.g., inflammatory responses). As an example, Arid5a, Regnase-1 and Roquin each target interleukin-6 (IL6) through a conserved structural motif; however, each of these proteins shapes the IL6-mediated response in different ways. There are likely to be other important RBPs and RNA targets affecting a wide range of biochemical pathways. However, the lack of structural information makes it difficult to generalize the modes of action of known RBPs to other RBPs with similar domains. For these reasons, we are working on the prediction of protein-nucleotide complexes starting from sequence information. This approach is challenging due to the flexibility of single-stranded nucleotides as well as the noisiness of some RNA binding sites. Our initial goal to address these issues was to integrate information from multiple sequence alignments of known protein-RNA complexes in order to predict nucleotide binding propensities for new RBPs. This strategy has been largely successful and our resulting tool, aaRNA (Li et al), and its companion, aaDNA, have been validated in third-party benchmarks as well as in small-scale collaborative studies with experimental groups (Masuda et al., Mino et al, Yokogawa et al). The next goal was to utilize predicted binding propensities to drive docking simulations of fully flexible single-stranded nucleotides. This strategy has also paid off, as evidenced by recent collaborative projects (Nyati, et al., Fukuda et al., Sakakibara, et al.) Our flexible docking simulations utilize a coarse-grained representation of the nucleotide chain and thus lack the resolution required to predict base specificity of a given RBP. To address this limitation, we are now developing an extension that can generate all-atom representations of the coarse-grained docked models (manuscript in preparation). With these tools in place we aim to identify new RBP-RNA interactions that are likely to play a role in regulation of immune reponses.

B and T cell repertoires are shaped by various factors, both intrinsic and extrinsic to the host, including genetics, age and antigen exposure. B and T cell repertoires thus contain an imprint of past and present antigens as well immune responses to these antigens. With the emergence of novel high-throughput B cell receptor (BCR) and T cell receptor (TCR) sequencing along with proteomics-based technologies, it is now possible to collect BCR and TCR sequences from individual donors on a large scale. Functional interpretation of the resulting Big Sequence Data is a major challenge, however, as there are few well annotated BCR or TCR sequences with which to compare the data. Given the fact that the human immune system can theoretically produce hundreds of billions of unique BCRs and hundreds of millions of unique TCRs, comparing receptors conventially at the sequence level between donors or between a donor and a reference set of annotated sequences, is unlikely to yield many functional hits. Moreover, even if a significantly similar sequence is detected, little is known about how small sequence variations affect the sensitivity, specificity or affinity to known antigens. To address these issues, we have developed a structural modeling approach that is efficient enough to handle large-scale sequence data sets and yet robust enough to identify hits across multiple donors (Shirai, Yamashita). In 2016, we succeeded in speeding up the structural modeling step to within five seconds per structure by utilizing several new features in MAFFT and deriving a new knowledge-based scoring function. Preliminary tests indicate that pairs of BCR or TCR models within the same structural cluster are far more likely than random pairs to target the same antigen (manuscript in preparation).

Multiple sequence alignment (MSA) is an important step in comparative analyses of biological sequences. MAFFT is one of the most popular programs for building MSAs. Since the first release of MAFFT in 2002, we have continuously improved its accuracy, speed and utility in practical situations, providing different options for newly emerging types of data and analyses. Recent features include: Inclusion of secondary structural information of non-coding RNAs and proteins, interactive selection of sequences for phylogenetic tree inference, etc. We are working on an extension to make more accurate options applicable to a larger number of sequences, in responding to the demands of large-scale analyses.

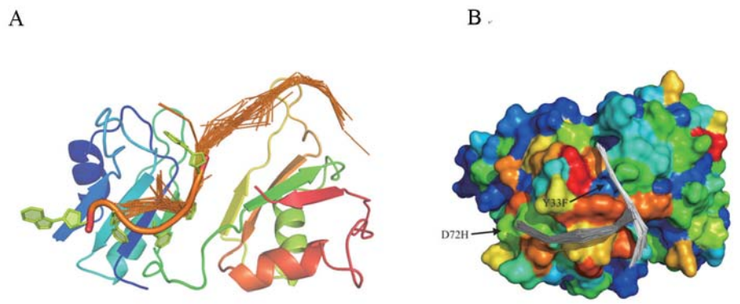


Figure.
A. Putative binding mode of mouse AUF-1 protein with the homology model of AUF-1 (Nyati et al).
B, Flexible docking of ssDNA to SLE antibody (Sakakibara et al).

Recent Publications

- Nyati K, et al. TLR4-induced NF-κB and MAPK signaling regulate the IL-6 mRNA stabilizing protein Arid5a. Nucleic Acids Res. 45, 2687-2703 (2017).
- Fukuda H, et al. Structural insight of APOBEC3G N-terminal domain reveals critical residues for its RNA association. Submitted (2016).
- Sakakibara S, et al. Clonal evolution and antigen-recognition of anti-nuclear antibodies in acute systemic lupus erythematosus. Submitted (2016).
- Yamada K.D, Tomii K and Katoh K. Application of the MAFFT sequence alignment program to large data—reexamination of the usefulness of chained guide trees. Bioinformatics 32, 3246-3251 (2016).
- Katoh K and Standley D.M. A simple method to control over-alignment in the MAFFT multiple sequence alignment program. Bioinformatics 32, 1933-1942 (2016).

Quantitative Immunology

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

The Quantitative Immunology Research Unit is a team of researchers with expertise in different scientific fields including immunology, bioinformatics and theoretical physics. Our aim is to understand how the immune system works by combining three different but closely interconnected approaches; (1) quantitative measurement of molecular dynamics, (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. We developed a novel algorithm to quantify the diffusion dynamics of single molecules without bias, even under high molecular density conditions. This highly quantitative technique can be used to describe the dynamics of the immune system's signaling pathways.

Data integration

The accumulation of high-throughput ("omics") datasets has 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.

In another attempt to utilize the power of mathematics in immunological research, we are developing a hierarchical model of the immune system. In this framework, we derive the macroscopic dynamics of the immune system from the stochastic and heterogeneous behavior of the individual cells involved in antigen presentation. This framework allows us to address one of the main questions in immunology, "how the balance between protective immunity and immune tolerance is achieved?".

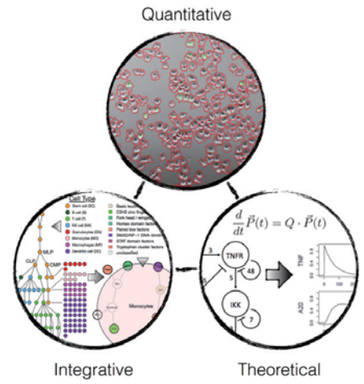
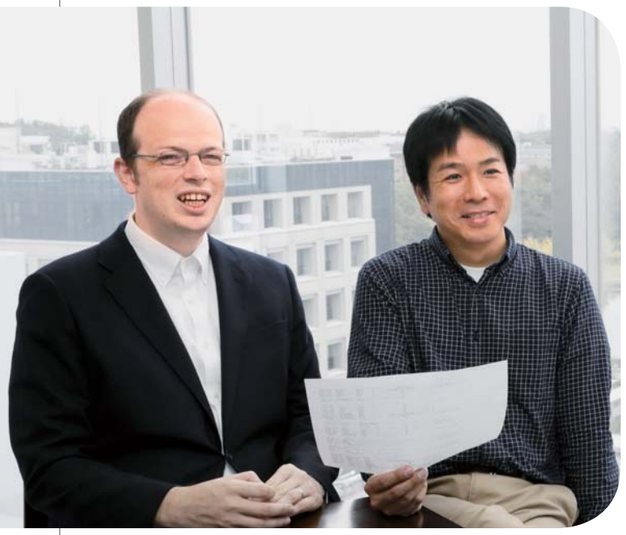


Figure.
The Quantitative Immunology Research Unit combines quantitative, integrative and theoretical approaches.

Recent Publications

- Kumagai Y, Vandenbon A, Teraguchi S, Akira S and 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 and 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 and 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).
- Patil A, Kumagai Y, Liang KC, Suzuki Y and Nakai K. Linking transcriptional changes over time in stimulated dendritic cells to identify gene networks activated during the innate immune response. PLoS Comp. Biol. 9, e1003323, doi:10.1371/journal.pcbi.1003323 (2013).
- Teraguchi S, Kumagai Y, Vandenbon A, Akira S and Standley DM. Stochastic binary modeling of cells in continuous time as an alternative to biochemical reaction equations. Phys. Rev. E, Stat. Nonlin. Soft Matter Phys. 84, 062903 (2011).

Immuno-Genomics



Assistant Professor	**Alexis Vandenbon**
	Hiromasa Morikawa
Research Assistant	**1**
Visiting Scientist	**1**

The ultimate goal of immunology is health care, elucidation of causes of diseases, and their treatment in human patients. To achieve this goal, a complete understanding of the immune network, the interactions and regulatory principles between cells and between gene products is required. In the Immuno-Genomics Research Unit, our aim is to establish and apply methodologies for extracting the maximum amount of information possible from limited experimental data, using integrative bioinformatics approaches. Here, we briefly introduce some of the research projects we are involved in.

The control of stimulus-induced dynamics in histone modifications in dendritic cells

The importance of transcription factors (TFs) and epigenetic modifications in the control of gene expression is widely accepted. However, the causal relationships between the dynamics in histone modifications, TF binding and gene induction in the course of a short-term response to pathogens are not well understood.

In collaboration with Dr. Kumagai of the Quantitative Immunology Research Unit, we have been studying the ordering of these events in dendritic cells (DCs) in response to lipopolysaccharide (LPS) stimulation. Genome-wide patterns in histone modification changes and gene induction were studied through bioinformatics analysis of newly obtained ChIP-seq, RNA-seq and TSS-seq time series data.

Although researchers often assume that activating histone modifications are a prerequisite for inducing transcription, our data only partly supported this view. Rather, LPS-induced accumulation of several histone modifications at promoters occurred within well-defined time windows, independent of the transcriptional induction of the promoters. For example, LPS-induced acetylation of H3K9K14 occurs rapidly, followed by methylation of H3K4, and finally methylation of H3K36.

Through integrative analysis with binding data for >20 TFs in LPS-treated DCs, we revealed potential links between the timing of TF activation and the timing of accumulation of histone modifications. Especially, binding by STAT1/2 coincided with induction of H3K9K14ac, and was followed by increases in H3K4me3. Analysis of TRIF, IRF3, and IFNR knock-out data further confirmed the link between STAT1/2 binding and the establishment of these histone modifications.

Establishment of a comprehensive cell type-specific network inference framework

Last year, we published Immuno-Navigator, a gene co-expression database for cells of the human and mouse immune system (Vandenbon et al., PNAS, 2016). The current database includes 4,639 human microarray samples, obtained from 19 cell types from 191 studies, and 3,434 mouse microarray samples, obtained from 24 cell types from 261 studies in total. We are currently preparing a larger dataset including >44,900 mouse and >38,700 human RNA-seq samples, covering more than 60 cell types.

At the same time, we are developing a new network analysis methodology in which biological networks are inferred simultaneously over extensive parts of the differentiation lineage tree as well as over stimulus-induced signaling pathways. The method leverages known relationships between progenitor cells and differentiated cell types as well as similarities in signaling pathways between different cell types. A conceptual scheme is shown in Figure 1.

Interdisciplinary collaborative research projects

In addition to projects that are mostly bioinformatics-driven, we are involved in several interdisciplinary projects covering several aspects of regulation of the immune system.

In collaboration with the Quantitative Immunology Research Unit we have analyzed the genome-wide patterns of RNA degradation in response to LPS stimulation of DCs, and employed these for the prediction of RNA secondary structure prediction (Kumagai et al., BMC Genomics, 2016). We also contributed to the further analysis of post-transcriptional regulation by Regnase-1 (Mino et al., *Cell*, 2015) and circulating serum micro-RNAs (Nosirov et al., Advances and Applications in Bioinformatics and Chemistry, 2016), to the elucidation of the role of Satb1 in establishing super-enhancers during the development of regulatory T cells (Kitagawa et al., Nature Immunology, 2017), and to the analysis of single-cell gene expression data of subsets of migratory T cells (Ikebuchi et al., Scientific Reports, 2016).

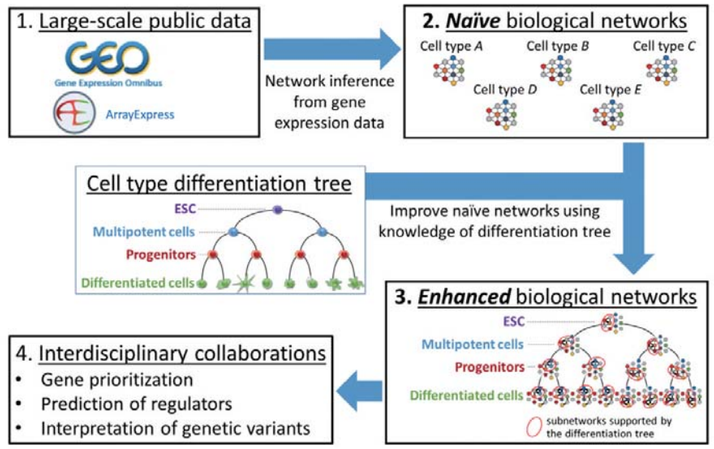


Figure.
Conceptual flow of our comprehensive cell type-specific network inference framework. A large amount of gene expression data (1) is used to infer biological networks using default, naïve methodologies (2). Using the prior knowledge of cell type differentiation, our methodology improves the inferred networks (3). These high quality networks can finally be used for downstream interdisciplinary collaborations (4).

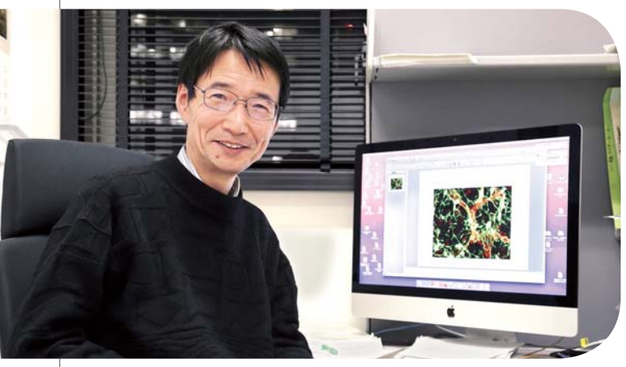
Recent Publications

- Kitagawa, Y,. et al. Guidance of regulatory T cell development by Satb1-dependent super-enhancer establishment. Nat. Immunol. 18, 173–183 (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 (2016).
- Vandenbon, A., et al. Immuno-Navigator, a batch-corrected coexpression database, reveals cell type-specific gene networks in the immune system. Proc. Natl. Acad. Sci. USA. 113, E2393–E2402 (2016).
- Ikebuchi, R., et al. A rare subset of skin-tropic regulatory T cells expressing Il10 / Gzmb inhibits the cutaneous immune response. Sci. Rep. 6, 35002 (2016).
- Mino, T., et al. Regnase-1 and Roquin Regulate a Common Element in Inflammatory mRNAs by Spatiotemporally Distinct Mechanisms. Cell 161, 1058–1073 (2015).

New Principal Investigators from FY2017

IFReC welcomed four new principal investigators with a new framework for governing structure in 2017. We expect them to add new impetus to IFReC.

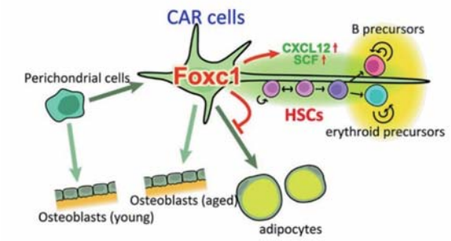
Laboratory of Stem Cell Biology and Developmental Immunology



Takashi Nagasawa, MD/PhD

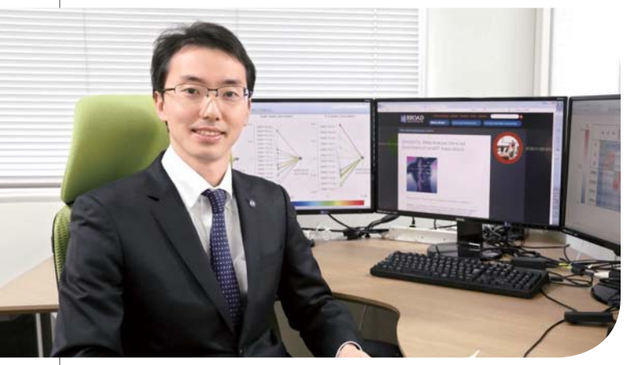
We isolated a chemokine, CXCL12 (SDF-1/PBSF) as a molecule that stimulates the growth of B cell precursors (*PNAS* 1994) and found that CXCL12 and its receptor CXCR4 are essential for colonization of bone marrow by hematopoietic stem cells (HSCs)(*Nature* 1996; *Immunity* 2003), maintenance of a pool of HSCs in bone marrow (*Immunity* 2006), development of immune cells, including B cells, pDCs and NK cells (*Nat. Rev. Immunol.* 2006), vascular formation and cardiogenesis (*Nature* 1998).

Additionally, we identified a population of reticular cells expressing CXCL12 at high levels, termed CXCL12-abundant reticular (CAR) cells within bone marrow (*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 (*Immunity* 2010). Recently, 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 (*Nature* 2014). We are studying the roles of CXCL12-CXCR4 signaling and CAR cells in the spatiotemporal regulation of lymphohematopoiesis during homeostasis and diseases.



Conceptual Figure

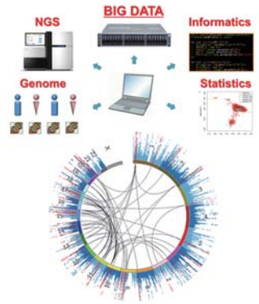
Laboratory of Statistical Immunology



Yukinori Okada, MD/PhD

Statistical genetics is a research field that evaluates causality of human genetic variations on diseases, using statistical and bioinformatics approaches. Recent developments of sequencing technologies have provided human genome data of hundreds of thousands of the subjects, and successfully identified comprehensive catalogues of genetic susceptible 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 theme of our laboratory is to develop such methods and apply to the latest large-scale disease genome and omics data. We have demonstrated that the disease risk genes were significantly enriched in overlap with the target genes of the drugs currently used for treatment of the diseases, and could be promising resources of drug repositioning (e.g. CDK4/6 inhibitors for rheumatoid arthritis, Okada Y et al. *Nature* 2015). Construction of the novel analytical methodologies of statistical genetics, such as the HLA imputation method and the miRNA network-based GWAS analysis (Okada Y et al. *Nat Genet* 2015, *Sci Rep* 2016), is also the focus of our laboratory. Through such translational approaches, we would like to empirically show the value of statistical genetics to dissect disease immunology and novel drug discovery.



Laboratory of Molecular Immunology

Sho Yamasaki, PhD

The immune system uses a wide range of receptors for the protection against pathogens and their clearance. Innate immune receptors elicit rapid responses, while a more complex one is triggered by adaptive immune receptors.

The analysis of the C-type lectin family of innate immune receptors has made significant progress in the past decade.

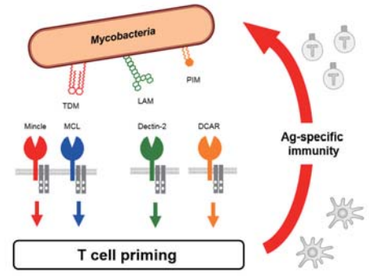
We have found that these receptors can sense both "damaged self" and "non-self pathogens". Notably, our team showed that Mincle, MCL, Dectin-2 and DCAR are C-type lectin receptors that recognize *M.* tuberculosis. Our objective is to identify and study novel immunoreceptors and their ligands, in order to elucidate the mechanisms underlying ligand recognition as well as their potential roles in immune disorders. Based on these results, we also aim to design new methods to orient the immune responses.

To this end, our research centers on the following axes:

1) Significance and mechanisms of recognition of "aberrant self" or "non-self pathogens" by C-type lectin family receptors.

2) Identification of "self" through the TCR as well as its role in T cell development.

3) Study of newly discovered T cell subsets involved in autoimmune diseases.

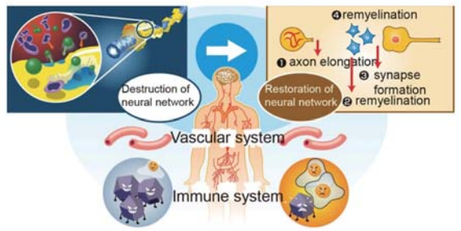


Laboratory of Molecular Neuroscience

Toshihide Yamashita, MD/PhD

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. In this study, we 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. Further, we analyzed the mechanism by which the spatiotemporal dynamics in those biological systems control a series of processes.

Particularly, the ultimate goal of this study was to elucidate the control mechanism exerted by the associations among the nervous system, immune system, and vascular system. Additionally, we aimed 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.



Joint Research Chair of Innovative Drug Discovery in Immunology

Kunihiro Hattori (Visiting Professor)

Ryusuke Omiya (Visiting Associate Professor)

Junichi Hata (Visiting Researcher)

As well as other researchers and technicians.

Members of Chugai Lab
Drs. Kunihiro Hattori, Junichi Hata, and Ryusuke Omiya (left to right)

The laboratory was set up on the basis of the comprehensive collaboration agreement for cutting-edge research formed between Osaka University and Chugai Pharmaceutical Co., Ltd.

The Chugai researchers in this laboratory and IFReC researchers will promote close exchanges of information and comprehensive collaboration including performing joint feasibility studies prior to advancing joint research. The synergy of IFReC's recent research results with Chugai's proprietary antibody engineering technologies and molecular library etc. has high potential for the discovery of seeds for new drugs.

Interview with Dr. Hattori

I don't know if it is the tradition of Osaka University largely influenced by Professor Kishimoto, but the researchers at IFReC are all thoroughly devoted to pure basic research. It may be that they have not had the opportunity to view their research from the perspective of drug discovery.

I hope that this new laboratory, set up under the comprehensive collaboration agreement between Chugai and Osaka University, will act as a pathway to drug discovery based on IFReC's cutting-edge research.

Our job is to not simply receive experiment data from the university and make drugs but is to utilize the drug discovery technologies developed over many years at Chugai to collaborate with and accelerate research as well as create a win-win relationship in which we strategically acquire intellectual property and share knowhow on efficient application methods and research processes.

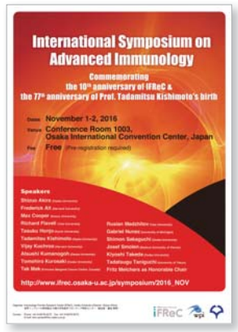
Following the global success of the monoclonal antibody Actemra® commercialized under the direction of Professor Kishimoto, we seek second and third drugs from seeds originating from Osaka University and in the near future expect to expand the laboratory located here to 20 researchers.

Symposia & Seminars

International Symposium on Advanced Immunology

- **Date :** November 1-2, 2016
- **Venue :** Conference Room 1003, 10th floor of Osaka International Convention Center

This symposium was held for commemorating the 10th anniversary of IFReC and the 77th anniversary of Prof. Tadamitsu Kishimoto's birth. In the symposium, the world's leading scientists discussed current progress in elucidating immune reactions and mechanisms that mediate and regulate immune responses. The participants had an excellent opportunity to exchange information and ideas to accelerate and further the progress of immunology to the future discovery of new therapies for immune diseases.



Nov. 1

Opening remarks

Shojiro Nishio (President of Osaka University)
Hiroo Imura (Former President of WPI Program)
Shizuo Akira (Director of IFReC)

Speaker	Title
Shimon Sakaguchi (Osaka University)	Control of immune responses by regulatory T cells
Vijay K. Kuchroo (Harvard University)	Single cell genomics identifies novel regulators of autoimmunity and anti-tumor immunity
Richard Flavell (Yale University)	Non-coding RNAs in the regulation of inflammation
Tasuku Honjo (Kyoto University)	Cancer immunotherapy by PD-1 blockade
Tak W. Mak (Princess Margaret Cancer Centre, Canada)	Beyond Checkpoint Blockade: Emerging Strategies in Immunotherapy
Atsushi Kumanogoh (Osaka University)	Involvement of semaphorins in pathogenesis of autoimmune, allergy and inflammatory diseases
Josef S. Smolen (Medical University of Vienna)	Insights into pathways to inflammatory rheumatic diseases - novel therapies and response prediction
Tadamitsu Kishimoto (Osaka University)	IL-6, Tocilizumab and Arid5a; Immunopathology and Therapy of Autoimmune Diseases

Nov. 2

Speaker	Title
Frederic Alt (Harvard University)	Recurrent DNA Break Cluster Genes in Neural Development, Diversification and Disease: Potential analogies to Lymphocyte Rearrangement Processes
Tomohiro Kurosaki (Osaka University)	Fate decisions of germinal center B cells into the memory B cell or plasma cell compartment
Max Cooper (Emory University)	Evolution of Lymphocyte Lineages
Kiyoshi Takeda (Osaka University)	Regulation of intestinal homeostasis by epithelial barriers
Gabriel Nuñez (University of Michigan)	Role of the Microbiota and Immunity in the Control of Pathogens at the Intestinal Barrier
Tadatsugu Taniguchi (University of Tokyo)	Recent advances in the regulation of inflammation and its associated diseases
Ruslan Medzhitov (Yale University)	Inflammation, Homeostasis and Disease
Shizuo Akira (Osaka University)	Identification of a novel monocyte subset involved in lung fibrosis

Closing talk & remarks by Fritz Melchers

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.


IFReC Seminar

IFReC

PATHOLOGY OF BEHCET'S DISEASE

3:00 - 3:40 pm

"Immunopathogenesis of Behcet's Disease"

Haner Direskeneli, M.D.

(Professor of Rheumatology, Chief of the Division of Rheumatology, Marmara University, School of Medicine Hospital, Istanbul)

3:40-4:20 pm

"Inflammatory signaling in familial Behcet's Disease"

Junya Masumoto, M.D., Ph.D.

(Professor, Department of Pathology, Ehime University Proteo-Science Center and Graduate School of Medicine)

4:20-5:00 pm

"Natural killer cells in Behcet's Disease"

Gunnur Deniz, Ph.D.

(Professor, Head of Department of Immunology, Director of Aziz Sancar Institute of Experimental Medicine, Istanbul University)

DATE Friday, March 24, 2017

TIME 3:00-5:00pm

VENUE Meeting Room #1, IFReC Research Building 1F

HOST Ceveyir COBAN (Malaria Immunology, IFReC)

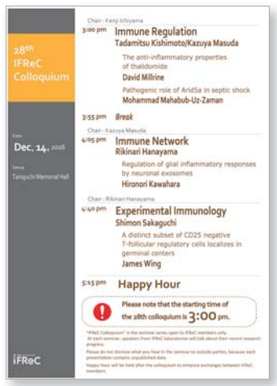
Date	Speaker	Affiliation	Title
2016			
Apr. 28	**Klas Kärre**	Karolinska Institutet, Sweden	MHC class I dependent education of NK cells: implications for immunotherapy to tumors
Jun. 17	**Masaru Kanekiyo**	NIAID, NIH, USA	Self-assembling nanoparticle-based immunogen design: mimicry of virus and beyond
Jul. 8	**Stephan Gasser**	National University of Singapore	DNA damage and immune recognition of cancer cells
Sep. 27	**Suresh Kumar Verma**	Center for Translational Medicine, Temple University, USA	Interleukin 10 in regulation of cardiac hypertrophy and heart failure
Oct. 20	**James Di Santo**	Institut Pasteur, France	Transcriptional regulation of innate lymphoid cell development and plasticity
Oct. 31	**Gabriel Nunez**	University of Michigan School of Medicine, USA	Role of the microbiota in the regulation of immunity against systemic infections
Nov. 4	**Richard A. Koup**	NIAID, NIH, USA	Impact of HIV and SIV on lymph node structure and function
Nov. 15	**Jian Han**	HudsonAlpha Institute for Biotechnology iCubate Inc., and iRepertoire Inc., USA	Towards the ultimate diagnostics: learning how to diagnose diseases from our immune system by NGS of immune repertoire
2017			
Jan. 13	**Gaetan Burgio**	John Curtin School of Medical Research, Australian National University	A voyage into the discovery of the genetic mechanisms for host resistance to malaria
	Richard Culleton	NEKKEN, Nagasaki University, Japan	A voyage into the discovery of the genetic mechanisms for malaria parasite resistance to hosts
Jan. 26	**Dennis Klinman**	The National Cancer Institute (NCI), NIH, USA	Intra-tumoral delivery of TLR agonists for cancer therapy
Jan. 27	**Daniela Verthelyi**	The Food and Drug Administration (FDA), USA	Modeling Zika virus in mice: what have we learned so far?
Feb. 1	**Mikael Martino**	European Molecular Biology Laboratory / Australian Regenerative Medicine Institute, Monash University, Australia	Promoting tissue regeneration by modulating the immune system an example with bone regeneration
Feb. 2	**Anne O'Garra**	The Francis Crick Institute, UK	Systems approaches to studying the immune response in tuberculosis: strategies to improve mouse models of human disease
Feb. 3	**Lewis L. Lanier**	University of California, San Francisco, USA	Tracking the fate of memory Natural killer cells
Feb. 22	**Ye Htun Oo**	University of Birmingham Liver and Hepatobiliary Unit, UK	Exploring human intrahepatic Treg and IL-17 secreting cells to develop translational Treg cell therapy
Mar. 23	**John C. Reed**	Global Head, Roche Pharm Research & Early Development	Bolstering immune responses
	David M. Lee	Global head, Immunology, Inflammation & Infectious Diseases, Roche Pharma Research & Early Development	Restoring immune homeostasis
Mar. 24	**Haner Direskeneli**	School of Medicine Hospital, Marmara University, Istanbul, Turkey	Immunopathogenesis of Behcet's Disease
	Junya Masumoto	Ehime University Proteo-Science Center and Graduate School of Medicine, Japan	Inflammatory signaling in familial Behcet's Disease
	Gunnur Deniz	Aziz Sancar Institute of Experimental Medicine, Istanbul University, Turkey	Natural Killer cells in Behcet's Disease

IFReC Colloquia

■ Date : 25 th Colloquium : April 20, 2016
26 th Colloquium : June 8, 2016
27 th Colloquium : August 10, 2016
28 th Colloquium : December 14, 2016
29 th Colloquium : February 8, 2017

■ Venue : Taniguchi Memorial Hall, Osaka University

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.



	Speaker	Title
25st	**Kosuke Fujimoto** (Mucosal Immunology)	Regulation of gut homeostasis by the ulcerative colitis-associated gene RNF186
	Alexis Vandenbon (Immuno-Genomics Unit)	Immuno-Navigator: a co-expression database for cell type-specific network inference in the imune system
	Yoshiko Murakami (Immunoglycobiology)	Paroxysmal nocturnal hemoglobinuria caused by PIGT mutations: Atypical PNH
26st	**Miwa Sasai** (Immunoparasitology)	Some ATG proteins play a critical role in non-autophagic cell-autonomous immunity to vacuolar pathogens
	Alison J. Hobro (Biophotonics)	Multimodal label-free imaging of cellular responses to stress
	Takashi Satoh (Host Defense)	NF-IL6 licenses pro-fibrotic monocyte differentiation from its progenitor
27st	**Akiko Nakai** (Immune Response Dynamics)	A novel molecular mechanism of chemoattractant receptor signaling
	Syoji Kobashi (Information Systems)	In vivo 3-D macrophage labeling and tracking method using time-lapse MR images
	Michelle Sue Jann Lee (Malaria Immunology)	Plasmodium infection modulates bone homeostasis
28rd	**David Millrine** (Immune Regulation)	The anti-inflammatory properties of thalidomide
	Mohammad Mahabub-Uz-Zaman (Immune Regulation)	Pathogenic role of Arid5a in septic shock
	Hironori Kawahara (Immune Network)	Regulation of glial inflammatory responses by neuronal exosomes
	James Wing (Experimental Immunology)	A distinct subset of CD25 negative T-follicular regulatory cells localizes in germinal centers
29th	**Kazuya Takeda** (Immune Regulation)	Tracking allergen-reactive IgE^{+} B cells in patients with eosinophilic chronic rhinosinusitis
	Aya Nakae (Brain-Immune Interaction)	Newly developed animal post-operative persistent pain model and its behavior and pituitary changes after experimental pain in humans
	Masaki Maruyama (Brain-Immune Interaction)	Dynamics of capsaicin-induced skin inflammation and its influence on decision behaviors
	Tristan Nakagawa (Brain-Immune Interaction)	The representation of capsaicin-induced tissue inflammation in the brain
	Hiroki Kato (Nuclear Medicine)	^{11}C-acetate PET in patients with multiple sclerosis: a pilot study

Events

The 6th NIF Winter School on Advanced Immunology

■ Date : January 22-26, 2017

■ Venue : Grand Copthorne Waterfront Hotel, Singapore

The sixth Winter School on Advanced Immunology was jointly organized with Singapore Immunology Network (SIgN). Forty-four young researchers, who were competitively selected from 85 applicants and 16 world leading immunologists, got together in Singapore on 22-26 January 2017. Five young IFReC researchers participated in the school. The participants shared intriguing insights and findings in immunology, discussed new ideas and forged friendships that will fuel networking and future collaborations.
(Visit the following website for details; http://ifrec-sign-winterschool.org)

Lecturer	Title
Shizuo Akira IFReC, Japan	Functional diversity of macrophage/monocyte subsets revealed by gene targeting
Veronique Angeli National University of Singapore	Homeostatic function of arterial macrophages
Burkhard Becher University of Zurich, Switzerland	How T cells talk to myeloid cells in inflammation
Antonio Bertoletti Duke-NUS Medical School, Singapore	Immunotherapy with T-cell receptor redirected T cell targeting viral antigen in viral related tumors
Subhra Biswas Singapore Immunology Network	Immuno-metabolic re-programming of monocytes/macrophages
Ana Cumano Institut Pasteur, France	Lymphocyte commitment during development
Wendy Havran The Scripps Research Institute, USA	Immune crosstalk in the skin
Tomohiro Kurosaki IFReC, Japan	Selection of germinal-center B cells into memory B cell or plasma cell compartment
Paul Macary National University of Singapore	Analyzing the impact of antibody-mediated BloT5 neutralization on airway hypersensitivity
Laura Mackay Doherty Institute, Australia	Development and Function of Tissue-Resident Lymphocytes
Evan Newell Singapore Immunology Network	High-dimensional cellular immune profiling in health and disease
Christiane Ruedl Nanyang Technological University, Singapore	Intestinal dendritic cells
Shimon Sakaguchi IFReC, Japan	Control of immune responses by regulatory T cells
Federica Sallusto Institute for Research in Biomedicine, Switzerland	Challenges and opportunities of human immunology
Kazuhiro Suzuki IFReC, Japan	Control of lymphocyte trafficking and adaptive immune responses by adrenergic nerves
Filip Swirski Harvard Medical School and Massachusetts General Hospital, USA	Unleashing the inflammatory cascade in cardiovascular disease

Outreach Activities

Japanese Lessons

Japanese language classes are held for overseas researchers / students to alleviate any stress and inconvenience in research or daily life that may be caused by the language barrier.

We offer two lecture-style classes, "Class A: Elementary to Pre-intermediate" and "Class B: Intermediate to Advanced". Class members are expected to learn verb and adjective conjugations to have basic knowledge of Japanese grammar in Class A , to learn grammar, vocabulary of intermediate/ advanced level, Kanji etc. to improve each member of skills in Class B.

Comment by Ms. Kaori Tajima (Japanese instructor)

Many researchers attend the classes because at IFReC Japanese is necessary when carrying out daily research activities in the laboratory. In order for the participants to start using Japanese sooner in communication with their colleagues, we learn words such as experiment, analysis, business trip, thesis/paper etc. at an early stage. Many of the participants eke out time for the classes from their full schedule so words such as busy, overtime, deadline, are often used in class. The participants find the classes a welcome break from the laboratory and emphasis is placed on listening and speaking using pair and group work to encourage networking with researchers from other laboratories.

At the end of a two year course, most communication in the class is in Japanese. This is the time when the participants can really start to enjoy their life in Japan by taking what they have learnt in the classes and using it to broaden their weekend activities.

Science Cafe

The series of science cafes is a long-lasting IFReC outreach activity to promote communication among researchers and the general public. It also enhances people's understanding of immunology researches and the researchers involved in them.

IFReC science cafe was held in Osaka University Suita campus in FY2016. About 70 participants enjoyed a novel topic in immunology in a relaxing atmosphere.

"Science Café on the Edge" at 2016 Icho Festival

<Forefront of immune therapy! Development of vaccine and adjuvant >

- Date : May 1, 2016
- Venue : Techno Alliance Building (Osaka University, Suita Campus)
- Guest : Etsushi Kuroda (Vaccine Science, IFReC)

Super Science High School Students Fair

- Date : August 10-11, 2016
- Venue : Kobe International Exhibition Hall
- Host : MEXT, JST
- Support : Boards of Education (Kobe Prefecture and Kobe City)

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

The SSH Student Fair FY2016 was held in Kobe and 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.

Students Visit

Nara Prefecture super science high school, science tour

- Date : August 18, 2016

IFReC welcomed thirty students from Nara Prefectural Nara Senior High School and Unebi Senior High School. They heard a lecture by Prof. Kurosaki (Lymphocyte Differentiation), and visited Akira Lab., Arase Lab. and Kishimoto Lab. to try some experiments and to talk with researchers.

Osaka Prefectural Tennoji High School

- Date : October 7, 2016

IFReC welcomed seventeen students from Osaka Prefectural Tennoji High School. They heard a short lecture from an IFReC Assistant Professor, Yutaro Kumagai (Quantitative Immunology), introducing immunology and innate immunity and tried some experiments using nucleotide database. They also visited the Center for Information and Neural Networks (CiNet), and listened to IFReC Professor Yoshichika Yoshioka (Biofunctional Imaging) and Associate Professor Aya Nakae (Brain-Immune Interaction) explain their research.

The Forum Commemorating the 10th Anniversary of WPI "Toward the future of Science in Japan"

In celebration of the 10th anniversary of WPI program, a lecture meeting for general citizens was organized by MEXT, JSPS, and WPI institutes. The nine leading researchers from all the WPI institutes were invited as the speakers, and the meeting was highly acclaimed by the audience.

- **Date :** December 17, 2016
- **Venue :** Lecture Hall, 3F MEXT East Building

Greetings	
13:30–13:45	**Yasunao Seki** (MEXT) **Yuichiro Anzai** (JSPS) **Hiroo Imura** (Former WPI President)
Keynote Lecture	
13:45–14:00	**"Toward the free cross-border science"** **Toshio Kuroki** (WPI Program Director)
Lectures Part 1	**"Mathematics, Physics, and Earth Science"**
14:00–14:20	**Hitoshi Murayama** (Director, Kavli-IPMU, University of Tokyo)
14:20–14:40	**Shigeru Ida** (Vice Director, WPI-ELSI, Tokyo Tech)
14:40–15:00	**Motoko Kotani** (Director, WPI-AIMR, Tohoku University)
Lectures Part 2	**"Life Science"**
15:20–15:40	**Ryuichiro Kageyama** (Vice Director, WPI-iCeMS, Kyoto University)
15:40–16:00	**Masashi Yanagisawa** (Director, WPI-IIIS, University of Tsukuba)
16:00–16:20	**Shimon Sakaguchi** (Vice Director, WPI-IFReC, Osaka University)
Lectures Part 3	**"Chemistry and Material Science"**
16:40–17:00	**Masakazu Aono** (Director, WPI-MANA, National Institute for Materials Science)
17:00–17:20	**Kenichiro Itami** (Director, WPI-ITbM, Nagoya University)
17:20–17:40	**Seiji Ogo** (Group Leader, WPI-I2CNER, Kyushu University)
Closing	
17:40–17:50	**"The future of Japan led by Science"** **Ryoji Noyori** (President of WPI Program, Nobel Laureate 2001)
17:50–18:30	**Freewheeling discussion by audience and speakers**

AAAS 2017 Annual Meeting

- **Date :** February 16-20, 2017
- **Venue :** Hynes Convention Center (Boston, U.S.A)

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 2017 Annual Meeting was held in Boston 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 300 participants visited the booth and gained interest in the WPI program and world leading researches in Japan.

Research Projects

Support Programs for Fusion Researches

One of the missions of the WPI program is to generate novel research fields through fusion of existing research fields. Under this mission, IFReC has been committed to the creation of innovative immunology fields through integration with imaging and bioinformatics technologies for comprehensive understanding of immune dynamics. To facilitate fusion research, IFReC launched the Research Support Program for Combined Research Fields in October 2009. This program provides financial support for research proposals combining different research areas. The project teams must consist of researchers from different fields or backgrounds. The IFReC board of representatives screened the projects based on proposals and the projects selected are given ¥3 million a year for three years. IFReC also started the Dual Mentor Program in October FY2012 with the aim of helping young researchers engage in fusion research at IFReC. In a selected project, a graduate student or a young postdoc, who obtained a Ph. D degree less than two years previous, is supervised by two mentors, whose specialization lies in different research fields, and is provided financial support of ¥3 million a year for three years by IFReC.

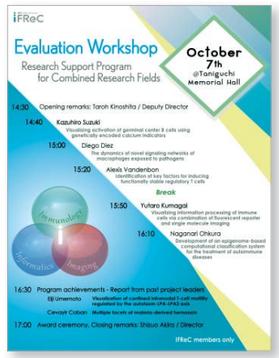
These programs have effectively encouraged IFReC researcher's interest in fusion research. By FY2016, a total of 27 projects had been selected for the program. Many interdisciplinary approaches were made and 36 papers successfully produced, contributing to the advancement of the fusion research at IFReC.

The papers of fusion research published by IFReC members, including those produced from this program, accounted for more than 30% of the total papers produced at IFReC in FY2015. This demonstrates that our continued efforts for the development of fusion research have come to fruition over the years of WPI program.

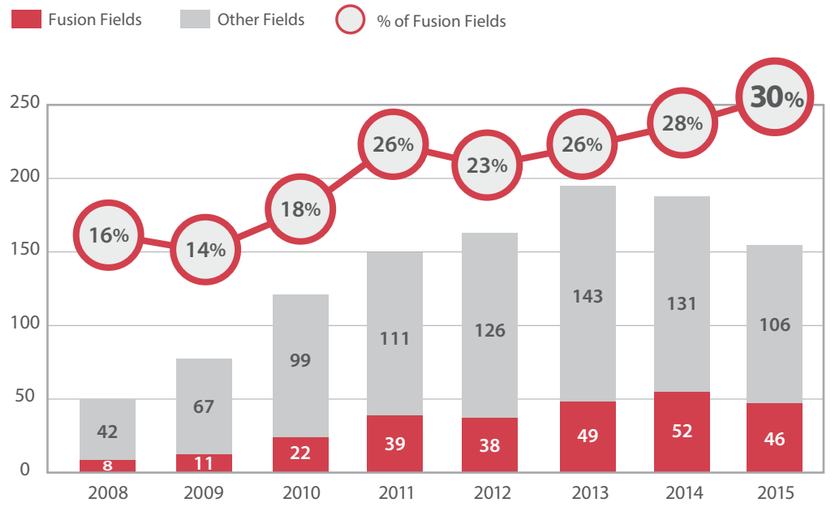
Evaluation Workshop

All the Combined Research Program and Dual Mentor Program projects are required to present their results once a year to IFReC members. At an annual Evaluation Workshop, IFReC PIs evaluate each project on the basis of specific criteria, such as "Achievement of the interdisciplinary research project mission ", Innovation of methodology" and "Prospects". The evaluators have sometimes added severe comments to encourage improvement. Each project leader is given the evaluation result after the workshop. In the workshop, a comment sheet was provided to the audience and the comments from the audience were also given to the project leaders.

In the 2016 workshop, five project leaders of the Research Support Program presented their research outcomes in front of IFReC members. The projects that gained a high score from IFReC PIs were awarded "Best Project Award." Also, two of the past project leaders, selected by the director according to their outcome, reported on their outstanding interdisciplinary research achievements in the workshop.



Papers in Fusion Research Fields



2013 – 2016

Project Leader	Title	Collaborators
Yutaro Kumagai	Visualizing information processing of immune cells via combination of fluorescent reporter and single molecule imaging	Jun Kozuka, RIKEN QBiC Shunsuke Teraguchi, IFReC
Kazuhiro Suzuki	Visualizing activation of germinal center B cells using genetically encoded calcium indicators	Yoshihiro Baba, IFReC
Naganari Okura	Development of an epigenome-based computational classification system for the treatment of autoimmune diseases	Alexis Vandenbon, IFReC Shota Nakamura, RIMD
Alexis Vandenbon	Identification of key factors for inducing functionally stable regulatory T cells	Shimon Sakaguchi, IFReC Hiromasa Morikawa, IFReC Naganari Ohkura, IFReC
Diego Diez	The dynamics of novel signaling networks of macrophages exposed to pathogens	Rikinari Hanayama, IFReC Yutaro Kumagai, IFReC Shunsuke Teraguchi, IFReC

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. Seven researchers used this support program in FY2016.

Young Scientist Support Program for Research Abroad

Name	Country	Conferences attended
Kazuya Masuda	USA	Gene expression and signaling in the immune system
Michelle Lee Sue Jann	Australia	International Congress of Immunology 2016
Szandor Simmmons	Germany	1. Institutes Seminar Series of the DRFZ 2. 46th Annual Meeting of the DGFI 3. Collaboration meetings with scientist of the Max Planck Institute
Alexis Vandenbon	Singapore	15th International Conference on Bioinformatics (InCoB2016)
Kouyuki Hirayasu	Italy	16th Annual Meeting of the Society for Natural Immunity
Alison Jane Hobro	USA	SCIX conference
Yutaro Kumagai	Singapore	15th International Conference on Bioinformatics (InCoB2016)

Since the start of this program, IFReC has provided support for 49 visits overseas by young researchers.

Support provided for 43 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. It 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**
4. **South Building, Research Institute for Microbial Diseases**
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
- DNA chip center

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.

FY2016 Kishimoto Fellowship Recipients

Position of recipient	Name (initials)	Nationality	Host researcher	Period
Specially Appointed Researcher	T. N.	Germany	Seymour	Apr. 1, 2015 - Mar. 31, 2017
Specially Appointed Researcher	P. L.	Australia	Coban	Oct. 1, 2015 - Sep. 30, 2016
Specially Appointed Researcher	D. H.	Australia	Sakaguchi	Oct. 16, 2015 - Oct 15, 2016
Specially Appointed Researcher	J. P.	Vietnam	Kishimoto	Apr. 1, 2016 - Mar. 31, 2018
Specially Appointed Researcher	N.T.	Slovenia	Quantitative Immunology Research Unit	Oct. 1, 2016 - Mar. 31, 2018

Major Awards

Shimon Sakaguchi　The Crafoord Prize

Shimon Sakaguchi was awarded the Crafoord Prize in Polyarthritis 2017 for his discoveries relating to regulatory T cells, which counteract harmful immune reactions in arthritis and other autoimmune diseases. The award ceremony was held at the Royal Swedish Academy of Sciences.

Sakaguchi is the fourth Japanese prize winner, of which three are currently or used to be IFReC researchers.

Shimon Sakaguchi, other laureates, and crown princess Victoria at the ceremony on May 18, 2017.

Tadamitsu Kishimoto　The King Faisal International Prize

Tadamitsu Kishimoto was awarded the King Faisal International Prize for Medicine 2017. The awarded topic is "Biologic Therapeutics in Autoimmune Diseases". The award ceremony was held at Riyadh, the Kingdom of Saudi Arabia.

The King Faisal International Prize is launched by the King Faisal Foundation, and is an award including Science and Medicine. Many prize winners have gone on to receive other prestigious prizes, such as the Nobel Prize.

King Salman bin Abdulaziz Al-Saud of Saudi Arabia and Tadamitsu Kishimoto at the award ceremony on April 4, 2017.

Five IFReC researchers　Highly Cited Researchers 2016

Highly Cited Researchers are researchers with papers that have a large number of citations from all over the world as selected by Thomson Reuters. In 2016, some 3,000 researchers from 21 fields were selected as being some of the most influential researchers in the world.

Six selections were from IFReC for a total of five researchers (Director Akira were selected for two categories).

Biology & Biochemistry

- Shizuo Akira, Director of IFReC

Immunology

- Shizuo Akira, Director of IFReC
- Shimon Sakaguchi, Deputy Director of IFReC
- Ken Ishii, Vaccine Science, IFReC
- Kiyoshi Takeda, Mucosal Immunology, IFReC
- Masahiro Yamamoto, Immunoparasitology, IFReC

Kiyoshi Takeda　Erwin von Bälz Prize/Osaka Science Prize

Kiyoshi Takeda (second left) at the award ceremony of the Bälz Prize
(Photo : Katsumi Yanagiya)

Kazuhiro Suzuki

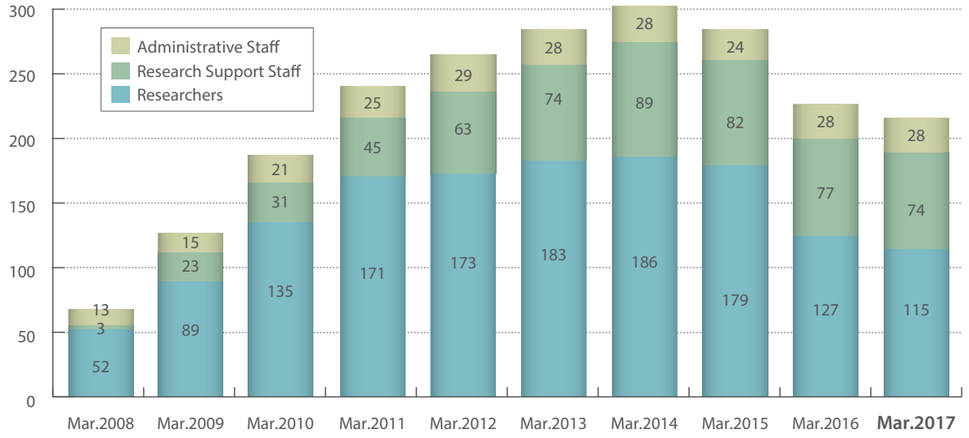
Medical Research Encouragement Prize of the Japan Medical Association

Astellas Awards for the Best Biomedical Research

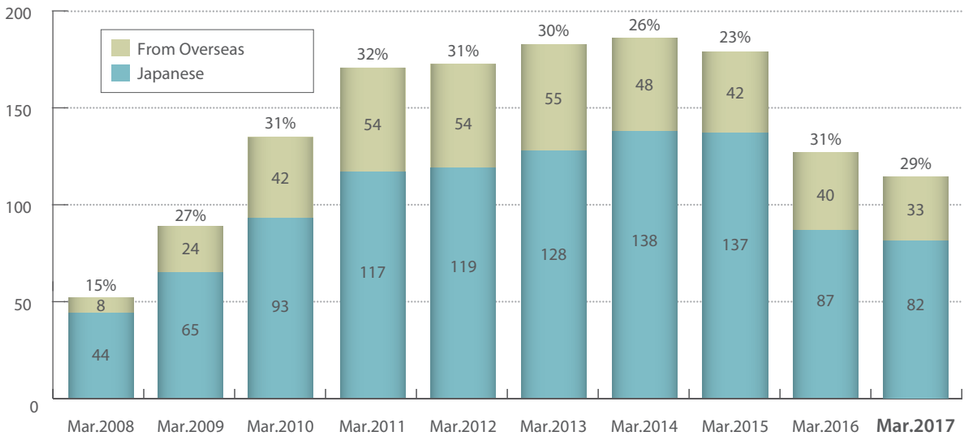
Kazuhiro Suzuki with the certificates of commendation for the prizes

Composition

Number of IFReC Staff

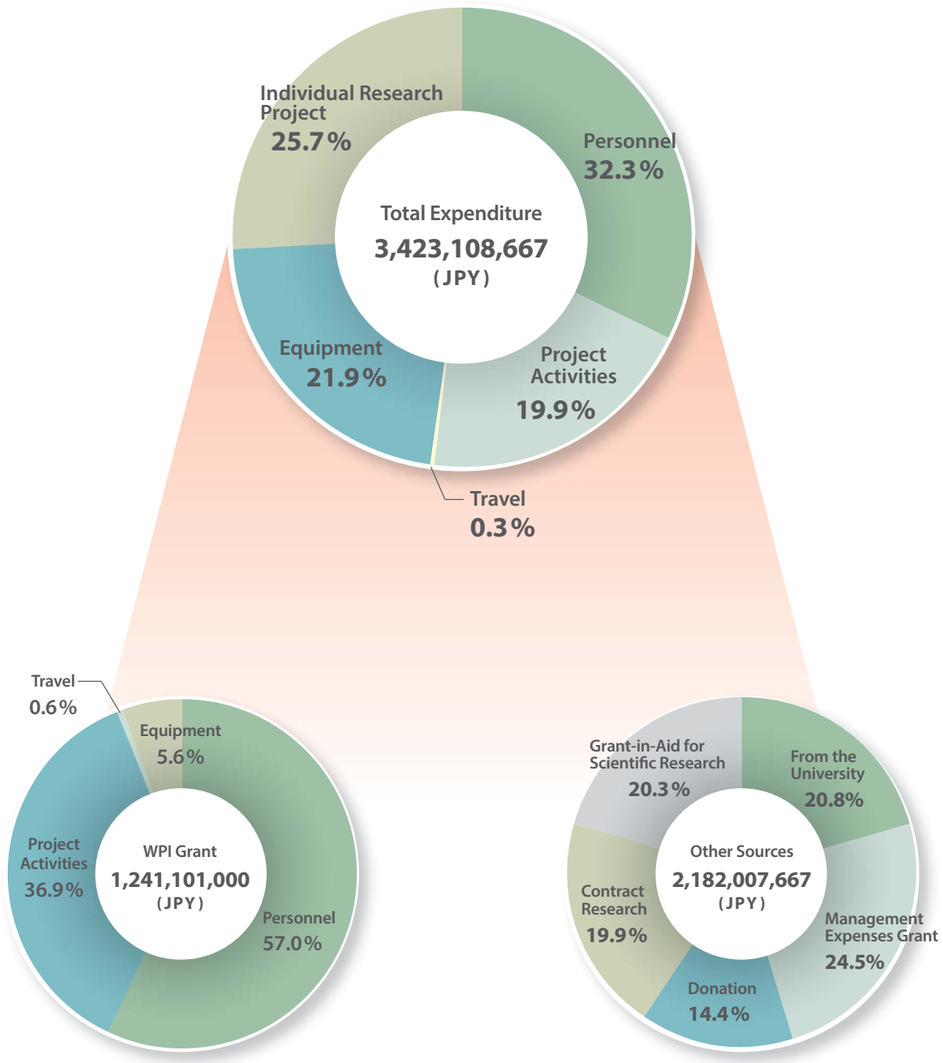


Number of Researchers



Finance

Breakdown of total expenditure at IFReC in FY2016



Selected Articles

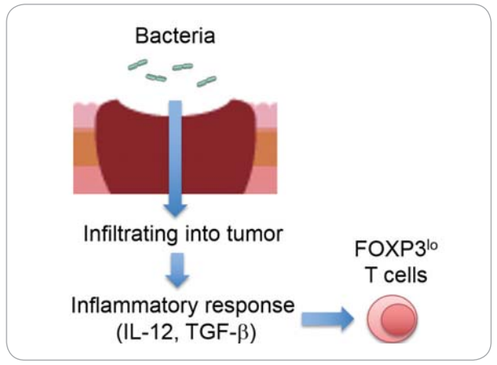
Two FOXP3+CD4+ T-cell subpopulations distinctly control the prognosis of colorectal cancers.

Nat Med. 22:679-84 (2016). doi: 10.1038/nm.4086.

Takuro Saito T, Nishikawa H, Wada H, Nagano Y, et al.

CD4+ T cells that express the forkhead box P3 (FOXP3) transcription factor function as regulatory T (Treg) cells and hinder effective immune responses against cancer cells. Abundant Treg cell infiltration into tumors is associated with poor clinical outcomes in various types of cancers. However, the role of Treg cells is controversial in colorectal cancers (CRCs), in which FOXP3+ T cell infiltration indicated better prognosis in some studies.

Shimon Sakaguchi and his group showed that CRCs, which are commonly infiltrated by suppression-competent FOXP3hi Treg cells, can be classified into two types by the degree of additional infiltration of FOXP3lo nonsuppressive T cells. Functionally distinct subpopulations of tumor-infiltrating FOXP3+ T cells contribute in opposing ways to determining CRC prognosis. Depletion of FOXP3hi Treg cells from tumor tissues, which would augment antitumor immunity, could thus be used as an effective treatment strategy for CRCs and other cancers, whereas strategies that locally increase the population of FOXP3lo non-Treg cells could be used to suppress or prevent tumor formation.



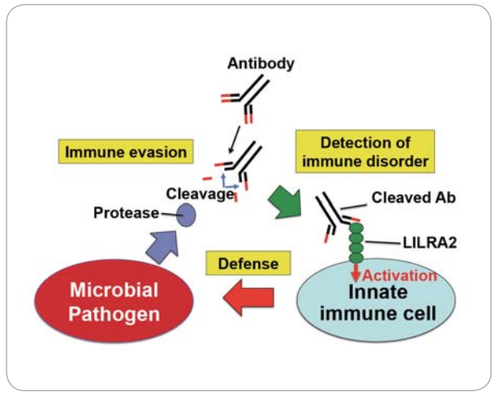
Microbially cleaved immunoglobulins are sensed by the innate immune receptor LILRA2.

Nat Microbiol. 1:16054 (2016). doi: 10.1038/nmicrobiol.2016.54.

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

Hisashi Arase group found that immunoglobulins disrupted by microbial pathogens are specifically detected by leukocyte immunoglobulin-like receptor A2 (LILRA2), an orphan activating receptor expressed on human myeloid cells. The microbially cleaved immunoglobulins but not normal immunoglobulins stimulated human neutrophils via LILRA2. In addition, stimulation of primary monocytes via LILRA2 inhibited the growth of L. pneumophila. When mice were infected with L. pneumophila, immunoglobulins were cleaved and recognized by LILRA2. More importantly, cleaved immunoglobulins were detected in patients with bacterial infections and stimulated LILRA2-expressing cells.

Their findings demonstrate that LILRA2 is a type of innate immune receptor in the host immune system that detects immunoglobulin abnormalities caused by microbial pathogens.



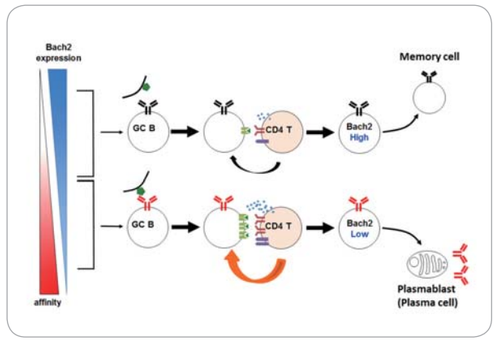
Regulated selection of germinal center cells into the memory B cell compartment.

Nat Immunol. 17:861-869 (2016). doi: 10.1038/ni.3460.

Shinnakasu R, Inoue T, Kometani K, Moriyama S, et al.

How memory cells are selected and generated during germinal-center (GC) reactions remains unclear.

Tomohiro Kurosaki and his group found that light-zone (LZ) GC B cells with B cell antigen receptors (BCRs) of lower affinity were prone to enter the memory B cell pool. Mechanistically, cells in this memory-prone fraction had higher expression of the transcriptional repressor Bach2 than that of their counterparts with BCRs of higher affinity.Haploinsufficiency of Bach2 resulted in reduced generation of memory B cells, independently of suppression of the gene encoding the transcription factor Blimp-1. Bach2 expression in GC cells was inversely correlated with the strength of help provided by T cells. Thus, they propose an instructive model in which weak help from T cells maintains relatively high expression of Bach2, which predisposes GC cells to enter the memory pool.



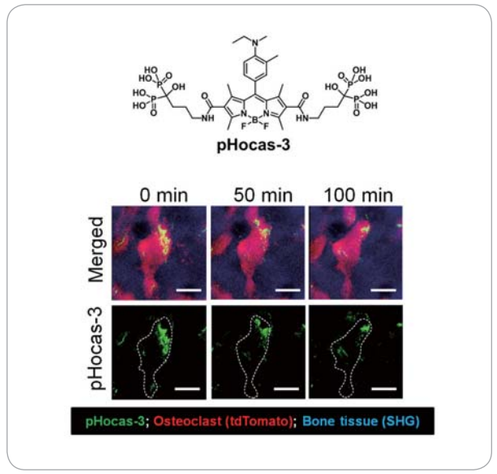
Real-time intravital imaging of pH variation associated with osteoclast activity.

Nat Chem Biol. 12:579-585 (2016). doi: 10.1038/nchembio.2096.

Maeda H, Kowada T, Kikuta J, Furuya M, Shirazaki M, Mizukami S, et al

Intravital imaging by two-photon excitation microscopy (TPEM) has been widely used to visualize cell functions. However, small molecular probes (SMPs), commonly used for cell imaging, cannot be simply applied to intravital imaging because of the challenge of delivering them into target tissues, as well as their undesirable physicochemical properties for TPEM imaging.

Kazuya Kikuchi and Masaru Ishii groups designed and developed a functional SMP with an active-targeting moiety, higher photostability, and a fluorescence switch and then imaged target cell activity by injecting the SMP into living mice. The combination of the rationally designed SMP with a fluorescent protein as a reporter of cell localization enabled quantitation of osteoclast activity and time-lapse imaging of its in vivo function associated with changes in cell deformation and membrane fluctuations. Real-time imaging revealed heterogenic behaviors of osteoclasts in vivo and provided insights into the mechanism of bone resorption.



Arid5a exacerbates IFN-γ-mediated septic shock by stabilizing T-bet mRNA.

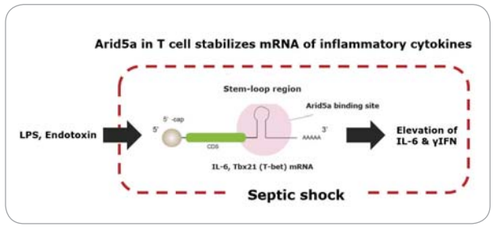
Proc Natl Acad Sci USA. 113:11543-11548 (2016).

Zaman MM, Masuda K, Nyati KK, Dubey PK, et al.

Adenine-thymine (AT)-rich interactive domain containing protein 5a (Arid5a) is an RNA-binding protein that has been shown to play an important immune regulatory function via the stabilization of IL-6 and STAT3 mRNA. However, the role of Arid5a in the overwhelming and uncontrolled immune response that leads to septic shock is unknown.

Tadamitsu Kishimoto group reported that Arid5a-deficient mice are highly resistant to lipopolysaccharide (LPS)-induced endotoxic shock and secrete lower levels of major proinflammatory cytokines, including IFN-γ, IL-6, and TNF-α, than WT mice in response to LPS. Their previous study suggests that Arid5a control the IL-6 level in vivo in response to LPS by stabilization of IL-6 mRNA. They also observed that neutralization of IFN-γ and IL-6 significantly recovered the mice from endotoxic shock.

They conclude that Arid5a regulates the augmentation of IL-6 and IFN-γ in response to LPS, which possibly works synergistically for amplification of various other cytokines that ultimately cause the development of septic shock in mice.



Identification of an atypical monocyte and committed progenitor involved in fibrosis.

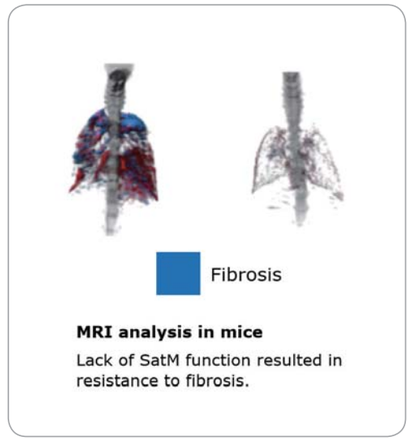
Nature 541: 96-101(2017). doi : 10.1038/nature20611.

Satoh T, Nakagawa K, Sugihara F, et al.

"Pulmonary fibrosis is one of a family of related diseases called interstitial lung diseases. As the lung tissue becomes scarred, it interferes with a person's ability to breathe. Most cases of pulmonary fibrosis have no known cause. These cases are called idiopathic pulmonary fibrosis." (American Lung Association)

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.

Shizuo Akira group identified a new type of macrophage, Segregated nucleus Atypical Monocytes (SatM). They found SatM have a bi-lobed segmented nuclear shape, and are 'disorder-specific monocyte/macrophage subtypes' corresponding to fibrosis. It may also now be possible to develop novel, more specific therapeutic targets for this intractable disease in the future.



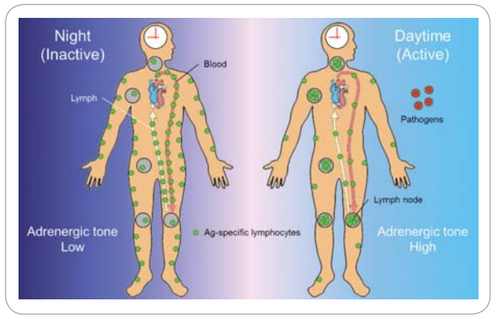
Adrenergic control of the adaptive immune response by diurnal lymphocyte recirculation through lymph nodes.

J Exp Med. 213:2567-2574 (2016).

Suzuki K, Hayano Y, Nakai A, Furuta F, and Noda M, et al.

It has long been proposed that various aspects of immune responses are influenced by nervous system activity. However, the cellular and molecular basis for neural regulation of immunity are largely unclear.

Kazuhiro Suzuki group revealed that neural inputs to β2-adrenergic receptors (β2ARs) expressed on lymphocytes generate the diurnal variation in the frequency of lymphocyte egress from lymph nodes, which is reflected in the magnitude of the adaptive immune response. 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 promote adaptive immune responses. This diurnal variation of lymphocyte trafficking may have evolved to maximize the efficiency of host defense against pathogens.



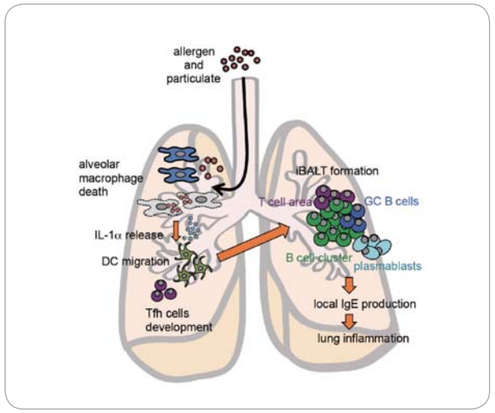
Inhaled fine particles induce alveolar macrophage death and interleukin-1α release to promote inducible bronchus-associated lymphoid tissue formation.

Immunity 45:1299-1310 (2016). doi: 10.1016/j.immuni.2016.11.010.

Kuroda E, Ozasa K, Temizoz B, Ohata K, Koo CX, et al.

Particulate pollution is thought to function as an adjuvant that can induce allergic responses. However, the exact cell types and immunological factors that initiate the lung-specific immune responses are unclear.

Ken Ishii group found that upon intratracheal instillation, particulates such as aluminum salts and silica killed alveolar macrophages (AMs), which then released interleukin-1α (IL-1α) and caused inducible bronchus-associated lymphoid tissue (iBALT) formation in the lung. IL-1α release continued for up to 2 weeks after particulate exposure, and type-2 allergic immune responses were induced by the inhalation of antigen during IL-1α release and iBALT formation, even long after particulate instillation. Recombinant IL-1α was sufficient to induce iBALTs, which coincided with subsequent immunoglobulin E responses, and IL-1-receptor-deficient mice failed to induce iBALT formation. Therefore, the AM-IL-1α-iBALT axis might be a therapeutic target for particulate-induced allergic inflammation.



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The articles were published between April, 2016 and March, 2017. The data were acquired using Web of Science Core Collection™ on May 12, 2017, and sorted by alphabetical order of the first authors.

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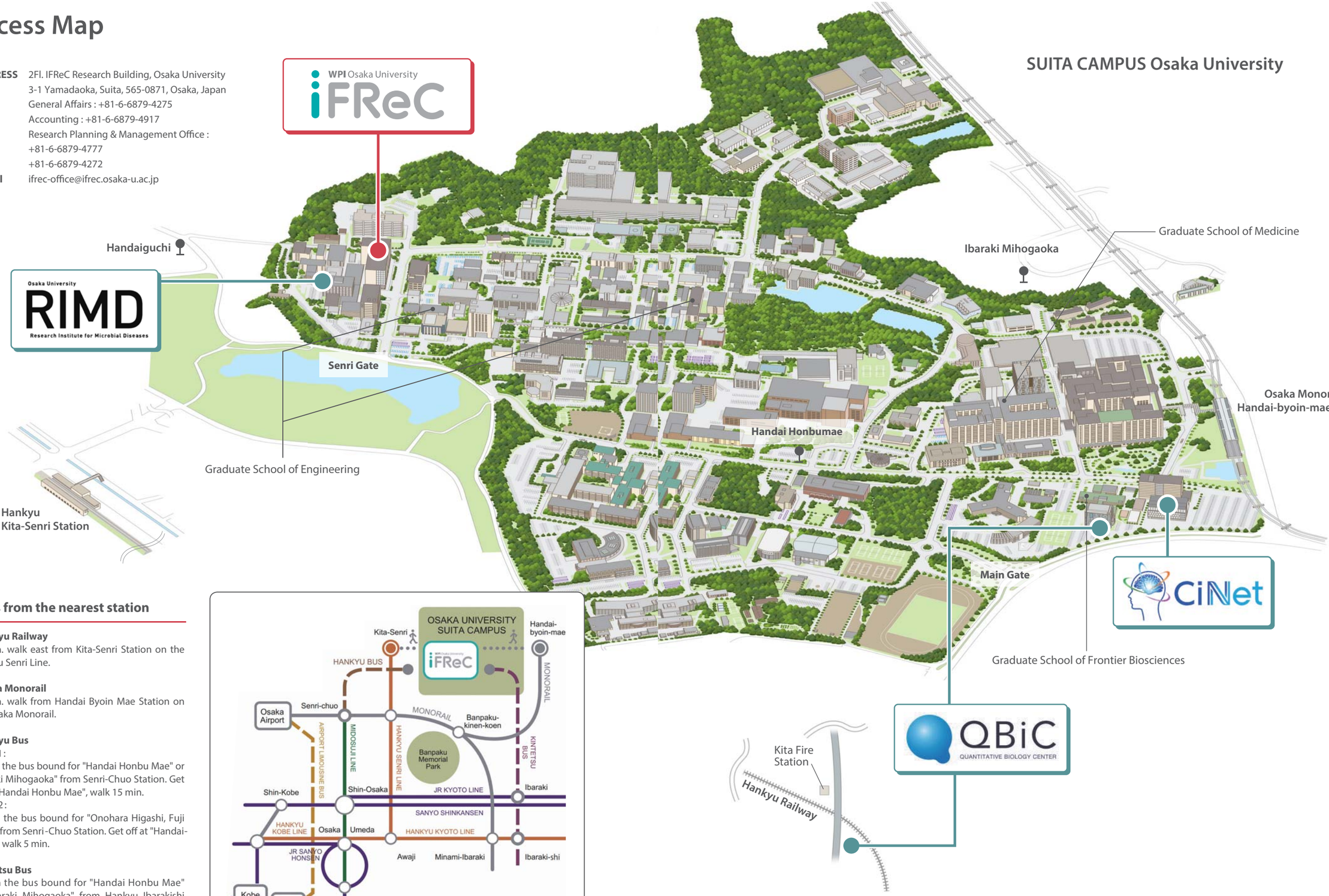

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