World Premier International
Research Center

Osaka University

Immunology Frontier Research Center

Annual Report
of IFReC
FY 2013

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WPI Osaka University
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Osaka University
Immunology Frontier Research Center

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 2013.

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 Committee, the Program Director and the Program Officers.

In the WPI interim evaluation carried out at the end of fiscal year 2011, IFReC was awarded a score of 'A' and it was noted that IFReC should be made a permanent part of the University as an International Level Research Institute. In alignment with this view, we are currently finalizing the future direction of IFReC at Osaka University with the University Executive Board as well as the Ministry of Education, Culture, Sports, Science and Technology (MEXT). We expect that an important decision will be made as to the continuation of IFReC beyond 2017 at the WPI program committee meeting to be held in fall 2014.

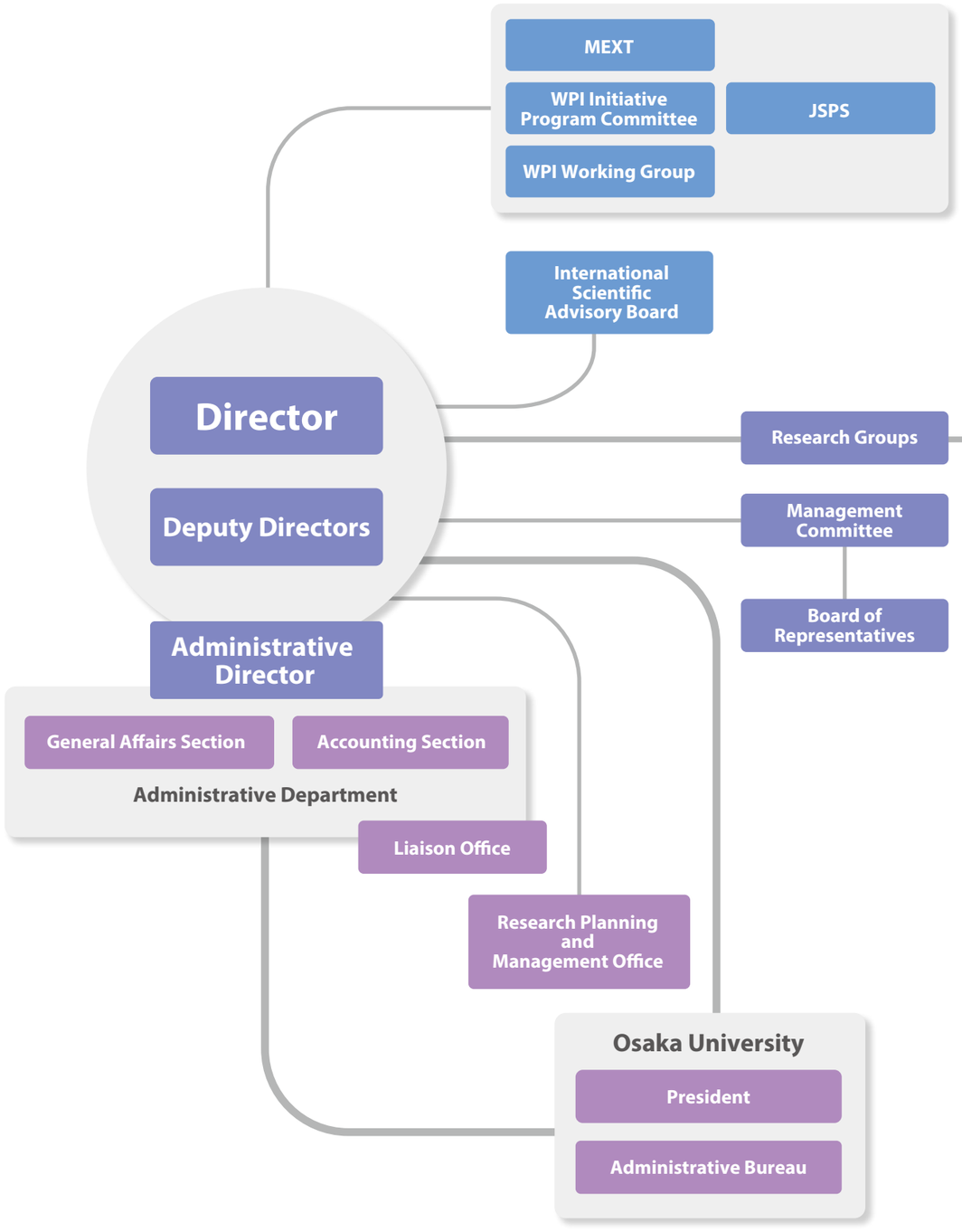
To this end, IFReC must also demonstrate its worth as a World Premier Research Institute. However, it is not sufficient to simply maintain the level of research. IFReC has been running an original research grant 'Research Support Program for Combined Research Fields' in order to promote collaborative studies between different fields. In 2013, two Combined Research Units were set up to advance this program. Both units comprise a group of young, talented researchers from different fields and we expect that lively debate will pave the way to new areas of research.

As an approach to nurturing young researchers, the third NIF Winter School, which was begun in 2011, was held in January, 2014 on Awaji Island. The young researchers, selected from a highly competitive field of candidates, spent one week living and debating together in an 'Immunology Boot Camp' that is fast becoming an institution of its own. The school prides itself on offering not only productive educational content but also an opportunity for the young researchers to form a global network.

We are committed to continuing contributions to scientific advances through immunology research and education and the evolvement of a Visible Research Center that leads the world.

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

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
Molecular Immunology	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
Immune Regulation	Tsuneyasu Kaisho
Immune Network	Rikinari Hanayama
Immunoparasitology	Masahiro Yamamoto

Imaging Group

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

Informatics Group

Information Systems	Yutaka Hata
Systems Immunology	Daron M. Standley

Units for Combined Research Fields

Quantitative Immunology Research Unit	Yutaro Kumagai, Shunsuke Teraguchi, Diego Diez
Next Generation Optical Immune-imaging	Noriko Takegahara, Kazuaki Tokunaga

Common Facility

- 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

Overseas

- Pohang University of Science and Technology, Korea
- Convergent Research Consortium for Immunologic Disease, Seoul St. Mary's Hospital, the Catholic University of Korea
- Indian Institute of Science Education and Research, India
- Maurice Wilkins Center, The University of Auckland, New Zealand

Committee and Advisory Board for IFReC

World Premier International Research Center Initiative (WPI)

Program Committee Members List

As at April, 2013

<Chair> **Hiroo Imura**	President, Foundation for Biomedical Research and Innovation
Toshiaki Ikoma	Executive Vice President & CTO, Canon Inc.
Hiroto Ishida	President Emeritus, Kanazawa Gakuin University
Shinichiro Ohgaki	Professor Emeritus, The University of Tokyo
Tsutomu Kimura	Chairman, Tokyo Metropolitan Government Board of Education
Kiyoshi Kurokawa	Academic Fellow, National Graduate Institute for Policy Studies
Makoto Kobayashi	Director, Research Center for Science Systems, Japan Society for the Promotion of Science (JSPS), Nobel Laureate in Physics (2008)
Masatoshi Takeichi	Director, RIKEN, Kobe Institute
Michiharu Nakamura	President, Japan Science and Technology Agency(JST)
Ryoji Noyori	President, RIKEN, Nobel Laureate in Chemistry (2001)
Hideo Miyahara	Professor Emeritus, Osaka University
Robert Aymar	Senior Counsellor, "Administrateur Général"(CEO), French Atomic Energy Authority(CEA)
Rita Colwell	Professor, University of Maryland
Richard Dasher	Professor, Stanford University
Ian Halliday	Professor Emeritus, University of Edinburgh
Chuan Poh Lim	Chairman, Agency for Science, Technology and Research, Singapore
Matthew Mason	Director, Robotics Institute, Carnegie Mellon University

Program Director

Toshio Kuroki	Senior Advisor, Research Center for Science Systems (JSPS)

Working Group Leaders and Assigned Members

As at April, 2013

<Chair, Program Officer> **Takehiko Sasazuki**	University Professor, Institute for Advanced Study, Kyushu University
Hiroshi Kiyono	Dean and Professor, Division of Mucosal Immunology, Department of Microbiology and Immunology, Institute of Medical Science, The University of Tokyo
Nagahiro Minato	Dean and Professor, Graduate School of Medicine, Kyoto University
Kazuhiko Yamamoto	Professor and Chairman, Department of Allergy and Rheumatology, Graduate School of Medicine, The University of Tokyo
Günter J. Hämmerling	Professor and Chairman, Division of the Molecular Immunology, German Cancer Research Center Heidelberg DKFZ, DEU
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/ Chairman, The Singapore Immunology Network, FR
Diane Mathis	Morton Grove-Rasmussen Professor of Immunohematology/ Head, Division of Immunology, Department of Microbiology and Immunobiology, Harvard Medical School, Boston, USA

International Advisory Board

As at April, 2013

Jeffrey Ravetch	Professor, Laboratory of Molecular Genetics and Immunology, The Rockefeller University	**Immunology**
Chris Goodnow	Professor, John Curtin School of Medical Research and Australian Phonemics Facility, The Australian National University	**Immunology**
Richard Locksley	Professor, Departments of Medicine and Microbiology/ Immunology, University of California, San Francisco	**Immunology**
Anne O'Garra	Division Head, Division of Immunoregulation, The National Institute for Medical Research	**Immunology**
Lewis Lanier	American Cancer Society Professor Chair, Department of Microbiology & Immunology, University of California, San Francisco	**Immunology**
Kiyoshi Takatsu	Director, Toyama Prefectural Institute for Pharmaceutical Research	**Immunology**
Kayo Inaba	Professor, Graduate School of Biostudies, Kyoto University	**Immunology**
Yasuyoshi Watanabe	Director, CLST, RIKEN Kobe Institute	**Imaging**
Masamitsu Iino	Professor, Graduate School of Medicine, The University of Tokyo	**Imaging**
Yale Goldman	Professor, Pennsylvania Muscle Institute, University of Pennsylvania	**Imaging**
Akinori Kidera	Professor, Graduate School of Integrated Science, Yokohama City University	**Informatics**
Hiroyuki Toh	Deputy Director, AIST-CBRC	**Informatics**
David Westhead	Professor, School of Biochemistry and Molecular Biology, Leeds University	**Informatics**
Vladimir Brusic	Principal Associate, Dana-Farber Cancer Institute, Harvard Medical School	**Informatics**
Mo Jamshidi	Lutcher Brown Endowed Chair and Professor, Department of Electrical and Computer Engineering, University of Texas, San Antonio	**Informatics**
Philip Chen	Professor, Faculty of Science and Technology, University of Macau	**Informatics**
Takeshi Yamakawa	Board Vice-Chairman and Director, Fuzzy Logic Systems Institute	**Informatics**

The WPI Program Committee conducts follow-up activities on progress being made by the WPI institutes with an eye to developing them into "highly visible research centers". They carry out the interim evaluation in close cooperation with a Program Director, Program Officer and the working group of WPI established to evaluate IFReC. The working group conducts site visits and compiles the results in a Site Visit Report, which is used by the Program Committee along with the results of a hearing on each center to prepare the interim evaluation.

IFReC Advisory Board Members evaluate scientific activities by reviewing PI's scientific achievements.

Administrative Office of IFReC

General Affairs Section

- Employment/acceptance of researchers and staff procedures
- Support for international researchers
- Social insurance/Employment insurance
- Public dormitory for Osaka University workers
- Procedures related to international students
- Management of work hours
- Procedures related to patents
- Issuing various certifications

Liaison Office

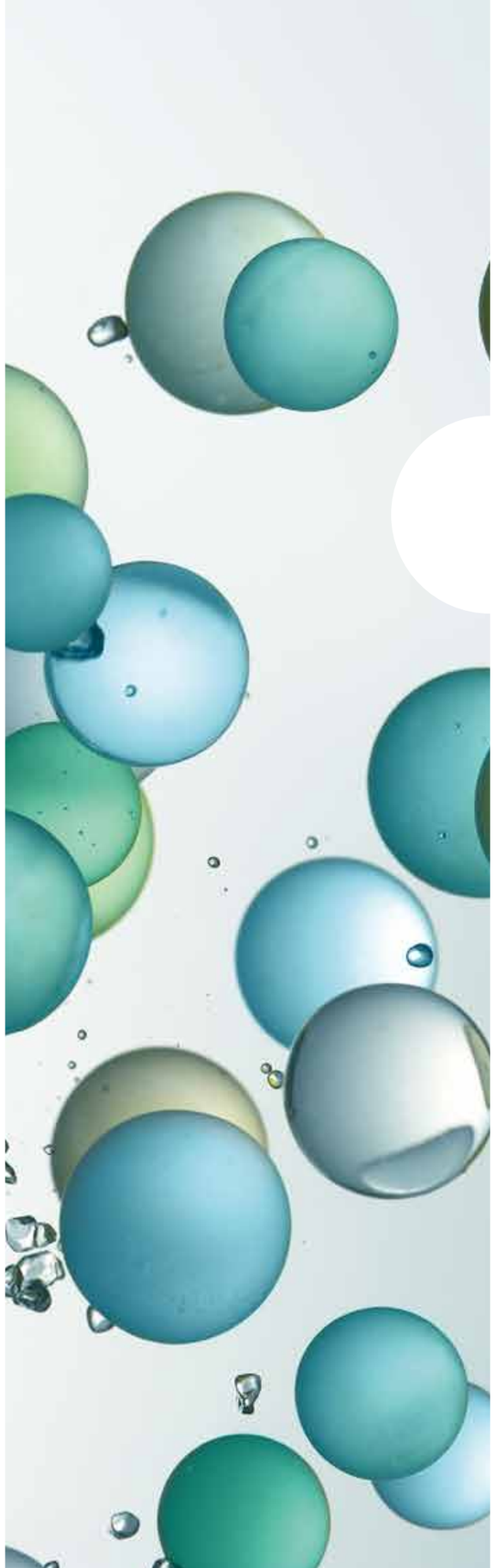
- General support for researchers from overseas
- Assistance for each international PI's lab
- Providing support for development of an international research environment

Accounting Section

- Budget drafting/implementation/management
- Purchasing procedures
- Acceptance and implementation of external funding
- Payment of payroll, travel expenses and honorariums
- Health insurance procedures
- Management of buildings and assets
- RI 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, Dual Mentor Program, 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.)



Laboratories

- Immunology
- Imaging
- Informatics
- Units for Combined Research Fields

Host Defense

Shizuo Akira, MD/PhD

Professor	Shizuo Akira
Associate Professor	Tatsuya Saitoh
Assistant Professor	Takashi Satoh
Postdoctoral Fellow	5
Research Assistant	9
Visiting Scientist	4
Support Staff	1

The immune system plays a critical role in host defense against microbes. Pattern-recognition receptors (PRRs) sense microbes ranging from bacteria to fungi, protozoa and viruses, and induce innate immune response, an evolutionally conserved host defense response. After sensing microbial components, PRRs stimulate production of inflammatory factors such as cytokines/interferons by dendritic cells to support induction of acquired immune response, another immune response mediated by T cells and B cells, resulting in elimination of invading microbes. However, aberrant activation of the immune responses often causes massive inflammation, leading to the development of autoimmune diseases. Therefore, both activation and inactivation of immune responses must be strictly controlled. To gain a deeper understanding of the immune system, we have examined regulatory mechanisms of innate and acquired immune responses, and novel PRRs capable of sensing microbial components.

1. Cathepsin D potentiates poly IC-induced innate immune response

RIG-I-like receptors (RLRs) sense viral dsRNA to exert the antiviral immune response and mediate the adjuvant effect of polyinosinic-polycytidylic acid (poly IC), a synthetic dsRNA. We revealed the regulatory mechanisms of immune response induced by poly IC (Zou et al., *Immunity*, 2013). Poly IC is taken up by dendritic cells (DCs), and induces lysosomal destabilization, which in turn activates an RLR-dependent signaling pathway. Upon poly IC stimulation, cathepsin D is released into the cytoplasm from the lysosome to interact with IPS-1, an adaptor molecule for RLRs. This interaction facilitates cathepsin D-dependent cleavage of caspase-8 and the activation of NF-κB, resulting in enhanced cytokine production. Further recruitment of RIP-1 to this complex initiates the necroptosis of a small number of DCs. HMGB1 released by dying cells enhances IFN-β production in concert with poly IC. These findings indicate that cathepsin D-triggered necroptosis is a mechanism that propagates the adjuvant efficacy of poly IC (Figure 1).

2. ZAP mediates host defense against murine leukemia virus

When host cells are infected by an RNA virus, PRRs recognize the viral RNA and induce the antiviral immune response. However, the PRR that recognizes murine leukemia virus (MLV) is not fully understood. We found that zinc-finger antiviral protein (ZAP) acts as an RNA sensor and induces the degradation of the MLV transcripts by the exosome, an RNA degradation system, on RNA granules (Lee et al, *PNAS*, 2013). The loss of ZAP greatly enhances the replication efficiency of MLV. ZAP localizes to RNA granules, where the processing-body proteins assemble. ZAP induces the recruitment of the MLV transcripts and exosome components to the RNA granules. The CCCH-type zinc-finger domains of ZAP, which are RNA-binding motifs, mediate its localization to RNA granules and MLV transcripts degradation by the exosome. Thus, ZAP is the cytosolic RNA-sensing PRR that induces elimination of intracellular RNA viruses including MLV.

3. Malt1-dependent cleavage of Regnase-1 regulates immune response of CD4$^+$ T Cells

Regnase-1 is an RNase that destabilizes a set of mRNAs through cleavage of their 3' UTRs. Although loss of Regnase-1 leads to development of an autoimmune disease characterized by T cell activation, one role of Regnase-1 in regulation of T cell-mediated immunity has remained unclear. We found that Regnase-1 regulates the mRNAs of a set of genes, including c-Rel, Ox40, and Il2, through cleavage of their 3' UTRs, and is essential for preventing aberrant cell autonomous generation of effector CD4$^+$ T cells (Uehata et al, *Cell*, 2013). Regnase-1 is cleaved by Malt1 after T cell receptor (TCR) stimulation and enhances T cell activation. Consistently, Malt1 protease activity is involved in control of the mRNA stability of T cell effector genes. These findings indicate that dynamic control of Regnase-1 expression is critical for modulation of T cell activation (Figure 2).

In the future, we will further our research to achieve the goal of a comprehensive understanding of the immune system and to develop an effective treatment for immune-related inflammatory diseases.

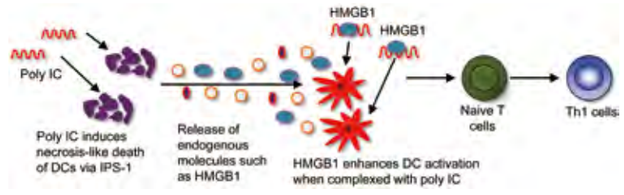


Figure 1. Poly IC potently activates dendritic cells.

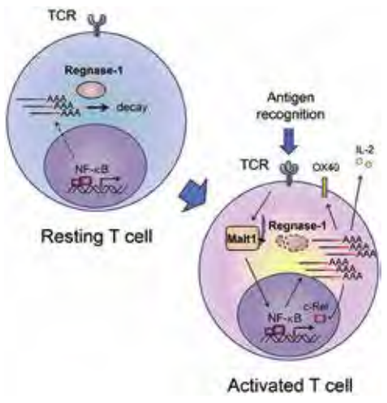


Figure 2. Regnase-1 controls T cell response.

Recent Publications

- Zou J, Kawai T, Tsuchida T, Kozaki T, Tanaka H, Shin KS, Kumar H, Akira S. Poly IC triggers a cathepsin D- and IPS-1-dependent pathway to enhance cytokine production and mediate dendritic cell necroptosis. ***Immunity*** 38:717-28, 2013.
- Uehata T, Iwasaki H, Vandenbon A, Matsushita K, Hernandez-Cuellar E, Kuniyoshi K, Satoh T, Mino T, Suzuki Y, Standley DM, Tsujimura T, Rakugi H, Isaka Y, Takeuchi O, Akira S. Malt1-induced cleavage of regnase-1 in CD4$^+$ helper T cells regulates immune activation. ***Cell*** 153:1036-49, 2013.
- Lee H, Komano J, Saitoh Y, Yamaoka S, Kozaki T, Misawa T, Takahama M, Satoh T, Takeuchi O, Yamamoto N, Matsuura Y, Saitoh T, Akira S. Zinc-finger antiviral protein mediates retinoic acid inducible gene I-like receptor-independent antiviral response to murine leukemia virus. ***Proc. Natl. Acad. Sci. USA.*** 110:12379-84, 2013.
- Maruyama K, Uematsu S, Kondo T, Takeuchi O, Martino MM, Kawasaki T, Akira S. Strawberry notch homologue 2 regulates osteoclast fusion by enhancing the expression of DC-STAMP. ***J. Exp. Med.*** 210:1947-60, 2013.
- Akira S, Misawa T, Satoh T, Saitoh T. Macrophages control innate inflammation. ***Diabetes Obes Metab.*** 15 Suppl. 3:10-18, 2013.

Immunoglycobiology

Taroh Kinoshita, PhD

The main topic in our laboratory has been biological and medical significance of glycosylphosphatidylinositol-anchored proteins (GPI-APs). At least 150 different cell surface human proteins including many with immunological functions are GPI-APs. We aim to understand molecular mechanisms of biogenesis of GPI-APs, their biological functions and molecular pathogenesis of acquired and inherited GPI deficiencies.

1. Molecular pathogenesis of GPI-anchor deficiencies.

Hyperphosphatasia mental retardation syndrome (HPMR), also Mabry syndrome, is a recently-identified inherited GPI-anchor deficiency caused by mutations in *PIGV* or *PIGO*, which are involved in biosynthesis of GPI-anchor in the endoplasmic reticulum (ER). We, in collaboration with Dr. Krawitz and his colleagues in Germany, and with Dr. Hansen in Denmark and Dr. Abou Jamra in Germany, reported that *PGAP2*, which is involved in fatty acid remodeling of GPI-APs in the Golgi apparatus, is the third gene responsible for HPMRS (Hansen L et al, ***Am. J. Hum. Genet.***, 92:575, 2013; Krawitz P et al, ***Am. J. Hum. Genet.***, 92:584, 2013). We also found the first individual with inherited GPI-deficiency caused by mutations in *PIGW*, involved in inositol-acylation. The individual had hyperphosphatasia, intellectual disability and intractable seizures, fulfilling diagnosis of HPMRS.

In collaboration with groups in Germany and England, we studied mutations in *PGAP3* gene found in several individuals with hyperphosphatasia and intellectual disability. All mutations caused partial loss of function of PGAP3 due to mislocalization of the mutant protein, nonsense-mediated mRNA decay, or decreased enzymatic activity. These mutations lead to inefficient fatty acid remodeling of GPI-APs in the Golgi and result in inefficient raft-association and release of GPI-APs. This work revealed the importance of maturation of the lipid part of GPI-APs in neuronal development and function (Howard MF et al, ***Am. J. Hum. Genet.***, 94:278, 2014). These results established that molecular mechanisms of hyperphosphatasia (release of alkaline phosphatase) in PIGV/PIGO/PIGW-HPMR, PGAP2-HPMR and PGAP3-HPMR are different (Figure1).

2. Roles of p24γ2(TMED5)in the ER-to-Golgi transport of GPI-APs

GPI-APs are transported from the ER to the Golgi via COPII coated transport vesicles. For efficient packaging of GPI-APs into the COPII coated vesicles in the ER exit sites, a cargo receptor that captures and recruits GPI-APs is required. We previously reported that a complex consisting of p24α2/TMED9, p24β1/TMED2, p24γ2/TMED5 and p24δ1/TMED10, acts as a cargo receptor of GPI-APs. We recently demonstrated that p24γ2/TMED5 is the subunit of this receptor that recognizes GPI-APs and that the α-helical domain but not GOLD domain of p24γ2/TMED5 is required for the recognition of GPI-APs. It is likely that the juxta-membrane region within the α-helical domain contains a binding site for the glycan part of GPI.

One of the goals of our research is to identify all the factors specifically involved in transport of GPI-APs from the ER to the cell surface membrane microdomains, and to understand functions of these factors. This will give us a complete picture of how GPI-APs are synthesized and expressed in functionally relevant subcellular locations, leading to full understanding roles of GPI-APs in various cells.

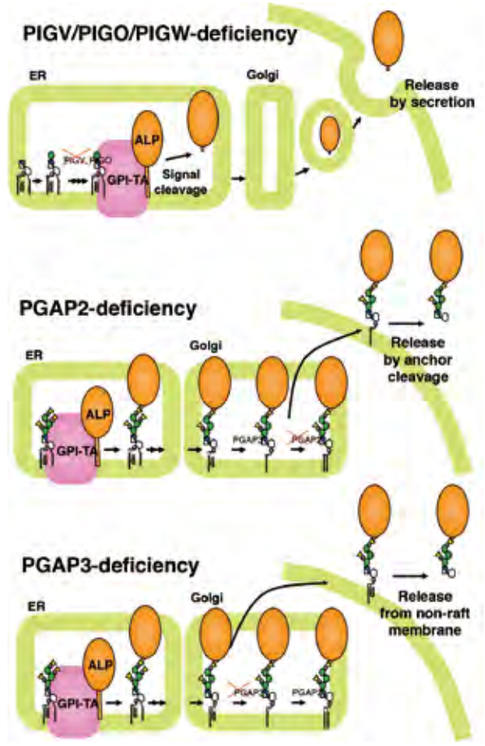


Figure1. Different mechanisms of hyperphosphatasia in PIGV/PIGO/PIGW-, PGAP2- and PGAP3-deficiencies.

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

Recent Publications

- Fujita M, Maeda Y, Ra M, Yamaguchi Y, Taguchi R, Kinoshita T. GPI-glycan remodeling by PGAP5 regulates transport of GPI-anchored proteins from the ER to the Golgi. ***Cell*** 139:352-65, 2009.
- Fujita M, Watanabe R, Jaensch N, Romanova-Michaelides M, Satoh T, Kato M, Riezman H, Yamaguchi Y, Maeda Y, Kinoshita T. Sorting of GPI-anchored proteins into ER-exit sites by p24 proteins is dependent on remodeled GPI. ***J. Cell Biol.*** 194:61-75, 2011.
- Murakami Y, Kanzawa N, Saito K, Krawitz PM, Mundlos S, Robinson PN, Karadimitris A, Maeda Y, Kinoshita T. Mechanism for release of alkaline phosphatase caused by glycosylphosphatidylinositol deficiency in patients with hyperphosphatasia-mental retardation syndrome. ***J. Biol. Chem.*** 287:6318-25, 2012.
- Kanzawa N, Shimozawa N, Wanders RJA, Ikeda K, Murakami Y, Waterham HR, Mukai S, Fujita M, Maeda Y, Taguchi R, Fujiki Y, Kinoshita T. Defective lipid remodeling of GPI anchors in peroxisomal disorders, Zellweger syndrome, and rhizomelic chondrodysplasia punctata. ***J. Lipid Res.*** 53:653-63, 2012.
- Wang Y, Murakami Y, Yasui T, Wakana S, Kikutani H, Kinoshita T, Maeda Y. Significance of GPI-anchored protein enrichment in lipid rafts for the control of autoimmunity. ***J. Biol. Chem.*** 288:25490-99, 2013.

Immunopathology

Atsushi Kumanogoh, MD/PhD

Professor	**Atsushi Kumanogoh**
Associate Professor	**Toshihiko Toyofuku**
Assistant Professor	**Hyota Takamatsu**
Research Assistant	**6**
Support Staff	**1**

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 (Figure 1). 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. The primary receptors for semaphorins are neuropilins and plexins, which have cell type-specific patterns of expression and are involved in multiple signalling responses. Beyond such basic implications, we are trying to apply the findings from this proposed study into other research fields and diagnosis/therapy for human diseases such as autoimmunity, allergy, immune deficiency, cancer/metastasis, and neurodegenerative diseases.

1. Sema4A and multiple sclerosis.

Multiple sclerosis (MS) is a demyelinating autoimmune disease of the CNS and a leading cause of lasting neurologic disabilities in young adults. Although the precise mechanism remains incompletely understood, approximately one-third of patients with multiple sclerosis (MS) respond poorly to interferon-beta (IFN-β) therapy. Serum Sema4A is increased in MS patients, and those who have high Sema4A do not respond to IFN-β therapy. We therefore investigated whether recombinant Sema4A abrogates the efficacy of IFN-β in mice with experimental autoimmune encephalomyelitis (EAE), an animal model of MS. Administration of Sema4A concurrently with IFN-β diminished the efficacy of IFN-β in EAE. These effects of Sema4A were attributed to promote Th1 and Th17 differentiation and to increase adhesive activation of T cells to endothelial cells, even in the presence of IFN-β.

2. Plexin-A1 is involved in Toll-like receptor-mediated microglial activation.

Semaphorins, which are identified as repulsive axon guidance molecules, play crucial roles in maintaining brain homeostasis by regulating microglial activity. To investigate the in vivo role of Plexin-A1 in lipopolysaccharide (LPS)-induced injury in the brain, we examined the neuroinflammatory changes induced by LPS administration in wild-type (WT) and Plexin-A1-deficient (-/-) mice. WT mice showed a significantly higher expression of COX-2, iNOS, IL-1β and TNF-α in the hippocampus, and a significantly greater ventricular enlargement and intracerebral infiltration of mononuclear cells. In contrast, Plexin-A1-/- mice administered LPS did not show a significantly increased expression of COX-2, iNOS, IL-1β or TNF-α in the hippocampus. Plexin-A1-/- mice administered LPS did not show significant increases in ventricle size or infiltration of leukocytes into the brain, as compared with the saline-treated group. In WT, but not in the Plexin-A1-/- primary microglia treated with LPS, Sema3A induced significantly more nitric oxide production than in the immunoglobulin G control. These results suggested the crucial role of the Sema3A-Plexin-A1 interaction in the Toll-like receptor 4-mediated signaling of the LPS-induced activation of microglia.

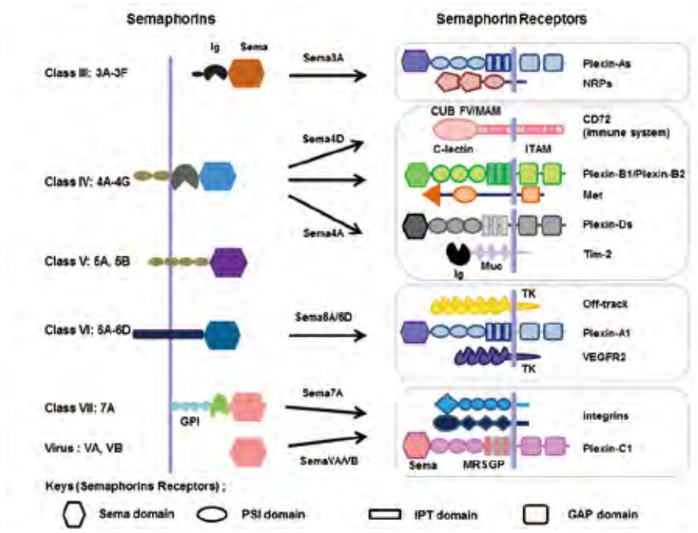


Figure 1.

Recent Publications

- Nojima S, Toyofuku T, Kamao H, Ishigami C, Kaneko J, Okuno T, Takamatsu H, Ito D, Kang S, Kimura T, Yoshida Y, Morimoto K, Maeda Y, Ogata A, Ikawa M, Morii E, Aozasa K, Takagi J, Takahashi M, Kumanogoh A. A point mutation in Semaphorin 4A associates with defective endosomal sorting and causes retinal degeneration. ***Nat. Commun.*** 4:1406. 2013.
- Kumanogoh A, Kikutani H. 2013. Immunological functions of the neuropilins and plexins as receptors for semaphorins. ***Nat. Rev. Immunol.*** 13: 802-14
- Hayashi M, Nakashima T, Taniguchi M, Kodama T, Kumanogoh A, Takayanagi H. Osteoprotection by Semaphorin 3A. ***Nature*** 485:69-74. 2012.
- Takamatsu H, Takegahara N, Nakagawa Y, Tomura M, Taniguchi M, Friedel RH, Rayburn H, Tessier-Lavigne M, Yoshida Y, Okuno T, Mizui M, Kang S, Nojima S, Tsujimura T, Nakatsuji Y, Katayama I, Toyofuku T, Kikutani H, Kumanogoh A. Semaphorins guide the entry of dendritic cells into the lymphatics by activating myosin II. ***Nat. Immunol.*** 11:594-600, 2010.
- Nogi T, Yasui N, Mihara E, Matsunaga Y, Noda M, Yamashita N, Toyofuku T, Uchiyama S, Goshima Y, Kumanogoh A, Takagi J. Structural basis for semaphorin signalling through the plexin receptor. ***Nature*** 467:1123-7. 2010.

Immunochemistry

Hisashi Arase, MD/PhD

Professor	Hisashi Arase
Assistant Professor	Tadahiro Suenaga Masako Kohyama Kouyuki Hirayasu
Postdoctoral Fellow	1
Research Assistant	2
Support Staff	3

Paired receptors that consist of inhibitory and activating receptors are involved in immune regulation. Inhibitory receptors play an important role to suppress immune response to self. However, some inhibitory receptors are utilized by several viruses to evade immune response. On the other hand, activating receptors are involved in host defense by recognizing viral proteins. Therefore, paired receptors seem to have evolved with pathogens and seem to play an important role in host-pathogen interactions (Arase et al. ***Science*** 2002; Shiratori et al. ***J. Immunol.*** 2005). Based on these findings, we have been extensively working on interactions between pathogens and various paired receptors. These studies are important to understand not only host-pathogen interactions but also mechanisms of immune regulation.

A) Interaction between PILR and herpes simplex virus (HSV)

a) Identification of HSV receptor

PILR is one of paired receptors that are mainly expressed on various immune cells. PILR consists of inhibitory PILRα and activating PILRβ. 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). Interestingly, specific *O*-glycan structures on CD99 were found to be required for the association with PILR (Wang et al. ***J. Immunol.*** 2008).

Because PILR is one of paired receptors, we addressed whether PILR interacts with certain pathogens. 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. This suggested that interaction between PILRα and gB plays and important role in HSV-1 infection. We showed that immune inhibitory receptors can be exploited by viruses to invade host cells for the first time (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).

B) Role of Siglec in varicella zoster virus (VZV) infection

VZV belongs to α-herpes virus similar to HSV, although cellular receptor that mediates membrane fusion during infection was unclear. We found that Siglec-4 (MAG, myelin associated glycoprotein), one of paired receptors, associates with VZV gB. Furthermore, Siglec-4 mediated VZV infection as well as membrane fusion. Interestingly, Siglec-4 also associated HSV gB and mediated HSV infection. Because Siglec-4 is specifically expressed in neural tissues, Siglec-4 seemed to be involved in neurotropic characteristics of HSV and VZV (Suenaga et al. ***Proc. Natl. Acad. Sci. USA.*** 2010).

C) PILRα plays an important role in neutrophil infiltration

The role of PILRα in host immune response has remained unclear, although PILRα is involved in HSV-1 infection. In order to elucidate the function of PILRα in immune response, we generated PILRα-knockout mice and analyzed the function of PILRα. Development of immune cells was normal in PILRα-deficient mice. However, 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. When we analyzed neutrophils from PILRα-deficient mice, we found that activation of integrin by chemokine stimulation is augmented in PILRα-deficient neutrophils (Wang et al. ***Nat. Immunol.*** 2012). These findings indicated that PILRα plays an important role in the regulation of inflammation by regulating integrin function (Figure 1).

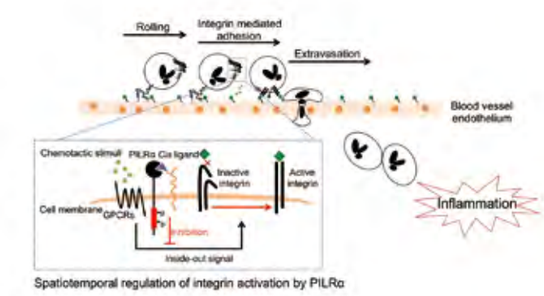


Figure 1. PILRα regulates neutrophil recruitment in inflammatory responses via modulating integrin activation. We found that PILRα, an inhibitory receptor containing an ITIM, negatively regulated neutrophil infiltration during inflammation. PILRα expressed on neutrophils constitutively associated in cis with its ligands, resulting in clustering of PILRα during stimulation with a chemoattractant. Clustering of PILRα enhanced ITIM-mediated signaling, thus modulating β2 integrin inside-out activation. These data demonstrate that neutrophil recruitment in inflammatory responses is regulated by PILRα via modulation of integrin activation (Wang et al. ***Nat. Immunol.*** 2013).

D) 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 can become targets for autoantibodies in autoimmune disease patients (Jin et al. ***Proc. Natl. Acad. Sci. USA.*** 2014). Misfolded proteins, which complexed with MHC class II molecules of disease-susceptible alleles but not disease-resistant MHC class II alleles, were recognized by autoantibodies. 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 complexed with MHC class II molecules are natural autoantigens for autoantibodies. Indeed, expression of MHC class II molecules is induced by inflammation during infections, and most autoimmune diseased tissues aberrantly express MHC class II molecules. Therefore, misfolded proteins, which normally would not be exposed to the immune system, can be targets for autoantibodies when they avoid protein degradation (Figure 2).

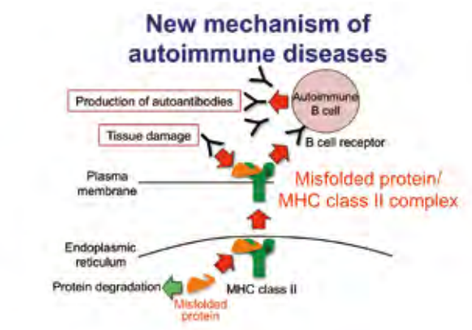


Figure 2. Misfolded proteins transported to the cell surface by MHC class II molecules are targets for autoantibodies. Cellular misfolded proteins are transported to the cell surface by MHC class II molecules without processing to peptides when misfolded proteins are associated with MHC class II molecules at ER (Jiang et al. ***Int. Immunol.*** 2013). Furthermore, Misfolded proteins complexed with MHC class II molecules of disease-susceptible alleles are specifically recognized by autoantibodies. Of note, 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 complexed with MHC class II molecules are natural autoantigens for autoantibodies, which affects autoimmune disease susceptibility. (Jin et al. ***Proc. Natl. Acad. Sci. USA.*** 2014).

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Immune Regulation

Tadamitsu Kishimoto, MD/PhD

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A Summary of our Recent Research.

1. Arid5a controls IL-6 mRNA stability, which contributes to elevation of IL-6 level in vivo

Post-transcriptional regulation of IL-6 has been largely uncharacterized, with the exception of the RNase Regnase-1, which prevents autoimmunity by destabilizing IL-6 mRNA. Here, we identified a novel RNA binding protein, AT-rich interactive domain 5a (Arid5a), which stabilizes IL-6 but not TNF-α mRNA through binding to the 3′ untranslated region (UTR) of IL-6 mRNA. Arid5a was enhanced in macrophages in response to LPS, IL-1β and IL-6. Arid5a deficiency inhibited elevation of IL-6 serum level in LPS-treated mice, and suppressed IL-6 levels and the development of T_H17 cells in experimental autoimmune encephalomyelitis (EAE). Importantly, Arid5a inhibited the destabilizing effect of Regnase-1 on IL-6 mRNA. These results indicate that Arid5a plays an important role in promotion of inflammatory processes and autoimmune diseases.

2. P38 MAP kinase signaling pathway regulates stabilization of Arid5a mRNA.

In the current study, we observed that through TLR4 signaling, Arid5a mRNA is induced rapidly and degrades quickly in macrophages, B cells and dendritic cells. Interestingly, we found that through TLR4 signaling, chemical inhibitors of P38 MAP kinase, enhanced the degradation of Arid5a mRNA levels in macrophages, B cells and dendritic cells. Thus, we identified P38 MAP kinase signaling as a key pathway for regulating Arid5a mRNA stability. Our findings thus indicate that p38 MAP kinase signaling is linked to the induction of Th17 cells and autoimmune disease through regulating Arid5a mRNA stability and its function in post-transcriptional control of IL-6 level in vivo *(research in progress)*.

3. Aryl hydrocarbon receptor-mediated induction of miR-132/212 cluster enhances T_H17 cell differentiation

Aryl hydrocarbon receptor (AHR) has critical roles in autoimmune diseases such as multiple sclerosis (MS) by controlling Interleukin 17 (IL-17)-producing T helper cells (T_H17 cells) and Regulatory T cells (T_{reg} cells). Although various transcription factors and cytokines have been identified as key participants in T_H17 generation, the role of microRNA is poorly understood. We found that miR-132/212 cluster is induced by AHR activation under T_H17-inducing, but not T_{reg} cell-inducing conditions. miR-132/212 cluster deficiency abrogated enhancement of T_H17 cell differentiation by AHR activation. We identified Bcl-6, a negative regulator of T_H17 cell differentiation, as a target of miR-132/212 cluster.

4. Type-I interferon controls its own production in immune homeostasis by inducing PPAR-γ expression and an inhibitory PPAR-γ/IRF7 complex.

Type-I interferon is important for anti-viral immunity, but its over-production is linked to the development of autoimmunity. Type-I interferon production requires the transcription factor IRF7. How type-I interferon signals to attenuate its own production in immune homeostasis is not known. Here we show that type-I interferon induces expression of PPAR-γ, which forms an inhibitory interaction with IRF7, attenuating type-I interferon production via the virus-activated (MyD88-independent) pathways in fibroblasts and TLR-activated (MyD88-dependent) pathways in pDCs, and type-I IFN-dependent responses in autoimmunity (*manuscript under revision*).

5. AHR negatively regulates type 1 interferon production and the development of murine lupus.

In addition, we found that production of type I interferon and expression of interferon stimulation genes in aryl hydrocarbon receptor knockout mice are higher than in wild-type mice when we induced lupus-like disease by pristane treatment. Our results suggest that aryl hydrocarbon receptor is a critical negative regulator of TLR-mediated type I interferon production and inhibits type I interferon signaling in murine lupus. Now, we are evaluating protective effects of aryl hydrocarbon receptor agonists in murine lupus (*research in progress*).

6. Molecular Mechanisms of Thalidomide Anti-Inflammatory Effects.

Thalidomide is known to inhibit TLR4-mediated inflammatory cytokine production (including IL-6 and TNF-alpha). We investigated the mechanism underlying these properties by studying the recently identified Thalidomide binding protein, Cereblon. We demonstrated that Thalidomide derivatives potently inhibits the TLR4-TRIF pathway (which normally acts in synergy with the MyD88-dependent pathway for cytokine production), involving inhibition of IRF3 transcriptional activity and production of interferon-beta. We have also demonstrated that under homeostatic conditions cereblon exists in complex with Rabex-5, an established regulator of intracellular transport. Intriguingly, Treatment with Thalidomide derivatives was found to disrupt this complex. Rabex-5 was also found to be critical for TLR induced signal transduction. Thus, we reason that disruption of the Cereblon-Rabex-5 complex underlies Thalidomide's anti-inflammatory properties. *(manuscript submitted).*

7. Therapeutic targeting of the interleukin-6 receptor.

Our research is engaged in clinical studies on the effectiveness of anti-IL6R antibody (Tocilizumab) in autoimmune diseases.

(i) Tocilizumab can inhibit bone resorption and joint destruction in chronic rheumatoid arthritis (RA) patients by a large-scale randomized control trial. This effect is due to the inhibitory effect of IL-6 signal blockade on the expression of Rank-ligand and differentiation into osteoclasts of mononuclear cells.

(ii) A randomized placebo-controlled phase III trial confirmed that Tocilizumab is effective and safe in patients with systemic-onset juvenile idiopathic arthritis (JIA). The USA and EU approved the use of Tocilizumab for the treatment of JIA. In December 2012, large-scale clinical trials for JIA in Europe and the USA confirmed efficacy and safety of Tocilizumab.

Other autoimmune inflammatory diseases have been treated with Tocilizumab, including refractory relapsing polychondritis, AA amyloidosis, reactive arthritis, polymyalgia rheumatica, systemic sclerosis, polymyositis and acquired hemophilia A. The results confirmed efficacy and safety of Tocilizumab in these diseases.

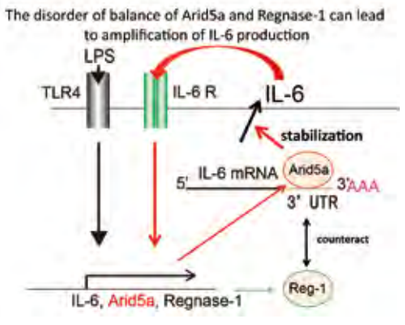

Figure 1. Arid5a stabilization of IL-6 mRNA

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- Masuda K, et al. Arid5a controls IL-6 mRNA stability, which contributes to elevation of IL-6 level in vivo. ***Proc. Natl. Acad. Sci. USA.*** 110:9409-14, 2013.
- Nakahama T, Kimura A, Nguyen NT, Chinen I, Hanieh H, Nohara K, Fujii-Kuriyama Y, Kishimoto T. Aryl hydrocarbon receptor deficiency in T cells suppresses the development of collagen-induced arthritis. ***Proc. Natl. Acad. Sci. USA.*** 108:14222-27, 2011.
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Mucosal Immunology

Kiyoshi Takeda, MD/PhD

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	Makoto Kinoshita
Postdoctoral Fellow	**1**
Research Assistant	**13**
Visiting Scientist	**1**
Support Staff	**2**

Dysregulation of innate immune responses causes immune disorders such as inflammatory bowel diseases. Our previous study using mice lacking Stat3 in their innate immune cell populations showed that TLR-mediated activation of innate immunity, when in excess, induces intestinal inflammation. Thus, innate immune cell activity in the intestine of normal mice is finely regulated to prevent excessive inflammatory responses. Since then, we have tried to identify unique subsets of innate immune myeloid cells that regulate gut immunity. As a result, we identified CD70^{high} CD11b^{+} CD11c^{+} dendritic cells that induce Th17 cells in the intestinal lamina propria. In addition, we have identified that CX3CR1^{high} CD11b^{+} CD11c^{+} myeloid cells suppress T cell growth via ICAM-1/VCAM-1-mediated interaction. Suppressive activity of the regulatory subset of myeloid cells (regulatory myeloid cells: Mreg cells) is mediated by anti-inflammatory cytokine IL-10. So far, we have characterized several intestinal innate myeloid cell subsets that mediate the murine gut homeostasis. However, in order to apply these basic findings to clinics to treat inflammatory bowel diseases, we need to know whether similar subsets of intestinal innate myeloid cells exist in the human intestine.

The human intestinal myeloid cell subset inducing Th17 cells.

We then tried to identify a human counterpart of myeloid cell subset that induces Th17 cell development in the intestinal lamina propria. We found that a CD14^{+} CD163^{low} cell subset in HLA-DR^{high} Lin^{-} cells, which was selectively present in the human intestinal lamina propria, expressed *TLR2*, *TLR4*, and *TLR5*, and produced IL-6, IL-1β and TNF-α in response to LPS. In vitro coculture with naïve T cells revealed that these cells induced Th17 cell differentiation. We also examined CD14^{+} CD163^{low} cells in Crohn's disease patients. CD14^{+} CD163^{low} cells from inflamed regions of mucosa of Crohn's disease patients expressed high levels of *IL-6*, *IL-23p19*, and *TNF* and strongly induced Th17 cells. Enhanced Th17-inducing activity of CD14^{+} CD163^{low} cells was also observed in non-inflamed intestine of a patient with Crohn's disease. Thus, we identified a human intestinal myeloid cell subset inducing Th17 cells and the Th17-inducing activity of the subset increases in Crohn's disease patients.

A role of a lymphoid tissue in the appendix.

We also analyzed the function of a lymphoid tissue in the appendix, which is thought to be a kind of remnant tissue in our body. Gut-associated lymphoid tissues (GALTs) such as Peyer's patches and isolated lymphoid follicles are known to be responsible for generation of IgA-secreting cells. In addition to these GALTs, there is a lymphoid cell cluster in the appendix, which is called a cecal patch. However, the function of the cecal patch remains unknown. We analyzed the role of the cecal patch in mucosal immune responses using germ-free mice colonized with microbiota after appendectomy. Appendectomized mice showed delayed accumulation of IgA^{+} cells in the large intestine, but not the small intestine, after colonization. Decreases in colonic IgA^{+} cells correlated with altered composition of fecal microbiota. IgA^{+} cells with photoconverted Kaede protein in the cecal patches migrated to the large and small intestines. In contrast, photoconverted IgA^{+} cells in Peyer's patch were preferentially recruited to the small intestine. Adoptive transfer of CD45.2^{+} cells of the cecal patch or Peyer's patches into CD45.1^{+} mice also indicated that IgA^{+} cells generated in the cecal patch migrate to the large and small intestines; IgA^{+} cells produced in Peyer's patches showed preferential migration to the small intestine. IgA^{+} cells in the cecal patch expressed higher levels of CCR10 than cells in Peyer's patches. In accordance, dendritic cells in the cecal patch, but not Peyer's patch, prominently induced CCR10 on cocultured B cells. Thus, we revealed that the cecal patch is a major site for generation of IgA-secreting cells that migrate to the large intestine.

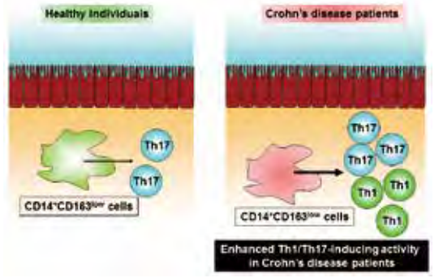

Figure 1. CD163low myeloid cells induced Th17 cells and its activity was enhanced in Crohn's diseases

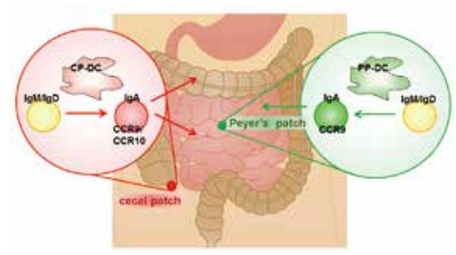

Figure 2. Cecal patch generates IgA-secreting cells migrating to the small and large intestines.

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- Masahata K, Umemoto E, Kayama H, Kotani M, Nakamura S, Kurakawa T, Kikuta J, Gotoh K, Motooka D, Sato S, Higuchi T, Baba Y, Kurosaki T, Kinoshita M, Shimada Y, Kimura T, Okumura R, Takeda A, Tajima M, Yoshie O, Fukuzawa M, Kiyono H, Fagarasan S, Iida T, Ishii M, Takeda K. Generation of colonic IgA-secreting cells in the cecal patch. ***Nat. Commun.*** 10:3704**,** 2014.

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Molecular Immunology

Hitoshi Kikutani, MD/PhD

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1) Molecular mechanisms in immune pathology caused by host-pathogen interaction: Epstein-Barr virus (EBV)-encoded latent membrane proteins (LMP) 1 and 2a affect B cell survival and selection

EBV infects human B cells and particularly targets the memory B cell population for persistent infection. Among various viral genes expressed in the EBV-infected B cells, LMP1 and 2a constitutively activate and mimic the CD40 and BCR signals, respectively. However, it has been largely unknown what impacts these virus-derived molecules have an in vivo activation and differentiation of B cells. In our laboratory, we generated transgenic mice conditionally expressing LMP1 or LMP2a in germinal center (GC) B cells to evaluate their effects on the humoral immune responses in vivo.

In the spleen of LMP2a Tg mice, normal GC was observed whereas titers of antigen-specific antibodies were significantly low. Sequencing analysis of the immunoglobulin gene of antigen-specific B cells revealed impaired affinity maturation in the LMP2a Tg mice after immunization (Figure 1). In addition, plasma cell differentiation was significantly accelerated in LMP2a Tg mice. These results indicate that EBV LMP2a reduced the threshold for selection of high affinity B cells, which may contribute to the latent infection of EBV in memory B cells.

Unlike LMP2a, the expression of LMP1 in B cells strongly inhibited GC formation. Interestingly, GC formation and antibody response were also impaired in chimera mice co-transferred with LMP1 Tg and wild-type-derived bone marrow cells, suggesting that LMP1$^+$ B cells provide inhibitory effect on neighboring wild type cells. Thus, LMP1 may contribute to EBV infection by suppressing host humoral responses.

2) Understanding of mechanisms in emergence of auto- and polyreactive B cells and their physiological and pathological roles

The circulating IgG$^+$ memory B cells from healthy individuals contain a significant proportion of polyreactive clones that are reactive to multiple self-components. Because their immunoglobulin genes have multiple somatic hypermutations (SHM), it is expected that polyreactive IgG$^+$ memory B cells are post-GC B cells. The inducers of polyreactive B cells have not been extensively investigated. Our analysis for mouse infection model of MHV68 (murine γ-herpesvirus 68) provides strong evidence that polyreactivity emerges and is selected from the splenic IgG$^+$ GC B cells harboring somatically mutated immunoglobulins. Therefore, MHV68 infection will provide an ideal experimental system for analysis of polyreactive B cell generation (Figure 2).

Is polyreactive antibody related to autoimmune diseases in humans? As we analyzed polyreactivity of plasma cells from systemic erythematosus lupus (SLE) patients and healthy donors, both contained a similar proportion of polyreactive clones, indicating that emergence of polyreactive clones is not correlated with disease activity of SLE. Characteristic differences, such as affinity to antigen of polyreactive antibodies between SLE and healthy donors remain to be elucidated.

On the other hand, several clones of anti-nuclear antibody (ANA), which is generally used for diagnosis, were isolated from high percentage (~5%) in plasma cells from acute SLE patients. These ANA clones are not polyreactive and recognize each nuclear autoantigen specifically. For further study, we plan to analyze the immunoglobulin genes encoding ANA utilizing next generation sequencing (NGS) technologies to understand dynamics of autoreactive B cells for their generation and selection in humans.

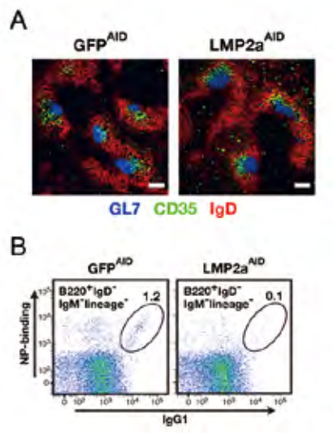

Figure 1.
EBV LMP2A reduces the threshold for selection of high-affinity B cells.
(A) The conditional expression of LMP2a by the AID (activation-induced cytidine deaminase) promoter-driven Cre did not alter GC size and structure in the spleen upon immunization.
(B) Generation of antigen-binding B cells was extremely impaired in the spleen of LMP2a Tg mice after NP-CGG/alum immunization.

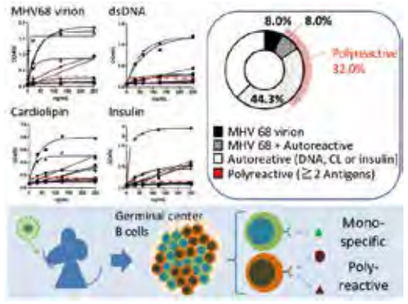

Figure 2.
Polyreactive clones emerges in the splenic IgG$^+$ germinal center B cells during MHV68 infection.
Single-cell-based cloning of immunoglobulins followed by ELISA revealed that ~30% of the splenic IgG$^+$ germinal center B cells showed polyreactivity. MHV68 infection will provide ideal experimental system for study of polyreactive B cell generation.

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Experimental Immunology

Shimon Sakaguchi, MD/PhD

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Postdoctoral Fellow	6
Research Assistant	4
Support Staff	8

Naturally occurring regulatory T (nTreg) cells, which specifically express the transcription factor FoxP3, are indispensable for the maintenance of immunological self-tolerance and homeostasis. One of the main projects in this laboratory is to understand the molecular basis of Treg cell development and function. In the previous year, we showed that Treg development was achieved by the combination of two independent processes, i.e., the expression of Foxp3 and the establishment of Treg-type CpG hypomethylation, both induced by TCR stimulation. We have continued the study and shown that developing nTreg cells in the thymus acquire a Treg-specific and stable hypomethylation pattern in a limited number of genes, which encode key functional molecules including FoxP3. This epigenetic change is acquired via TCR stimulation, beginning prior to FoxP3 expression. The Treg-specific DNA hypomethylated regions generally act as gene enhancers in steady state nTreg cells, contributing to the stable expression of Treg function-associated key genes including *Ctla4*, *Il2ra* and *Ikzf4* in addition to *Foxp3*. Upon TCR stimulation of mature nTreg cells, FoxP3 strongly represses many genes including *Il2*, contributing to Treg suppressive activity. Thus, the Treg-specific epigenome alteration can determine the heritable Treg-specific gene network including *Foxp3*-dependent gene regulation (Ohkura et al., *Immunity*, 2012; Ohkura et al., *Immunity*, 2013; Kitagawa et al., *Front. Immunol*. 2013; Morikawa et al., *PNAS*, 2014; Morikawa et al., *Immunol. Rev.*, 2014). Considering physiological presence of non-suppressive FoxP3$^+$ T cells in the immune system and loss of FoxP3 in Treg cells under certain immunological conditions, functional nTreg cells can be more accurately defined as a T cell subpopulation possessing the Treg-type epigenome, rather than Foxp3$^+$ T cells. This epigenome-based definition of Treg cells would enable better understanding of functional stability, plasticity, and heterogeneity of Treg cells (Figure).

This laboratory also studies human Treg cells and their roles in controlling physiological and pathological immune responses. We have previously shown that human FOXP3$^+$ T cells can be dissected into three subpopulations including suppressive and non-suppressive ones (Miyara et al., *Immunity,* 2009). The most differentiated and suppressive population is FOXP3-high CD25-high CD45RA-low cells, called effector Treg (eTreg) cells. To enhance immune responses by specifically depleting eTreg cells, we have been looking for a cell surface molecule that is selectively expressed by eTreg cells. One of the candidates has turned out to be the chemokine receptor CCR4. Indeed in vitro depletion of CCR4$^+$ cells from peripheral blood T cells selectively reduced eTreg cells and evoked immune responses against tumor-associated antigens in healthy individuals and also in cancer patients (Sugiyama et al., *PNAS*, 2013). In vivo administration of anti-CCR4 monoclonal antibody, which is now in use to kill CCR4+ leukemic cells in adult T-cell leukemia/lymphoma (ATLL) patients in Japan, also eliminated eTreg cells and evoked immune responses against leukemic cells by CD4 and CD8 T cells in patients. Based on these findings we plan to embark on a new immunotherapy of cancer this year by depleting eTreg cells and subsequently immunizing with cancer vaccine first in ATLL patients and then in patients with other cancers (Nishikawa and Sakaguchi, *Curr Opin. Immunol.*, 2013).

This laboratory previously established an animal model of autoimmune arthritis, called SKG mice, which possess a ZAP-70 gene mutation and spontaneously develop T cell-mediated autoimmune arthritis immunopathologically similar to rheumatoid arthritis (RA) in humans. This year, we have established another animal model of autoimmune disease by introducing a variety of mutations in the ZAP-70 gene in mice. One of such ZAP-70 gene knock-in mice spontaneously developed not only arthritis but also colitis, which are immunopathologically similar to RA and inflammatory bowel disease, respectively, in humans. We have investigated how the mutation affects the structure of ZAP-70 and the binding of ZAP-70 to TCRζ chains, and downstream signal transduction, and how it consequently alters thymic selection of autoimmune T cells and Treg cells and their respective functions. These spontaneous models of autoimmune disease due to TCR signal alteration are instrumental in understanding the pathogenetic mechanisms of autoimmune diseases in humans and devising new ways of their prevention and treatment.

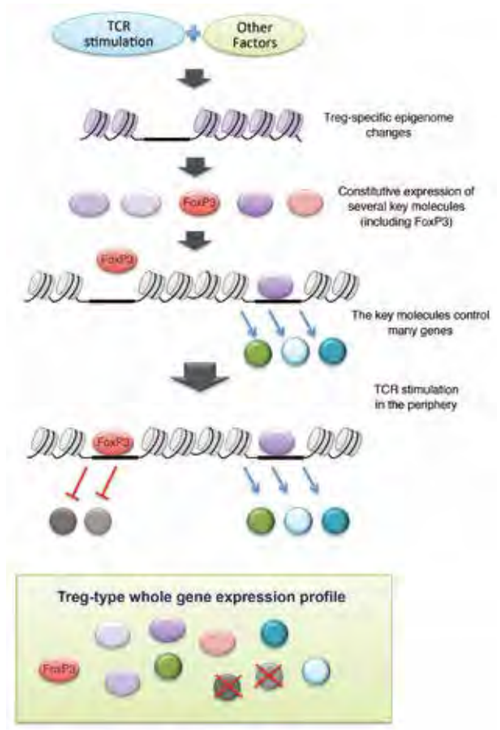

Figure.
Treg-type whole gene expression profile consists of several layers of gene regulations. TCR stimulation, possibly together with other unknown factors, induces Treg-specific epigenome changes that lead to the constitutive expression of several key molecules including FoxP3. These key molecules control many Treg cell-related genes including CTLA4, CD25, etc. TCR stimulation of Treg cells in the periphery not only triggers additional key gene expression but also activates FoxP3 to exert gene repression of many genes including Il2. These gene activations and FoxP3-dependent gene repressions at various layers construct the whole gene expression profile of Treg cells.

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- Sugiyama D, Nishikawa H, Maeda Y, Nishioka M, Tanemura A, Katayama I, Ezoe S, Kanakura Y Sato E, Fukumori Y, Karbach J, Jäger E, Sakaguchi S. Anti-CCR4 mAb selectively depletes effector-type FoxP3$^+$CD4$^+$ regulatory T cells, evoking anti-tumor immune responses in humans. ***Proc. Natl. Acad. Sci. USA.*** 110:17945-50, 2013.
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Cell Signaling

Takashi Saito, PhD

We have analyzed the mechanism and regulation of T cell activation and differentiation by analyzing function of genes and signaling molecules, whose expression are regulated during T cell activation and differentiation. Analyzing the genes altering during T cell development, we found that Bach2 changes the expression during T cell development. Bach2 is transcriptional repressor and has been thought to be specific for B cells, but we found it is expressed equally well in T cells and increase the expression along maturation of T cells. Bach2-deficient T cells reduce naïve T cells and enhances memory-type of T cells. The Bach2-/- naïve T cells highly express genes related to effector memory T cells, and rapidly produce Th2 cytokines upon T cell stimulation. Re-expression of Bach2 in effector memory T cells restored the characteristics of gene expression of naive T cells. These findings indicate that Bach2 suppresses effector memory-related genes to maintain the naive T cell state and regulates generation of effector-memory T cells (Figure 1).

To understand the integrative regulation for T cell activation by various different signals, we have analyzed the functional contribution of innate-related signals for T cell activation and function. We have previously shown that IRAK4, a critical kinase for innate signaling is involved in T cell activation and function. Therefore, we analyzed particularly the function of TLRs expressed on T cells and related signals. We found that effector Th1 but not Th2 cells were directly activated through TLR2 for IFNg production. Nucleic acids also induce T cell co-stimulation for cytokine production and proliferation, which we found were independent of TLR/RLRs and their signaling. Unlike innate cells, not only CpG but also other DNA could induce T cell co-stimulation particularly when complexed with LL37 or histones. Nucleic acids-mediated T cell co-stimulation is induced by a yet-unknown unique sensor, and physiologically induces IL-4 production in naïve T cells which results in the induction of Th2 differentiation (Figure 2).

Professor	**Takashi Saito**
Postdoctoral Fellow	**1**
Research Assistant	**1**

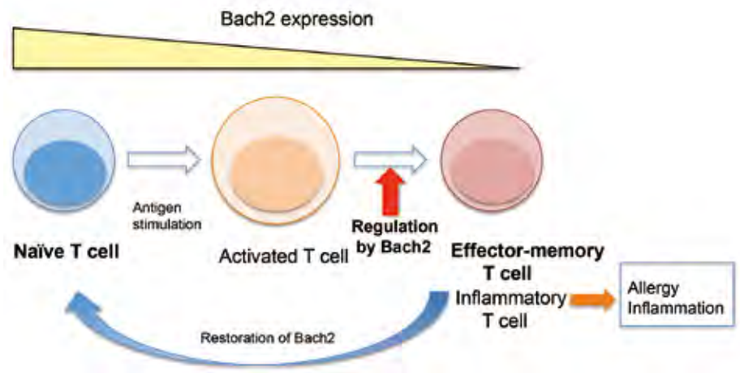

Figure 1. Regulation of generation of inflammatory T cells by Bach2.
Bach2 suppresses the expression of genes required to differentiate into Th2 and memory T cells, and maintain the naïve status of T cells. Bach2-deficient T cells express genes related to inflammatory/memory T cells, and. re-expression of Bach2 resulted in returning to the phenotype of naïve T cells

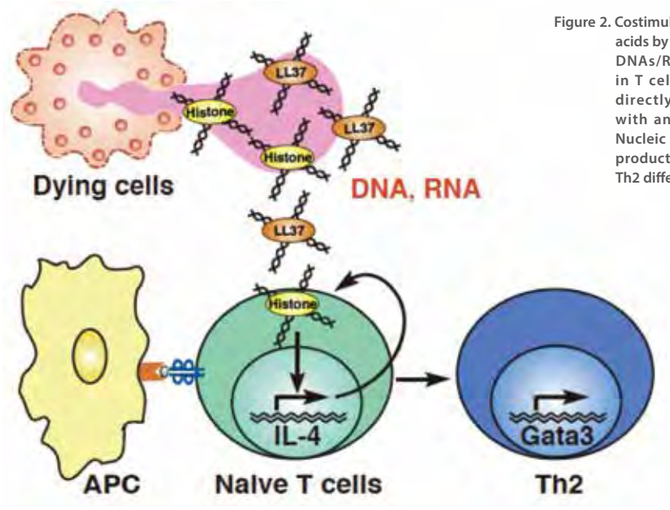

Figure 2. Costimulation of T cells by direct sensing of nucleic acids by T cells induces Th2 differentiation.
DNAs/RNAs are recognized by unique sensor in T cells. The nucleic acids from dying cells directly costimulate T cells when complexed with antimicrobial peptide LL37 or histones. Nucleic acid-mediated ostimulation induces IL-4 production from naïve T cells and consequently Th2 differentiation.

Recent Publications

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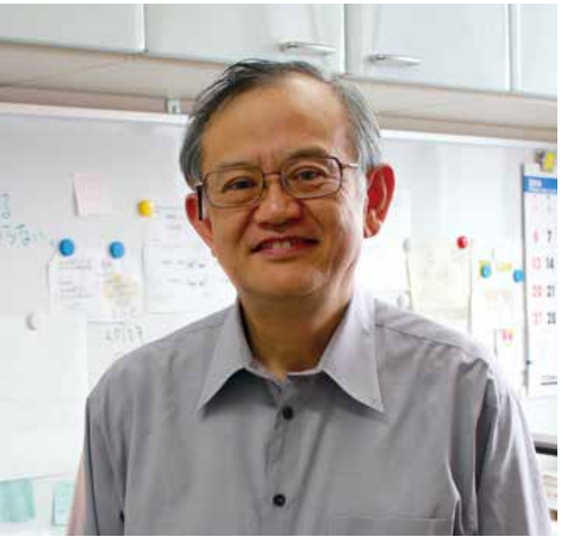
Lymphocyte Differentiation



Tomohiro Kurosaki, MD/PhD

Professor	**Tomohiro Kurosaki**
Associate Professor	**Yoshihiro Baba**
	Wataru Ise
Assistant Professor	**Takeshi Inoue**
	Masanori Matsumoto
	Shinya Tanaka
Support Staff	**4**

Introduction

B cells play an essential role in the regulation of immune responses. Upon first encountering their cognate antigens, B cells exert multiple functions including antibody production, antigen-presentation, and induction of T cell differentiation. In addition to protective roles against pathogens, B cells also have regulatory roles, e.g. serving as negative regulators as autoimmunity by secreting anti-inflammatory cytokines such as IL-10 and TGF-β. Our laboratory has now focused on deciphering when and where B cells produce IL-10, thereby dampening T cell dependent inflammation.

When B cells recognize the same antigen a second time, memory antibody responses can be induced by T cell help. These are typically seen in the response to T cell-dependent antigens and are characterized by the rapid production of high-titers of high-affinity antigen-specific antibody. Based upon our previous evidence that memory B cell responses require T cells help, we are now characterizing how memory T cells are generated and contribute to robust memory antibody responses.

B cell role for immune suppression

Calcium signaling is thought to be critical for multiple B cell function; however, supportive in vivo evidence has been lacking. Thus, our discovery that Stim1 and Stim2 proteins are required in BCR-medicated calcium mobilization, has allowed us to approach this question by taking a genetic approach. We generated mice with B cell-specific deletions of both Stim1 and Stim2 and found that; although both molecules are critically required for in vitro proliferation, they were dispensable for B cell development and antibody responses in vivo. However, the ablation of Stim1 and Stim2 in B cells caused defects in NFAT activation, B cell intrinsic IL-10 production, and suppression of an EAE model of autoimmune diseases. Furthermore, we demonstrated that plasmablasts, but not B cells, indeed secrete IL-10, which in turn acts on dendritic cells (DC) cells, thereby dampening T cell-mediated inflammation.

Interaction between memory T and B cells in humoral re-call responses responses

In primary immune responses, it is widely accepted that among several differentiated helper T cell subsets, follicular helper CD4 T cells (T_{FH} cells) are the major subset to deliver help to B cells. As a T_{FH} lineage regulator, a transcription factor, Bcl6, has recently been identified; it is highly expressed by T_{FH} cells and is required for their development. According to the current view, during a primary response, Bcl6 expression by T cells is induced by priming with dendritic cells and ICOS is a key co-receptor molecule for induction of Bcl6. The initial Bcl6 induction and subsequent cXCR5 expression allow CD4 T cells to migrate toward the T-B border, where T_{FH} cells interact with antigen-specific B cells. According to this model, cognate B cells are not required for the induction of Bcl6, but support the expansion of T_{FH} cells.

Although the importance of Bcl6 and its expression kinetics in naïve T cell differentiation have been well elucidated, its role and activation mechanisms in T_{FH} memory cells still remain obscure. We demonstrated its importance for maintenance and activation of T_{FH} memory cells. Indeed, Bcl6 in memory T_{FH} cells is rapidly induced upon re-challenge by soluble antigen and this response is mainly mediated through antigen-presentation by the memory B cells. Thus, memory B cells are the primary APCs to induce the rapid differentiation of memory T_{FH} cells toward effectors, further accelerating memory B cells responses during recall.

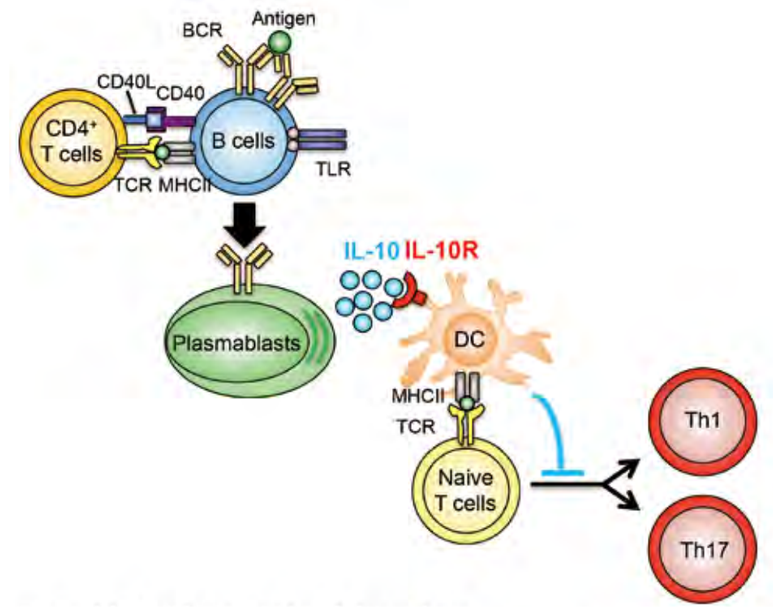


Figure 1. The model of regulatory function of plasmablasts
Plasmablasts are generated through the interactions between B cells and $CD4^+$ T cells. The plasmablast-derived IL-10 inhibits dendritic cell functions and thereby suppresses the generation of effector T cells such as Th1 and Th17 cells.

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Malaria Immunology

Cevayir Coban, MD/PhD

Associate Professor	**Cevayir Coban**
Research Assistant	**2**
Support Staff	**1**

Our lab is interested in the host-pathogen interactions in the context of Plasmodium infections, a disease known as malaria. We mainly focus on how *Plasmodium* parasites invade host cells, mostly erythrocytes, and cause inflammation in tissues due to infection-related events. We try to learn from these host-parasite interactions and transfer our understanding into the development of successful vaccines against malaria as well as other infectious diseases.

We have developed several new experimental approaches to reveal *Plasmodium*-host interactions. One of them is using new imaging technologies such as ultra-high field MRI and multi-photon live imaging microscopy. We have closely collaborated with other IFReC laboratories such as Biofunctional Imaging Lab. (Prof. Yoshioka) and Vaccine Science Lab. (Prof. Ken J Ishii) to visualize Plasmodium parasites and related events in vivo. For instance, we recently evaluated murine cerebral malaria pathogenesis, an experimental model for the deadliest complication of *Plasmodium falciparum* infection called cerebral malaria (*Zhao et al., Cell Host Microbe, 2014*).

Severe symptoms of human cerebral malaria such as sudden onset coma, death or neuro-disability are commonly observed in mice after *P. berghei*ANKA infection. In this experimental cerebral malaria model, multiple pathological events such as blood-brain-barrier disruption, vascular leakage, immune cell accumulation (especially CD8 T cell infiltration) occur in the brain. Although the brain is known to be affected severely, the location and spatiotemporal mechanism of how this occurs is poorly understood. We have identified in mice that the olfactory region is a vulnerable location for vascular leakage during experimental cerebral malaria. This discovery could only be possible by using an ultra-high field MRI in combination with multi-photon live imaging microscopy. We have shown that the olfactory bulb is the area physically disrupted and damaged functionally by *Plasmodium* parasites, followed by high fever (Figure 1A). Thus, we identified that there is an early symptom, olfaction loss, before the onset of coma. Given that trabecular small capillaries are a unique architectural structure for olfactory bulb, olfactory easily undergoes parasite accumulation and cell occlusion followed by micro-bleeding (Figure 1B).

In the search for the underlying mechanism(s) of pathology of experimental cerebral malaria via olfactory, we found that circulating parasites in the olfactory vessels are sensed by astrocytes around olfactory glomeruli at the early stage of infection, and may release CCL21and may have a role for the recruitment of pathological CD11c+ CD8 T cells into the brain. We further evaluated this novel understanding into a novel intervention strategy by blocking chemokine-receptor interactions when the early symptom of experimental cerebral malaria, olfaction loss, was evident. Given that even early detection of one day of malarial coma could dramatically increase treatment success; this previously unnoticed, truly overlooked location and detection of olfaction loss during malaria infection may provide early, cheap and easy diagnosis of cerebral malaria.

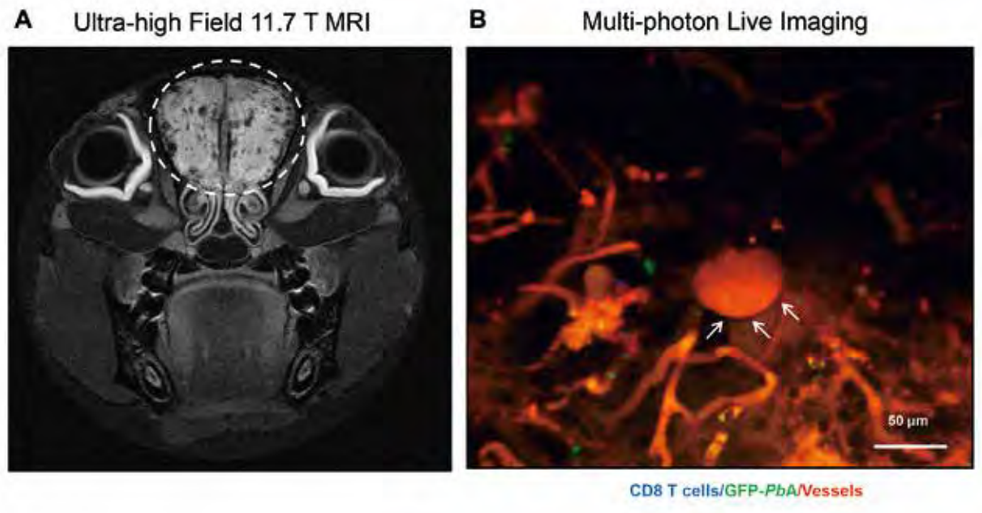

Figure 1.

(A) 11.7 T MRI of mouse head infected with *P. berghei*ANKA parasites. Diffusion weighted image, coronal section. White dotted circle is olfactory bulb; black dark spots are bleeding spots.

(B) Fresh micro-bleeding in olfactory bulb captured by multi-photon live imaging microscopy.

Recent Publications

- Zhao H, Aoshi T, Kawai S, Mori Y, Konishi A, Ozkan M, Fujita Y, Haseda Y, Shimizu M, Kohyama M, Kobiyama K, Eto K, Nabekura J, Horii T, Ishino T, Yuda M, Hemmi H, Kaisho T, Akira S, Kinoshita M, Tohyama K, Yoshioka Y, Ishii KJ, Coban C. Olfactory is an overlooked site for the initiation of cerebral malaria. ***Cell Host & Microbe.*** 15:551-63, 2014.
- Kobiyama K, Aoshi T, Narita H, Kuroda E, Hayashi M, Tetsutani K, Koyama S, Mochizuki S, Sakurai K, Katakai Y, Yasutomi Y, Saijo S, Iwakura Y, Akira S, Coban C, Ishii KJ. Nonagonistic Dectin-1 ligand transforms CpG into a multitask nanoparticulate TLR9 agonist. ***Proc. Natl. Acad. Sci. USA.*** 111:3086-91, 2014.
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Vaccine Science

Ken Ishii, MD/PhD

Professor	**Ken Ishii**
Associate Professor	**Etsushi Kuroda**
Assistant Professor	**Taiki Aoshi**
	Kouji Kobiyama
	Kohei Tetsutani
Postdoctoral Fellow	**2**
Research Assistant	**4**
Visiting Scientist	**5**
Support Staff	**5**

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 allergies as well as other non-communicable diseases.

<Basic and translational vaccine science>

Nucleic acids as an essential built-in adjuvant for successful vaccines: Our group and others have recently clarified that most successful vaccines, such as FLU and DNA vaccines possess DNA and/or RNA, which appear to act as essential "built-in" adjuvants (Ishii KJ et al *Nature* 2008, Koyama S et al, *Science Trans. Med.* 2010). In 2013, we published a book entitled "*Biological DNA Sensor: The Impact of Nucleic Acids on Diseases and Vaccinology*." (Elsevier 2013 edited by Ken J. Ishii and Jason Choon Kit.)(Figure.1), which defines the meaning of DNA sensing pathways and demonstrates the importance of the innate immune responses induced by double stranded DNA (dsDNA) through its influencing functions in disease pathology and immune activity of adjuvants for vaccines.

Old, but newly evolving adjuvant research; As we postulated that our immune system is substantially modulated by metabolic intermediates of nucleic acids (Ishii KJ et al *Curr. Op Immunol* 2008), we went further on identifying a key mechanism of the most commonly used adjuvant, aluminum salt, was due to nucleic acids as well as PGE_2, released as an alarmin (Marichal T et al *Nat. Med* 2011, Kuroda E et al *Immunity* 2011).

Taken together, we believe this is a new area of vaccine science and we propose that revealing the critical role in driving the responses mediated by many current vaccines of nucleic acid-sensing mechanisms (Desmet C and Ishii KJ *Nat Rev Immunol* 2012), as well as host-derived metabolites (Jounai N et al *Front Cell Infect Microbiol.* 2012) and particulate molecules (Kuroda E et al, *Int. Rev. Immunol* 2013) may demonstrate possibilities for the design of new vaccines.

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

A Ph-I clinical trial for novel-adjuvanted vaccine; We have been successful in developing a nucleic-acid-based adjuvant; humanized CpG-ODN for a travelers' malaria vaccine targeting a blood stage parasite antigen (Tougan T et al *Human Vac* 2013). Preclinical studies assessing safety and efficacy have been completed with GMP grade humanized CpG ODN and we conducted multiple pre-clinical studies to ensure safety and efficacy. As a result, we obtained approval from the IRB of Osaka University Hospital and PMDA (Japanese regulatory agency) and initiated an investigator driven GCP Phase-I clinical trial during 2013 in Osaka University Hospital.

Development of the second-generation adjuvants: CpG oligonucleotide (ODN), a synthetic TLR9 ligand, is a promising immunotherapeutic agent; however, it has not been successful to develop interferon (IFN)-inducing CpG ODN forming a stable nano-particle without undesirable aggregation. Here, we generated a nano size particle CpG ODN (K3) wrapped by a non-agonistic Dectin-1 ligand schzophyllan (SPG), namely K3-SPG. K3-SPG stimulates human PBMCs to produce a large amount of both type-I and type-II IFN. K3-SPG thus became a potent adjuvant, especially for CTL induction to co-administered protein antigens without conjugation that is attributed to its nature of nano-particle, rather than targeting Dectin-1. In addition, most of K3-SPG was rapidly trapped by MARCO+ macrophages in the draining lymph node, and then K3-SPG activated pDCs and CD8α+ DCs to exert its adjuvant effects. K3-SPG acting as an influenza vaccine adjuvant was demonstrated in vivo by both murine and non-human primate model. Taken together, K3-SPG may be used as IFN-inducer as well as CTL inducer for immunotherapeutic applications (Kobiyama K et al *PNAS* 2014) (Figure 2). This K3-SPG has been nominated for a JST-supported grant with a pharmaceutical company in Japan.

Clinical studies on seeking bio-marker(s) for safety as well as efficacy of adjuvanted vaccines were launched in 2012 (Adjuvant Data Base project supported by Ministry of Health, Labor and Welfare). 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 NIBIO. Preliminary results suggest serum miRNA may provide useful biomarkers to predict safety and immunogenicity of adjuvanted vaccines.

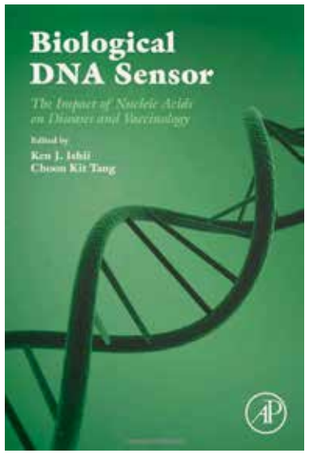

Figure 1. "*Biological DNA Sensor: The Impact of Nucleic Acids on Diseases and Vaccinology*." (Elsevier 2013 edited by Ken J. Ishii and Choon Kit Tang)

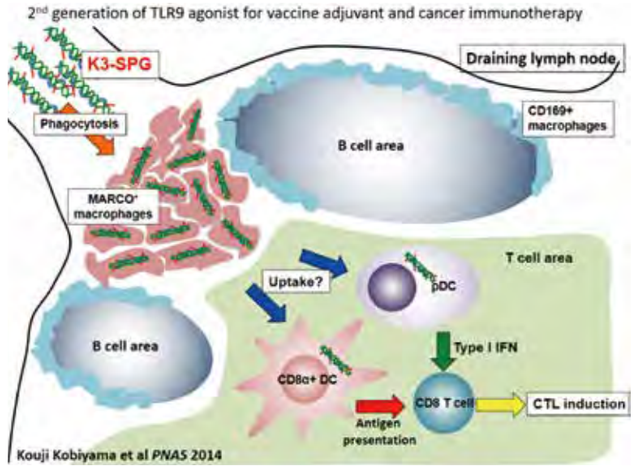

Figure 2. Nonagonistic Dectin-1 ligand transforms CpG into a multitask nanoparticulate TLR9 agonist. ***Proc Natl Acad Sci U S A.*** 2014 111(8):3086-91. (Direct submission)

Recent Publications

- Kobiyama K, Aoshi T, Narita H, Kuroda E, Hayashi M, Tetsutani K, Koyama S, Mochizuki S, Sakurai K, Katakai Y, Yasutomi Y, Saijo S, Iwakura Y, Akira S, Coban C, Ishii KJ. Nonagonistic Dectin-1 ligand transforms CpG into a multitask nanoparticulate TLR9 agonist. ***Proc. Natl. Acad. Sci. USA.*** 111:3086-91, 2014. (Direct submission)
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Immune Regulation

Tsuneyasu Kaisho, MD/PhD

Professor	**Tsuneyasu Kaisho**
Associate Professor	**Hiroaki Hemmi**
Assistant Professor	**Izumi Sasaki**
Research Assistant	**2**
Support Staff	**1**

Innate and adaptive immunity cooperate to achieve host defense and immune homeostasis. Ag presenting cells including dendritic cells (DCs) play critical roles in linking innate and adaptive immunity. DCs respond to a variety of immune adjuvants derived from pathogens or the host through the sensors such as Toll-like receptors (TLRs). Upon sensing, DCs produce proinflammatory cytokines or type I interferons (IFNs) and upregulate a set of costimulatory molecules, thereby inducing innate immune responses and supporting T cell activation and differentiation. These DC functions are critical not only for protective immunity against pathogens but also for the pathogenesis of autoimmune and inflammatory disorders.

DCs are heterogeneous and consist of various subsets, such as plasmacytoid DC (pDC) or conventional DC (cDC), which have subset-specific functions. We aim to clarify the molecular mechanisms for regulating these DC's functions and to develop novel immunoregulatory maneuvers based on the clarified mechanisms.

In vivo roles of a DC subset, XCR1-expressing DC

One cDC subset, the CD8α/103+CD11b- cDC, is characterized by the high ability to incorporate apoptotic or dead cells and crosspresent antigens to generate CD8 T cell responses. The cDCs are also featured by their high ability to produce proinflammatory cytokines in response to various TLR signals. Through these functions, cDCs contribute to anti-microbial or anti-tumor immunity. The cDCs are also considered to be important for maintaining immune homeostasis. However, whether or how the cDC subset is involved in developing various inflammatory diseases or maintaining the immune homeostasis remains unknown.

We have generated the in vivo system for tracking or ablating CD8α/103+CD11b- cDCs. We have focused on a chemokine receptor, XCR1, which is highly expressed in the cDC subset. The genes for a fluorescent protein, venus, and for the fusion protein of diphtheria toxin receptor (DTR) and venus were knocked into the XCR1 gene locus for generating the XCR1-venus and XCR1-DTRvenus mice, respectively. Venus expression was selectively detected in CD8α/103+CD11b- cDCs in various lymphoid and peripheral tissues. Eighty to ninety percent of CD8α/103+CD11b- cDCs were venus-positive and the venus-positive cells showed much higher ability to produce proinflammatory cytokines than the venus-negative cells among CD8α/103+CD11b- cDCs, indicating that venus-positive cells are functionally more mature than venus-negative cells. In the XCR1-DTRvenus mice, the XCR1-expressing DCs were efficiently ablated after injection of DT. By analyzing these mice, the XCR1-expressing DC was found to be critical for double-stranded RNA (dsRNA)-induced CD8 T cell responses (crosspresentation) of protein, cell-associated and bacteria-derived Ags (C. Yamazaki et al. 2013). Furthermore, XCR1-expressing DCs were also critical for NKT cell-mediated generation of tumor-specific memory CD8 T cell responses (K. Shimizu et al, 2013).

We are now analyzing the XCR1-DTRvenus mice for various models including immune tolerance or disease models. Based on the specific expression of XCR1 on CD8α/103+CD11b- cDCs, we are also generating and analyzing several knock-in mice for clarifying the novel in vivo functions of CD8α/103+CD11b- cDCs.

Critical roles of an Ets family transcription factor, Spi-B, in pDC

pDC is featured by the ability to produce large amounts of type I IFNs in response to signaling through TLR7 or TLR9, which can sense host- or microorganism-derived nucleic acids. This ability plays important roles in both protective immunity against viral infection and pathogenesis of certain autoimmune disorders such as SLE. It is still largely unknown how the activity is regulated. We have found that an Ets family of transcription factor, Spi-B, expressed abundantly in pDC, is critical for pDC function. Spi-B could transactivate the type I IFN promoters in synergy with a transcription factor, IRF-7, which is critical for TLR7/9-induced type I IFN gene expression. The synergy was most prominent with IRF-7 among IRF family members. Spi-B also associated with IRF-7 most strongly among IRF family members. Furthermore, Spi-B-deficient pDC and mice showed defects in TLR7/9-induced type I IFN production.

Spi-B was also highly expressed in the intestinal microfold cells (M cells), which are gate-keeping epithelial cells that initiate mucosal immune responses by uptaking and transcytosing luminal antigens. In Spi-B deficient mice, mature M cells were absent, indicating that Spi-B is a master regulator for M cell differentiation. We have found that Spi-B-deficient mice are defective for mucosal IgA responses. We are now studying how Spi-B is involved in IgA responses.

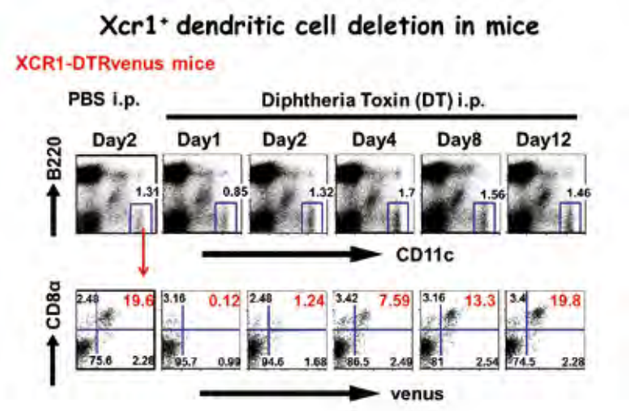

Figure. Xcr1+ dendritic cell deletion in mice. In the XCR1-DTRvenus mice, venus expressing cells in the CD8α/103+CD11b- cDCs are ablated *in vivo* after injection of DT. The mice are useful for clarifying the functions of XCR1-expressing DCs.

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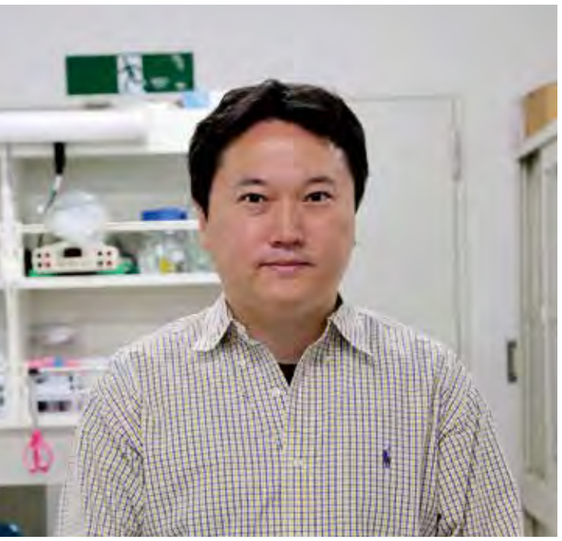
Immune Network



Rikinari Hanayama, MD/PhD

Associate Professor	**Rikinari Hanayama**
Postdoctoral Fellow	**3**
Research Assistant	**1**
Support Staff	**1**

Before joining IFReC, we were working on the molecular mechanisms for how macrophages remove apoptotic cells. We previously identified molecules that promote the engulfment of apoptotic cells by macrophages, and proved that the failure to remove apoptotic cells can lead to the development of lupus-like autoimmune diseases. Since we joined IFReC in November 2011, we have started to address new research projects on the mechanisms of exosome secretion and lysosomal fusion in macrophages and also the mechanisms that regulate hemophagocytosis by macrophages in hyper-inflammation.

1. Physiological functions of exosomes and their secretion mechanisms.

Exosomes are secreted small membrane vesicles, composed of a lipid bilayer with inserted transmembrane proteins, enclosing cytosolic components derived from the exosome-producing cells. Recently, exosomes have received much attention as messengers of intercellular communication networks, allowing the exchange of proteins and lipids between the exosome-producing cells and target cells. In particular, the findings that exosomes carry both antigenic materials and peptide-MHC complexes suggested their possible roles in triggering various immune responses, such as antigen exchange and immune cell activation (Figure 1). Exosomes were also shown to carry mRNAs and microRNAs inside them, raising the possibility that exosomes transfer genetic information between cells. However, it is not clear whether these processes occur under physiological conditions. The only way to conclusively demonstrate the physiological roles for exosomes would be to specifically inhibit or increase their secretion in vivo, and demonstrate that this affects the physiological outcomes. Therefore, our research is currently aimed at addressing three basic questions:

1) What are the molecular mechanisms of exosome secretion?
2) What are the physiological functions of exosomes?
3) How do exosomes travel from donor cells to the target cells in vivo?

To identify the regulator of exosome secretion, we established a shRNA-based high throughput screening system to sort out the cells with impaired exosome secretion. Using this assay system, we recently identified several genes that regulate the exosome secretion. We generated knockout mice of these genes to investigate the physiological functions of exosomes in immune systems. To clarify how exosomes travel in vivo, we generated a mouse model to visualize exosomes. As CD63 is one of the most specific protein markers for exosomes, we generated a conditional transgenic mouse line in which exosomes are specifically labeled with a CD63-EGFP fusion protein in a given immune cell population. Using this transgenic mouse, we are trying to clarify the behavior of exosomes in vivo.

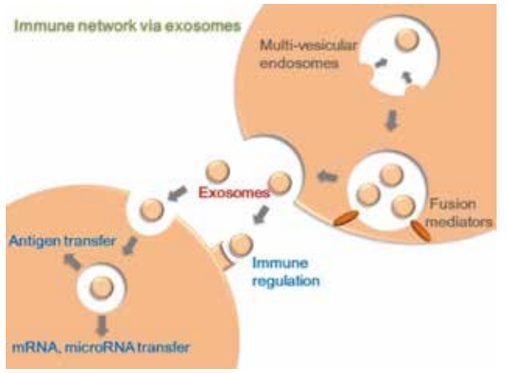


Figure 1. Exosomes: novel signaling entities that regulate complex immune system.

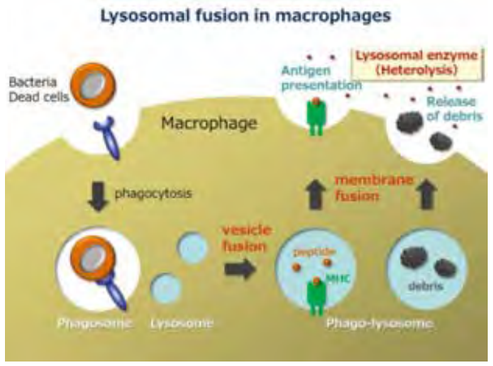


Figure 2. Lysosomal fusion events in macrophages.

2. Molecular mechanisms of lysosomal fusion in macrophages.

During inflammation, macrophages phagocytose many dead cells and/or bacteria into phagosomes and digest them into a series of peptides by the fusion of phagosomes with lysosomes. These peptides bind to MHC molecules and are transported to the surface of macrophages by the fusion of phago-lysosomes with cell plasma membrane. Using a similar mechanism, undigested debris in phago-lysosomes can be released from macrophages. During these processes, lysosomal enzymes are also secreted, causing the degradation of the surrounding tissues. This process is called heterolysis, but its molecular mechanisms as well as its relevance to the development of chronic inflammation have been unclear (Figure 2).

We have recently identified a novel protein that can be a mediator of lysosomal fusion in macrophages. It is a type II transmembrane protein, carrying multiple phospholipid-binding domains in the cytoplasmic region. It is highly expressed in various types of phagocytes, particularly inflammatory macrophages, but not in T and B lymphocytes. We found that this protein is specifically localized to lysosomes and the cell plasma membrane and mediates fusion between the membrane and lysosomes (and phago-lysosomes) upon calcium stimuli, causing the release of undigested debris, the secretion of lysosomal enzymes and MHC-mediated antigen presentation. Our findings will help to elucidate the molecular mechanisms of heterolysis that can be a critical process for the development of chronic inflammation.

3. Molecular mechanisms of hemophagocytosis.

Macrophages eat the apoptotic cells but not live cells by recognizing the "eat me" signal exposed only on the apoptotic cells. This process is strictly regulated. However, under special conditions, macrophages go crazy and start to eat the live blood cells. It is called hemophagocytic syndrome. It is often caused by severe hyper-inflammation which can occur with systemic infection, autoimmune diseases and malignancy. It is classified as one of the cytokine storm syndromes with high levels of inflammatory cytokines and also macrophage activation syndrome that is caused by overwhelming activation of T cells and macrophages. Familial diseases are caused by the deficiency of perforin, munc13 or syntaxin which lead to the impaired killing of virus-infected cells by NK cells and CTL and eventually cause hyper-inflammation. However, how these hyper-inflammation induce hemophagocytosis by macrophages remains completely unknown. To clarify the molecular mechanisms of this process, we established an in vitro assay system of hemophagocytosis. Using this assay, we have recently identified a receptor on macrophages that promotes the hemophagocytosis. We hope that our findings will help to clarify the pathophysiology of hemophagocytic syndrome that currently has no effective treatment.

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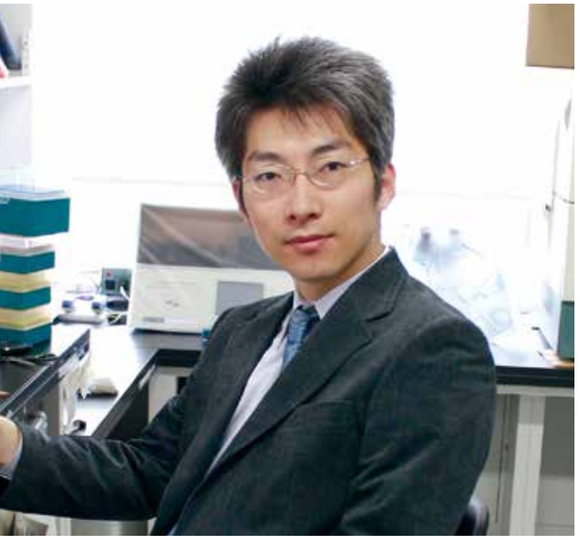
Immunoparasitology



Masahiro Yamamoto, PhD

Professor	**Masahiro Yamamoto**
Assistant Professor	**Miwa Sasai**
Postdoctoral Fellow	**1**
Support Staff	**1**

The host immune system produces inflammatory cytokines in response to infection of an intracellular protozoan pathogen *Toxoplasma gondii*. *T. gondii* is an obligatory intracellular protozoan parasite and the causative agent of toxoplasmosis in humans and animals. Although the infection of *T. gondii* in healthy animals largely results in opportunistic and latent infection, reactivation of this pathogen in immuno-compromised individuals suffering from AIDS or being treated by chemotherapy often leads to lethal encephalitis. Eradication of *T. gondii* critically requires IFN-γ, which indirectly stimulates anti-*T. gondii* immune responses by robust induction of nearly 2000 genes known as IFN-γ-inducible genes. IFN-γ activates anti-parasite programs involving IFN-γ-inducible GTPases such as p47 and p65 immunity-related GTPases called IRGs and GBPs, respectively. Mice deficient in IRGs such as LRG-47 (also known as Irgm1), IGTP (Irgm3) or IIGP1 (Irga6), or GBPs displayed loss of resistance to *T. gondii* infection.

The immunity-related GTPases have been implicated in cellular immunity against bacteria via autophagy. Autophagy is a homeostatic fundamental cellular process, in which cytoplasmic cargos included in double-membrane structures called autophagosomes are transported to lysosomes. The autophagic process involves a series of autophagy proteins, among which Atg5 was recently linked to the IFN-γ-mediated parasiticidal effect against *T. gondii*. Myeloid-specific ablation of Atg5 in mice culminated in decreased recruitment of an IRG to *T. gondii* in IFN-γ-activated macrophages and led to high parasite susceptibility in vivo. Furthermore, other autophagy proteins such as Atg7 and Atg16L1 have recently been shown to play a nondegradative role in the IFN-γ-mediated anti-viral programs against murine norovirus, prompting us to explore the functions of autophagy proteins other than Atg5 in the IFN-γ-mediated anti-*T. gondii* cellular innate immune responses.

Here we have characterized the role of essential autohpagy proteins, Atg7, Atg16L1, Atg9a and Atg14 in IFN-γ-mediated inhibition of *T. gondii* proliferation using mouse embryonic fibroblasts (MEFs) lacking these proteins. MEFs lacking Atg7 or Atg16L1 exhibit defects in reduction of *T. gondii* by IFN-γ treatment and recruitment of an IRG Irgb6 and GBPs to the infected parasites. In sharp contrast, Atg9a- or Atg14-deficient cells show normal suppression of *T. gondii* growth and the accumulation of immunity-related GTPases comparable to wild-type cells in response to IFN-γ stimulation, indicating differential participation of autophagy regulators in the mouse system. IFN-γ stimulation leads to strong inhibition of *T. gondii* proliferation in human cells. However, the contribution of human immunity-related GTPases and autophagy proteins to the inhibition remains unknown. To analyze the role of ATG16L1 and GBPs in human cells, we have generated ATG16L1- or GBPs-deficient human cells using Cas9/CRISPR-induced genome editing. Although IFN-γ-induced inhibition of *T. gondii* proliferation are normal in human cells devoid of ATG16L1 or GBPs, IFN-γ-induced recruitment of GBPs to the parasites is defective in ATG16L1-deficient human cells. Thus, these results demonstrate that IFN-γ-mediated recruitment of IRGs and GBPs to *T. gondii* is differentially regulated by autophagy proteins in mice, and the mechanism for the ATG16L1-dependent GBP recruitment to the parasite may be conserved in humans.

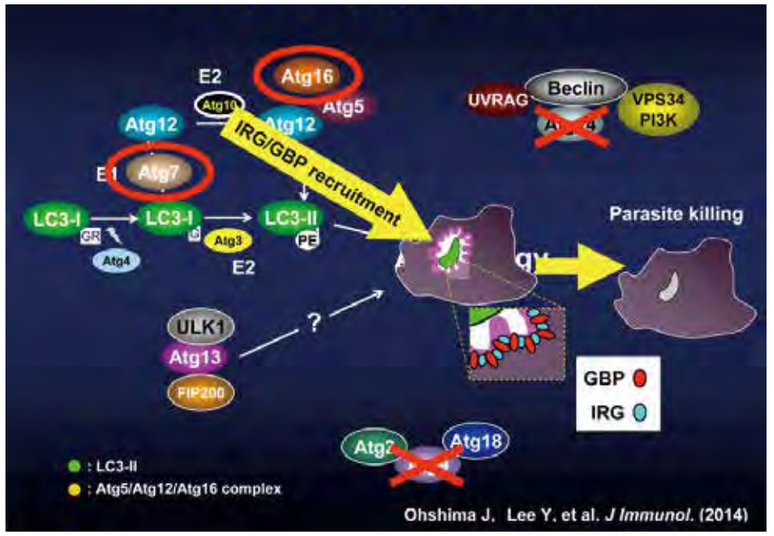


Figure. Autophagy proteins differentially participate in IFN-γ-induced anti-*T. gondii* cell-autonomous immunity

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Single Molecule Imaging

Toshio Yanagida, PhD

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	Tomoyuki Yamaguchi
	Yutaka Komai
Postdoctoral Fellow	**1**
Research Assistant	**1**
Guest Professor	**Takashi Jin**
	Tomonobu Watanabe

Theoretical and experimental studies of the mechanisms for self/non-self discrimination by Treg cells

$CD25^{+}CD4^{+}$ regulatory T cells (Treg) play a key role in the discrimination of self and non-self antigens. Previously we showed that T cells require co-signaling via CD28 or IL-2 for their proliferation and that Treg are able to inhibit the co-signaling using CTLA-4^{high} IL-2^{low} expression and predominant interactions to antigen-presenting cells (APC). More recently we used theoretical modeling and in silico simulations to assess whether this inhibitory mechanism is sufficient to suppress responses to self antigens. The results indicated that the dissociation of T cells from APC is regulated by specific Treg to discriminate self and non-self antigens in addition to inhibiting T cell-proliferation on shared APC. In vitro experiments showed that specific Treg actually upregulate the expression of adhesion molecules on APC with down-regulating co-stimulatory ligands . Interestingly the model also predicted that Treg are able to enhance the proliferation of conventional T cells (Tconv) in the presence of excessive co-signaling, which was confirmed in vitro. Thus, specific Treg inhibit both the dissociation and proliferation of T cells on APC for self-specific suppression. Therefore, the dissociation of T cells from APC might be a crucial step to induce appropriate the expansion of T cells .

Distinguishing the cell state using Raman spectroscopy

We used Raman spectroscopy, which can report changes in the cell state without labeling, as a non-invasive, single cell method. Significant differences in Raman spectra were observed at the cytosol and nucleus in different cell-lines from mouse, indicating that Raman spectra reflect differences in the cell state. Moreover, observations before and after the induction of differentiation in neuroblastoma and adipocytes demonstrated that Raman spectra can detect even subtle changes in the cell state. Differences in Raman spectra were also observed between T cells and B cells, indicating that Raman spectra can detect subtle changes between these two cell types (Figure. 1A). Details of the differences were made when Raman spectroscopy was coupled with principal component analysis (PCA), as different cell types were positioned in different regions of the PCA score plot (Figure. 1B). Thus, the present study strongly indicates that Raman spectral morphology, in combination with PCA, can be used to establish cellular fingerprints that distinguish and identify cellular states. We are now trying to visualize the transition of the T cell state after stimulation.

Chronic in vivo imaging in mouse organs using an implanted chamber

To enhance the fusion between imaging and immunology, more accessible procedures for optical microscopy are required. Two-photon microscopy is the only modality that can unveil cell dynamics in living animals, but it requires invasive surgical preparation which makes it difficult to use the same animal on different days, which is necessary when investigating disease development over time. We therefore have developed an implantable optical window and surgical procedure suitable for chronic in vivo imaging.

T cell transfer model mice, which were injected with T cells harvested from Fucci (Fluorescent Ubiquitination-based Cell Cycle Indicator) mice, were used to visualize T cell migration and proliferation during homeostatic proliferation and antigen stimulation. Fucci probes label individual nuclei in G1 phase nuclei red and nuclei in S/G2/M phases green. To image the same region on different day, the vasculature was visualized by sulfo-rhodamine B and fluorescein (Figure .2(c)). The half-life of both angiographic agents was about 10 min, which means they were available every hour for imaging. The implantable optical window system we developed is the only system that can observe chronically immunological events in lymph node follicles and spleen cortices for more than two weeks. We expect this system to be capable of observing any immunological event in both regions.

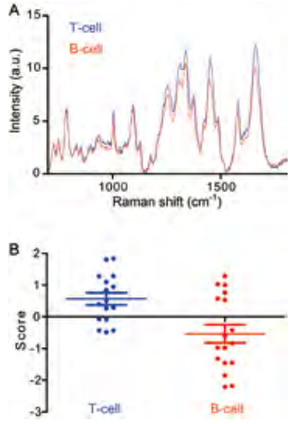

Figure 1.
(A) Raman spectrum of the fingerprint region from a T-cell (blue) and a B-cell (red).
(B) Score plot of DAPC results from the Raman spectrum.

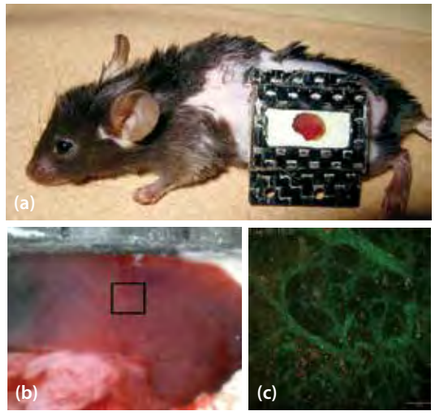

Figure 2.
(a) Spleen window in mouse.
(b) The spleen can be seen through the window. Superimposed box corresponds to the microscopic view.
(c) Images of angiography by sulfo-rhodamine B and fluorescein on days 14 and 15, respectively, after antigen stimulation. White bar corresponds to 100 μm.

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Biofunctional Imaging

Yoshichika Yoshioka, PhD

Professor	**Yoshichika Yoshioka**
Assistant Professor	**Masaki Fukunaga** **Yuki Mori** **Fuminori Sugihara**
Visiting Scientist	**2**
Support Staff	**2**

Our group has developed new highly sensitive and specific in vivo visualization techniques with magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) to unveil the functions of integrated immune system. We applied for two patents on MRI visualization techniques this year (on the MRI contrast probe: 2013-173996, on the diagnosis and treatment: 2014-066193). This MRI contrast probe is a magnetic substance of biological origin, biodegradable, and has a short half-life in animal bodies. The other MRI probe was also developed to sense pH using organic polymer (Okada et al., 2013).

MRI is widely employed for the diagnosis of immune diseases such as multiple sclerosis (MS: human autoimmune disease of the central nervous system). However, the size and number of the MRI lesions have had only modest correlations with neurological impairments in MS. Furthermore, such alterations reflect relatively advanced pathogenesis and not earlier symptoms like the breakdown of the blood–brain barrier (BBB) or edema accumulation. Auto reactive T cell accumulation in the fifth lumbar cord (L5) was recently indicated in experimental autoimmune encephalomyelitis (EAE) mice, an MS model. We investigated the event in EAE mouse precisely during the disease progression by using MRI at 11.7 T (Mori et al., 2014).

Figure 1 shows T_2-weighted MRI (T_2W MRI) of a lower spinal cord of the same EAE mouse during the disease progression. We found a marked increase of T_2W MRI signal intensities in L4 and L5 during the development of disease. At the same time, the sizes of L4 and L5 changed significantly. We investigated the entire spinal cord and found no detectable alterations in its higher levels or the brain even at the peak phase. These results show the localized occurrence of edematous inflammation in the lower spinal cord. This inflammation coincided with the increase of auto reactive T cell number in the region.

Diffusion-weighted MRI (DWI) shows the signal decrease in the dorsal marginal layers of L4 and L5 during the onset phase and further decrease in L3 and L4 during the peak phase, but recovered in L5, although the clinical scores remained high. During remission, the signal approached the pre-clinical level and returned there by late remission. These results suggest that changes in the DWI signal might precede clinical scores and, therefore, act as an effective marker for disease prognosis.

We found the spinal arteries (the black regions of spine in Fig. 1) also changed during the disease progression. Angiographic images of MRI (MRA) showed occluded and thickened vessels, particularly during the peak phase, followed by reperfusion in the remission phase (Figs. 1 & 2). Moreover, we showed that the demyelination regions of some MS patients, where inflammation occurs, had increased lactic acid content (Fig. 3), suggesting the presence of ischemic events.

We found that the bifurcation position of spinal arteries gradually shifted caudally with EAE development (Fig. 2). At the peak phase, a significant dislocation of over 2 mm between the preclinical and onset phases was observed. We hypothesize that enlargement of the lower spinal levels caused this shift by dragging the lumbar vessels to the lower side because there were some vessels that did not shift with the disease development.

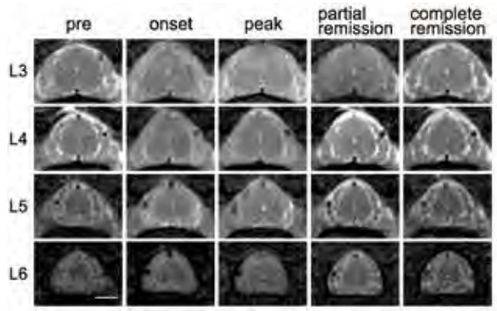

Figure 1. T_2W MRI of spinal cord (L3–L6) in the same EAE mouse at different stages of EAE. The spinal bodies at each stage are shown inside the dotted yellow polygons. Scale bar equals 500 μm. The top of each image shows the dorsal side.

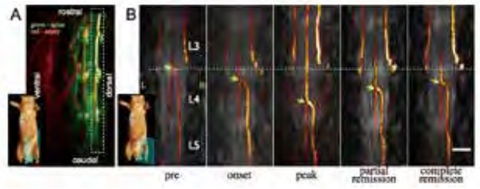

Figure 2. MRA of the dorsal spinal artery in an EAE mouse. (A) The sagittal plane of the vasculature in the lower lumbar cord. The region of interest is defined around the dorsal blood vessels by the dotted line. (B) Temporal alterations of the dorsal spinal artery in the coronal plane. The level of the spinal cord was registered using anatomical signals of the interspinal disks. A bifurcation in the spinal vessels is shown by the arrows. Dislocation of the bifurcation from the baseline (blue dotted line) was seen, and the lengths of the dislocation were correlated with the severity of the clinical scores. Scale bar equals 1 mm.

These results suggest that inflammation-mediated alterations in the lower lumbar cord change the homeostasis of the spinal cord and demonstrate that ultrahigh-field MRI enables the detection of previously invisible pathological alterations in EAE.

MRI and MRS are non-invasive and provide diverse information in vivo. The diverse information obtained by these techniques will enable direct visualization of the immune responses in order to clarify how the integrated and dynamic immune system actually works in the body and how immune cells behave under pathological conditions in vivo.

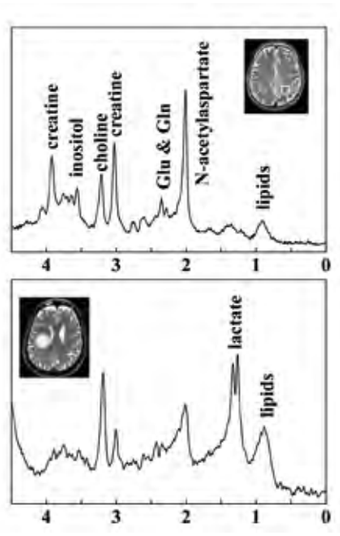

Figure 3. ^{1}H-MR spectra (TR/TE = 3000 ms/30 ms) at 3.0 T obtained from a healthy control (above) and a MS patient (below). Note the pronounced increases of choline and lactate in the MS patient compared with the control subject.

Recent Publications

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Immunology and Cell Biology

Masaru Ishii, MD/PhD

Professor	**Masaru Ishii**
Assistant Professor	**Junichi Kikuta** **Keizo Nishikawa** **Szandor Simmons**
Postdoctoral Fellow	**2**
Research Assistant	**2**
Visiting Scientist	**3**
Support Staff	**2**

The immune system is highly dynamic and a variety of cell types are migrating throughout the body and interacting with each other at specific sites with an appropriate time course. The mission of the lab is to understand the fundamental principle controlling cellular dynamics in this vivid small world inside our body, by means of our advanced imaging technology. The research activity of the lab is focusing on three specific topics, such as bone dynamics, immune and inflammatory systems, and cancer invasion, all of which are highly dynamic in vivo (see the figure). Investigations on immune systems have been so far based on the static histological and flow cytometry analyses and plausible 'cartoon' helped us to understand their dynamic nature in vivo, although the cartoons are sometimes incorrect. Recent advances in optical imaging technology have enabled us to shed light on the 'real' dynamic phenomenon that occurs inside the body. By using intravital 2-photon microscopy system, our lab has originally elaborated the system for visualizing the real cellular movements in various tissues and organs in their intact conditions.

In particular, our lab has pioneered a novel imaging technology for visualizing inside the bones, highly mineralized and the hardest tissues in the body, in their completely intact conditions. By using this technique, we have first elucidated the in vivo behaviors of osteoclasts, bone-destroying special macrophages resident in bones, 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 that sphingosine-1-phosphate (S1P) controls the migratory behavior of osteoclast precursors in concert with various chemokines (Nature 2009; J Exp Med 2010). S1P is preferentially enriched in blood plasma, and S1PR1, a cognate receptor for S1P, is expressed by bone marrow monocytoid-lineage cells, including osteoclast precursors (Nature 2009). Further examination demonstrated that osteoclast precursor monocytes express S1PR2, another cognate receptor for S1P, which counteracts the action of S1PR1 and negatively regulates migration toward S1P (J Exp Med 2010, J Immunol 2012). Intravital imaging of bone tissues dissected the finely tuned migratory behaviors of osteoclast precursors by these two reciprocally acting receptors, ensuring their correct positioning on the bone surface. We also showed the substantial contribution of S1P-mediated migratory control of bon cells by S1P by generating knockout mice deficient for endogenous S1P transporter (J Clin Invest 2012). Moreover, we have recently demonstrated that vitamin D, which is well-known as a bone-protecting factor, significantly blocks bone destruction by modulating S1P-mediated migration control of osteoclast precursor monocytes (PNAS 2013). Based on a series of studies, we proposed a new concept in which the migration and positioning of osteoclast precursor monocytes on the sites to be resorbed are critical points of action in the regulation of bone destruction.

By improving bone imaging techniques and using our originally developed reporter mouse strain in which a vacuolar type proton pump (a3 isoform of V-type H^+-ATPase) was specifically labeled with GFP, we recently succeeded in visualizing the function of fully differentiated osteoclasts adhering to bone surfaces in vivo (J Clin Invest 2013). This novel visualization identified two distinct mature osteoclast functional states; i.e., bone-resorbing (R) osteoclasts firmly adhering to bones and devouring the bone matrix by secreting acids, and non-resorbing (N) osteoclasts relatively loosely attached and wriggling along the bone surface. 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.

Chronic low-grade inflammation of adipose tissue plays a crucial role for the pathophysiology of obesity. Infiltration of several immune cells such as macrophages into adipose tissue was observed in obesity, although the initial factors triggering their migration have not been elucidated. Here, by using intravital multiphoton imaging technique, we analyzed the detailed time-courses of inflammatory processes in adipose tissues under high-fat and high-sucrose (HF/HS) diet. Mobility of macrophages was shown to be activated just 5 days after HF/HS diet, when the distinct hypertrophy of adipocytes and the accumulation of macrophages have not still become prominent. Significant increase of molecule A was detected in mature adipocyte fraction just 5 days after HF/HS diet. Recombinant molecule A stimulated chemotactic migration both in vitro and in vivo, as well as induced pro-inflammatory molecules both macrophage and adipocytes, such as TNF-a and CCL2. Finally, a neutralizing antibody targeting molecule A efficiently suppressed the HF/HS diet-induced initial inflammatory change, i.e., increased mobilization of adipose macrophages. In conclusion, time-lapse intravital multiphoton imaging of adipose tissues first identified the very early event exhibiting increased mobility of macrophages, which may be triggered by increased expression of molecule A and resultant to progression of chronic inflammation in situ.

Figure . Sheds lights on immune cell dynamics in vivo. Immune cells are high dynamic in the living body. We are elucidating the basic principle controlling the dynamic nature of immune cells by visualizing their in situ behaviors with advanced imaging techniques.

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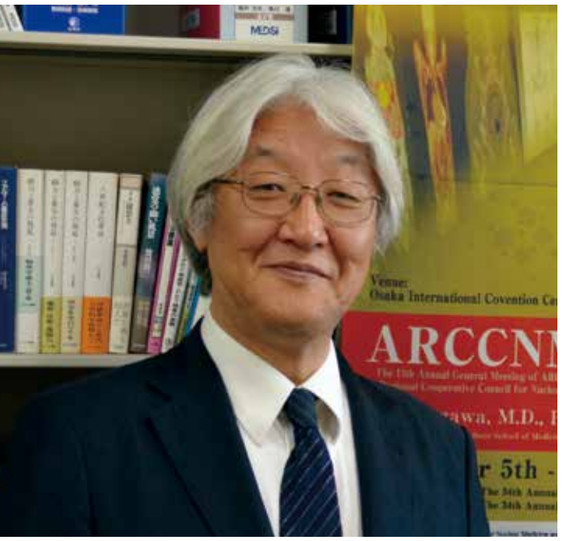
Nuclear Medicine



Jun Hatazawa, MD/PhD

Professor	**Jun Hatazawa**
Research Assistant	**1**
Support Staff	**3**

Nuclear medicine is a field of great potential which can evaluate the invivo dynamic imaging of the immune cells and molecules from small animals to humans.

The targets of nuclear medicine imaging are metabolic responses in inflammation, cancer, and immune related disorders by using specific tracers. To achieve these aims, we are developing a new methodology of in-vivo PET imaging, evaluation of patients receiving therapy with new metabolic based criteria, and new imaging modalities.

In addition to these researches, we began a twin study using ^{18}F-FDG PET in the 2013 fiscal year and are trying to evaluate genetic influence on glucose metabolism in various organs. From evaluation of diffusely increased ^{18}F-FDG uptake in thyroid gland, we guess that there are genetic influences on human immune function.

The principle of twin study

The twin study is an excellent method of estimating the extent of genetic and environmental influences on associations between multiple observed variables. The twin method is popular design in behavioral genetics and is also applicable to non-behavioral traits. Twins are divided into two major groups; monozygotic (MZ) twins and dizygotic (DZ) twins. The twin study is based on the assumption that MZ twins share all of their additive genes and DZ twins share on average half of their additive genes. In addition, we could expect MZ and DZ twin pairs to share on the whole their growth environment before and after birth to the same extent. So observed differences in the phenotype of interest between MZ twins of a pair must be due to non-genetic factors because MZ twins are genetically identical. The addition of DZ twins allows us to separate genetic from shared environmental factors. By comparing similarities in MZ twins with those in DZ twins, we can estimate the extent of genetic and environmental influences. If genetic influences are strong, similarities in MZ twins tend to be greater than those in DZ twins. However, when environmental influences are strong, the similarities in MZ twins tend to be the same as those in DZ twins, or the similarities in both MZ and DZ twins tend to be lower.

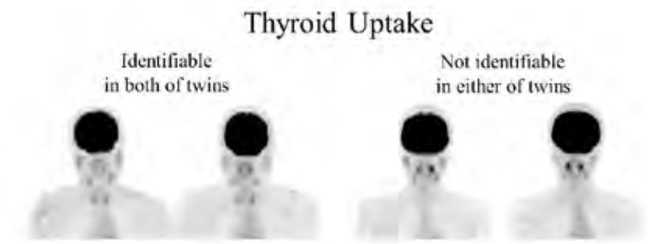
Our twin study was performed as a part of twin research at Osaka University. The twin registry of our research is composed of asymptomatic aged twin pairs, mainly over 60 years.

Genetic influence on diffusely increased ^{18}F-FDG uptake in thyroid gland in asymptomatic subjects

^{18}F-FDG accumulation in normal thyroid gland is usually not visualized in PET study. Incidence of diffuse increase in ^{18}F-FDG uptake has been reported to be 0.6%-6.6%. Diffuse thyroid uptake may be a normal variant due to genetic origin, while several reports suggested that diffuse thyroid uptake is associated with chronic thyroiditis.

We have so far evaluated thirty-eight pairs of volunteer twins (31 monozygotic (MZ) and 7 dizygotic (DZ) pairs, age ranging from 42 to 84 years, mean age 67.1 years). All of them underwent whole-body ^{18}F-FDG PET and laboratory tests on thyroid functions and serum anti-thyroid peroxidase (TPO) antibody, a marker of chronic inflammation. Diffuse thyroid uptake was defined as the presence of ^{18}F-FDG uptake in the whole thyroid gland greater than cervical background activity. As the result, Diffuse thyroid uptake was identified in both sides of the twins in 5 of 31 (16%) MZ pairs but none of DZ pairs (0%), and in either side of the twins in 6 of 31 (19%) MZ pairs and 2 of 7 (29%) DZ pairs. Among subjects with identifiable ^{18}F-FDG uptake (n=18), 13 subjects had euthyroid status. Four subjects had positive, but 14 subjects had negative anti-TPO antibodies. We found no apparent relationship between diffuse thyroid uptake and anti-TPO antibody levels or thyroid function.

Considering the higher concordance rate in MZ than in DZ pairs, diffusely increased ^{18}F-FDG uptake in thyroid gland in asymptomatic subjects is considered to be largely influenced by genetic rather than acquired factors. (In other words, our immune function might be influenced by not only environmental factors but also genetic factors.) We are planning further analysis.



	Identifiable in both of twins	Identifiable only in twins' one side
MZ (n=11)	5	6
DZ (n=2)	0	2

Recent Publications

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Chemical Imaging Techniques

Kazuya Kikuchi, PhD

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	Toshiyuki Kowada
	Yuko Kamikawa
Support Staff	**1**

Fluorogenic Probes for Quick No-Wash Live-Cell Imaging of Intracellular Proteins

Protein labeling by synthetic fluorescence probes is a powerful technique used to investigate protein function and localization in living cells. In this technique, objective proteins are fused with a protein (peptide) tag and are labeled by a fluorescent probe through the binding of the probe and tag. This method has attracted attention as an alternative to fluorescent proteins because it has the advantage of various bright fluorophores that can be incorporated into probes. In addition, the timing of the labeling can be easily controlled, allowing for precise spatiotemporal analyses of protein movement. On the other hand, unlabeled probes remaining inside cells have the potential to cause an increase in the background fluorescence and hinder the identification of labeled proteins. Therefore, rigorous washing of cells is required to remove free probes.

We developed novel fluorogenic probes for no-wash live-cell imaging of proteins fused to PYP-tag (Figure 1). Through the design of a new PYP-tag ligand, specific intracellular protein labeling with rapid kinetics and fluorogenic response was accomplished. The probes crossed the cell membrane, and cytosolic and nuclear localizations of PYP-tagged proteins without cell washing were visualized within a 6-min reaction time. The fluorogenic response was due to the environmental effect of fluorophore upon binding to PYP-tag. Furthermore, the PYP-tag-based method was applied to the imaging of methyl-CpG-binding domain localization. This rapid protein-labeling system combined with the small protein tag and designed fluorogenic probes offers a powerful method to study the localization, movement, and function of cellular proteins.

Multifunctional Core–Shell Silica Nanoparticles for Highly Sensitive ^{19}F MRI

^{19}F magnetic resonance imaging (MRI) is an especially powerful method for in vivo imaging of particular biomolecules, cells, and target tissues, because of negligible background signals. In ^{19}F MRI, fluorinated agents need to be delivered with high density per voxel to give signals of sufficient intensity. Therefore, highly sensitive ^{19}F MRI contrast agents are in great demand for their practical applications. However, we have faced the following challenges: 1) increasing the number of fluorine atoms decreases the solubility of the molecular probes, and 2) the restriction of the molecular mobility attenuates the 19F MRI signals.

To solve these issues, we developed a novel multifunctional ^{19}F MRI contrast agent, fluorine accumulated silica nanoparticle for MRI contrast enhancement (FLAME, Figure 2). FLAME is composed of a core micelle filled with liquid perfluorocarbon and a robust silica shell, and has superior properties such as high sensitivity, surface modifiability, biocompatibility, and sufficient in vivo stability. By modifying FLAME surface with ampicillin, gene expression of BL-tag as a reporter protein in living cells was successfully detected using ^{19}F MRI. Finally, the potential of FLAME for in vivo targeting was evaluated in tumor-bearing mice. FLAME was modified with polyethylene glycol (PEG) for effective delivery to tumors, because PEGylation reduces the uptake by increasing the electrostatic interactions with proteins and small molecules. As a result, strong ^{19}F MRI signals of FLAME-PEG were observed from the tumor site, whereas the ^{19}F MRI signals of non-PEGylated FLAME-COOH were detected only in the liver. These results demonstrate that FLAME can be a useful ^{19}F MRI contrast agent to overcome two major limitations of current ^{19}F MRI probes, that is, an impractical modifiability of the surface of nanoemulsions and a low sensitivity of small molecule-based probes.

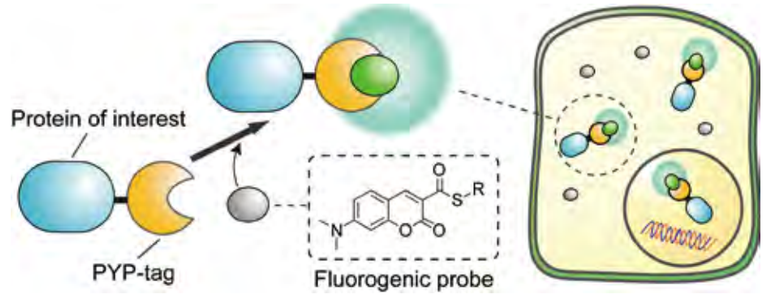

Figure 1. Principle of no-wash labeling system based on PYP-tag.

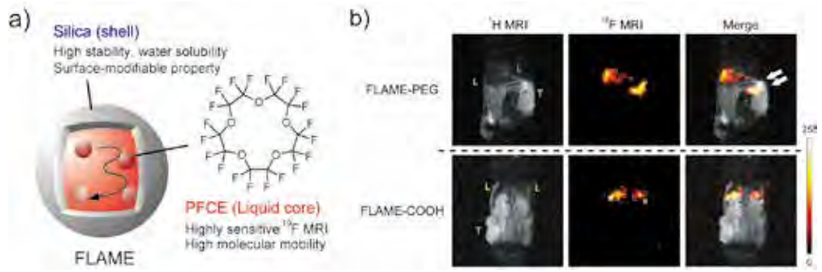

Figure 2. (a) Design and (b) in vivo tumor accumulation of FLAME.

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Biophotonics

Nicholas Isaac Smith, PhD

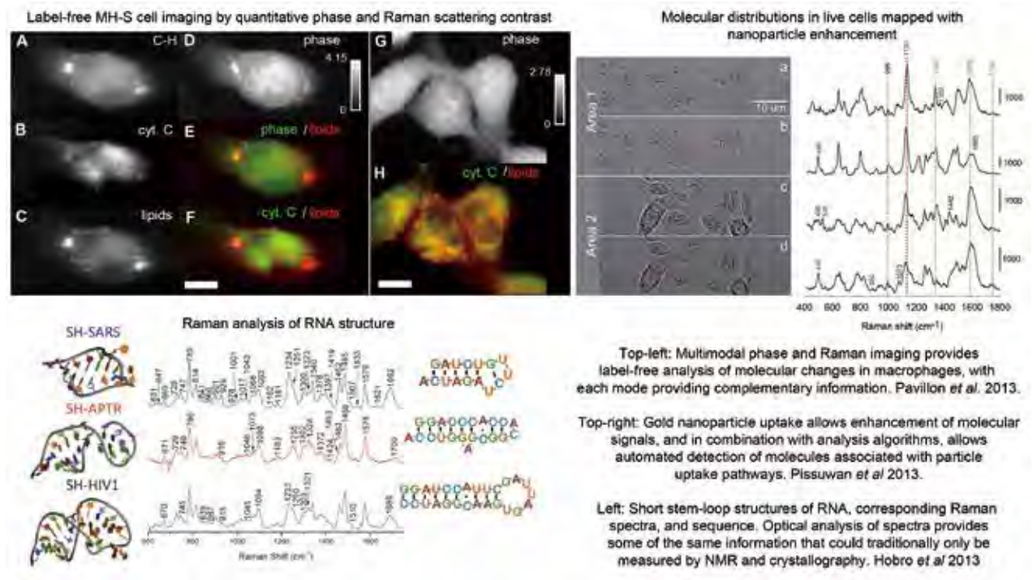
Associate Professor	**Nicholas Isaac Smith**
Postdoctoral Fellow	**3**

Raman spectroscopic imaging uses laser based imaging to determine the molecular composition of a sample and how the distributions of molecules change over time. Using only the physical scattering properties of the light beam, and by resonance with the endogenous molecules in a living cell or tissue sample, it is possible to apply Raman imaging to image molecular dynamics in living cells (Okada et al 2012). Without using labels, the technical aspects of imaging are challenging, but by collecting images based on the entire cellular ensemble of molecules and decoding the resulting spectra, we can see cell components at ~500 nm resolution, and with chemical information not available by other means. In the Biophotonics laboratory, we are particularly interested in how the application of such label-free techniques can reveal new information on the immune response, either in terms of new chemical components appearing, in new ways that cell component distributions change, or in how cell morphology changes during the response.

One challenge with Raman is that the imaging rate is limited by the scattering probability of the incident light, and it has been challenging to extract more raw signal from the cell samples. We then merged two different imaging modes, both label-free that can provide complementary information on the cell response (Pavillon et al 2014, Pavillon et al 2013, Pavillon and Smith patent submitted 2013). The additional mode uses coherent light and both imaging systems are spectrally isolated. This provides rapid and quantitative phase date, at speeds higher than what is achievable with spontaneous Raman imaging. The phase mode provides quantitative spatial data, with high time resolution and the Raman mode provides chemical specificity. Merging these two modes we get a multidimensional set of data which provides a previously unseen view of how macrophages respond to stimulation by LPS and other pathways. The phase imaging pathway gives higher sensitivity to proteins compared to Raman, and we found that the combination of these provides complementary imaging data. Since Raman spectra have hundreds or thousands of wavenumber channels, we can in theory separately map the distributions of a wide range of molecules in the cell from one measurement. Practically, if the sensitivity of all molecules is not equal, having an additional mode with higher sensitivity allows us to unravel some of the mixing of molecular distributions in the cells. This is important to determine which molecules are involved with, or changing during the immune response.

Using local enhancement from inert gold nanoparticles which are readily uptaken by macrophages, the sensitivity of Raman analysis can reach down to single molecule detection levels. This represents a type of nano-endoscopy where the trajectory and local environment can be probed during uptake or later cellular dynamics such as active transport (Ando et al 2011). We developed an automated process for detecting, classifying, and clustering signals from macrophage particle uptake (Pavillon et al 2013). With a robust system for treating large datasets, we were then able to look at how different types of particle surface charges modified the immune response (Pissuwan et al 2013), and charge effects on uptake, sequestration and local enhancement in the macrophage (Pissuwan et al 2014). With collaborators in the Dept. Applied Physics, we are also looking at multidimensional tracking of nanoparticles to try and understand how different proteins are involved in transport (Huang et al 2014).

Following the increasing interest of mRNA in the ensemble of cellular signaling molecules, we also created a method for quantitative analysis of the contributions of RNA bases to the Raman signature and thereby produce a metric for separating composition and structural features of the RNA (Hobro et al 2013). This has particularly good sensitivity to changes in RNA structure and can be used to study applications in Raman analysis of RNA. We additionally used the highly sensitive nature of spectroscopic detection of heme-based proteins, which resonate with the incoming laser field to develop a method for early malaria diagnosis (Hobro et al 2013), and are now looking for ways to expand those methods into other clinical applications.



Top-left: Multimodal phase and Raman imaging provides label-free analysis of molecular changes in macrophages, with each mode providing complementary information. Pavillon *et al.* 2013.

Top-right: Gold nanoparticle uptake allows enhancement of molecular signals, and in combination with analysis algorithms, allows automated detection of molecules associated with particle uptake pathways. Pissuwan *et al* 2013.

Left: Short stem-loop structures of RNA, corresponding Raman spectra, and sequence. Optical analysis of spectra provides some of the same information that could traditionally only be measured by NMR and crystallography. Hobro *et al* 2013

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Immune Response Dynamics

Kazuhiro Suzuki, MD/PhD

Our body consists of multiple organ systems which mutually communicate to coordinate responses to external stimuli and maintain homeostasis of internal environments. Thus, to understand biological events occurring in complex organ systems in our body, it is important to reveal the interconnection among multiple organ systems rather than focus on an isolated organ system. Keeping this notion in mind, we are investigating the communication between the nervous and immune systems. It has long been proposed that some aspects of immune responses were affected by activities of the nervous system. Indeed, the autonomic nervous system was shown to modulate the pathology of immune disorders, including rheumatoid arthritis and multiple sclerosis (Bellinger, *et al. Cell. Immunol.* 252: 27, 2008). Lymphoid organs are innervated by neurons releasing a variety of neurotransmitters, and immune cells express corresponding neurotransmitter receptors, of which stimulation affects a broad range of immune cell activities, including proliferation and cytokine production (Tracey. *Annu. Rev. Immunol.* 30: 313, 2012). However, the cellular and molecular basis by which the inputs from the nervous system are converted to the outputs from the immune system is still largely unclear.

Precise trafficking and positioning of immune cells are essential for homeostasis of the immune system and induction of immune responses, most part of which is orchestrated by a family of G protein-coupled receptors (GPCRs) that respond to chemoattractive molecules represented by chemokines. As well as chemokine receptors, many of the neurotransmitter receptors are also GPCRs. A recent study showed that different types of GPCRs form heteromeric complexes on the cell surface and cross-regulate their signaling (Fribourg, *et al. Cell* 147: 1011, 2011). This observation prompted us to investigate the relationship between neurotransmitter and chemokine receptors. We found that a group of neurotransmitter receptors expressed in lymphocytes form complexes with specific chemokine receptors on the cell surface. Stimulation of the neurotransmitter receptors in lymphocytes modulated the responsiveness of the associated chemokine receptors, which had a great impact on lymphocyte recirculation through lymphoid organs in mice. Moreover, alteration of lymphocyte dynamics by the neurotransmitters affected the disease course of mouse models of autoimmunity.

Associate Professor	**Kazuhiro Suzuki**
Research Assistant	**1**
Support Staff	**3**

In this study, we identified a lymphocyte-intrinsic mechanism by which neuronal inputs control migratory behaviors of lymphocytes and revealed a novel layer of immune regulation by the nervous system. However, it remains unclear how neurons interact with lymphocytes and control their trafficking in vivo. To solve this issue, we are setting up an experimental system to visualize the interface between the nervous and immune systems in a single frame by in vivo imaging using two-photon microscopy. This would help capture a real picture of the communication between the two organ systems. Another direction of our study is to clarify molecular mechanisms of the crosstalk between neurotransmitter and chemokine receptors. We have identified candidate molecules that could be involved in the crosstalk between the two classes of receptors. Functional analysis of these molecules would further promote our understanding of the molecular basis for neural regulation of immunity.

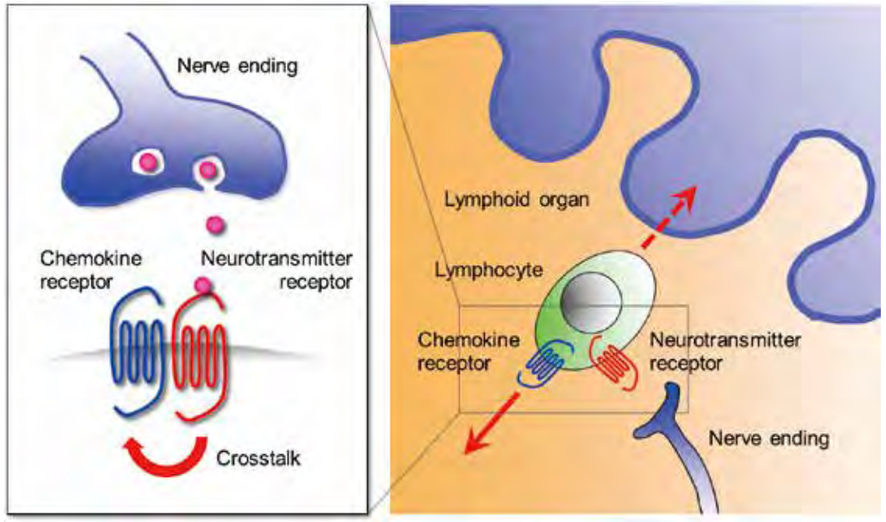

Figure. Control of lymphocyte dynamics by neuronal inputs. The crosstalk between the neurotransmitter and chemokine receptors regulates lymphocyte trafficking among lymphoid organs.

Recent Publications

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Information systems

Yutaka Hata, PhD

Professor	**Yutaka Hata**
Associate Professor	**Syoji Kobashi**
	Kei Kuramoto
Assistant Professor	**Manabu Nii**
Support Staff	**1**

We did the following studies in Information Systems.

Ultrasonic Imaging

1. Image Registration :

We proposed ultrasonic image registration for multi-frequency analysis (Figure 1). The goal of our research is the portable and real time brain diagnosis under the thick-skull. The choice of ultrasonic frequency is a trade-off between spatial image resolution and imaging depth. This study shows the usability of data synthesis by employing two different frequency ultrasounds. In the first part of this study, using Fast Fourier Transform, we conclude that the synthesized image was produced from two ultrasonic images of individual objects. The purpose of the second approach of the data synthesis is to investigate three methods of ultrasonic imaging. This approach is of particular interest for the design of further study intending to visualize any defects by ultrasonic methods. As for the results, the synthesized image with Wavelet transform has higher efficiency than the other synthesized images for the bone and the sulcus. In summary, this study indicates that ultrasonic synthesized imaging is useful to visualize the imitated brain area. This observation is encouraging for further studies of evaluating the brain in patients.

2. Ultrasonic Average Difference Imaging:

We proposed fuzzy average difference imaging for ultrasonic nondestructive testing. In our experiment, we employed a piece of wind turbine blade as a specimen. The specimen has holes on the rear side as artificial damage. We acquired ultrasonic waveforms from scanning lines on the surface of the specimen using an ultrasonic single probe. We made cross-sectional images of the specimen by correcting each scanning line wave data. We set scanning lines so that specimen constructions under the lines are the same as each other. Therefore the images show the same construction of inside the specimen, we can enhance the damage echoes by using average difference imaging. To extract the damage echoes from the images, we applied a damage extraction method aided by fuzzy logic and average difference imaging. As a result, we found the line image with all damage portions, and we estimated the depth of damage surface with high accuracy. Therefore fuzzy average difference imaging showed effectiveness for extracting difference portions on similar images.

Magnetic Resonance image processing

Hypoxic-ischemic encephalopathy is a typical cerebral disorder of newborn babies. For diagnosis of neonatal cerebral disorders, the measurement of cerebral volume and surface area is effective for quantitatively evaluating the morphological change. The measurement requires a brain segmentation process in magnetic resonance (MR) images. However, there are few studies for newborn brain segmentation. This study proposes an automated brain segmentation method for the newborn brain in MR images. The automated method can improve the diagnosis of the newborn cerebral disorders with efficiency and high accuracy. The proposed method first constructs fuzzy models using learning dataset (Figure 2). The fuzzy models express brain features by fuzzy membership functions. Next, the proposed method applies the deformable surface model based on the fuzzy models to the subject's head MR images, and estimates the subject's brain region. To validate the proposed method, it has been applied to 10 newborn subjects (revised ages are –1month and 1 month), and compare the segmentation result with those of the conventional methods. Leave-one-out-cross validation test was conducted. The mean accuracy was 93.6 ± 3.7%, the mean sensitivity was 98.9±0.5, and the mean G-metrics was 4.4 ± 0.8.

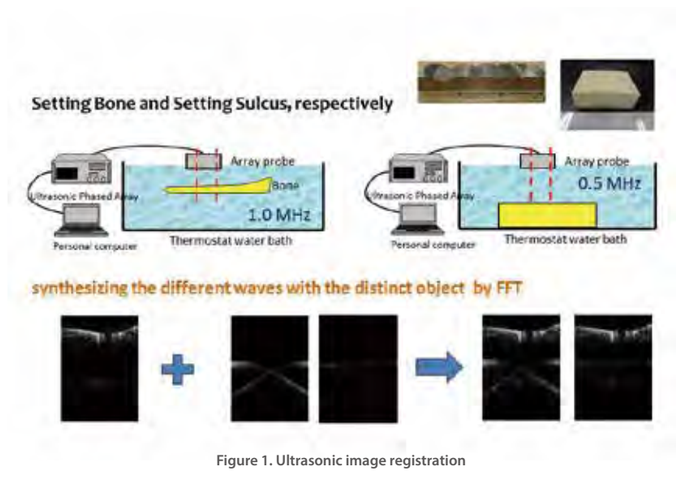

Figure 1. Ultrasonic image registration

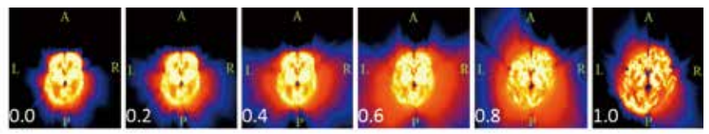

Figure 2. Growing fuzzy anatomical model of the neonatal brain.

Recent Publications

- Yagi N, Oshiro Y, Ishikawa T, Hata Y. YURAGI synthesis for ultrasonic human brain imaging. ***JACIII*** 17:74-82, 2013.
- Kobashi S, Kuramoto K, Wakata Y, Ando K, Ishikura R, Ishikawa T, Hirota S, Hata Y. Newborn brain MR Image segmentation using deformable surface model based on fuzzy knowledge models. ***IC-MED*** 5:115-24, 2013.
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- Oe K, Miwa M, Nagamune K, Sakai Y, Lee S. Y, Niikura T, Iwakura T, Hasegawa T, Shibanuma N, Hata Y, Kuroda R, Kurosaka M. Nondestructive evaluation of cell numbers in bone marrow stromal cells/beta-tricalcium phospate composites using ultrasound. ***Tissue Engineering, Part C: Methods*** 16:347-53, 2010.

Systems Immunology

Daron M. Standley, PhD

Associate Professor	**Daron M. Standley**
Associate Professor	**Kazutaka Katoh**
Assistant Professor	**Shide Liang**
	Alexis Vandenbon
Postdoctoral Fellow	**5**
Research Assistant	**2**
Support Staff	**1**

Our Laboratory uses computational methods to address biological questions that cannot easily be elucidated by experiment. We collaborate closely with a number of experimental groups and also develop software tools for general use. Some projects we are actively working on are described below.

Structures of protein-RNA complexes

Recent studies have revealed that control of gene expression at the mRNA level is essential for proper immune function. RNA-binding proteins (RBPs) have been shown to be important for controlling the stability of mRNAs coding for a number of essential proteins (e.g., Uehata, 2013). In order to reveal the mechanism by which RBPs recognize their RNA targets, we are developing in silico methods for predicting the structures of protein-RNA complexes. A key step in our approach is predicting RNA binding sites on the molecular surface of a protein structure of interest. To this end, we have developed a web-based tool called aaRNA, which out-performs established methods in three published benchmarks (Figure 1).

Antibody modeling and engineering

Antibodies are members of one of the largest protein superfamilies, and are currently the fastest growing class of therapeutic agents. To facilitate engineering of customized antibodies, we have developed a pipeline called Kotai Antibody Builder (http://kotaiab.org/). In a recent blind test of antibody structural modeling methods (AMA-II), our joint team with Astellas Pharma and the Institute for Protein Research (Shirai, 2014) succeeded in submitting the models of with the average lowest error, both in terms of whole variable region and the CDR-H3 loop (Figure 2).

Structures of TLR signaling complexes

TLR signaling is mediated by adaptor molecules that share a Toll/Interleukin-1 receptor (TIR) domain. A number of previous studies have proposed pairwise interactions between these TIR domain-containing proteins based on mutagenesis data; however, many of these models remain controversial. Using a novel flexible docking procedure, we constructed an atomic-resolution structure of the trimeric complex formed by one TRIF and two TRAM TIR domains based on mutagenesis and yeast two-hybrid (Y2H) data (Enokizono, 2013). The resulting complex helps explain how the TIR domain-mediated signal propagates.

Mechanism of STIM1 calcium sensing

The protein STIM1 is a key regulator of Store-operated Ca2+ entry (SOCE). Several studies in mice have indicated that SOCE is physiologically important in mast cells, T cells, B cells, and skeletal muscles. Interestingly, STIM1 responds cooperatively to Ca^{2+} depletion in the ER, resulting in calcium influx at precise Ca^{2+} concentrations. We dissected the mechanism of STIM1-mediated calcium signaling and found that loss of calcium promotes unfolding and dimerization in a cooperative manner. In vitro experiments and mathematical modeling could recapitulate the dissociation constant, free energy, Hill coefficient, and critical Ca^{2+} concentration of the in vivo reaction, supporting the proposed mechanism (Furukawa, 2014).

Multiple sequence alignment

Multiple sequence alignment (MSA) is at the core of many bioinformatics methods, including structural modeling, evolutionary analysis and function prediction. It is well established that structural information can be used to improve the accuracy protein MSAs; however, the optimal integration of sequence and structural information is far from trivial. Our approach to solving this problem (Katoh, 2013), which we call MAFFTash (http://sysimm.ifrec.osaka-u.ac.jp/MAFFTash/), compares very favorably with two established methods (Figure 3).

Macromolecular Dynamics

We use molecular dynamics (MD) to study proteins and protein complexes. Because we can observe the effects of ligand binding, point mutations, changes in temperature, etc. at high temporal and spatial resolution, MD is an important tool for understanding biomolecular function.

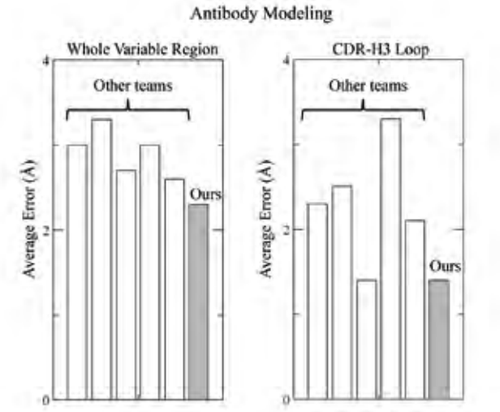

Figure 2. EOur performance in a recent antibody modeling contest.

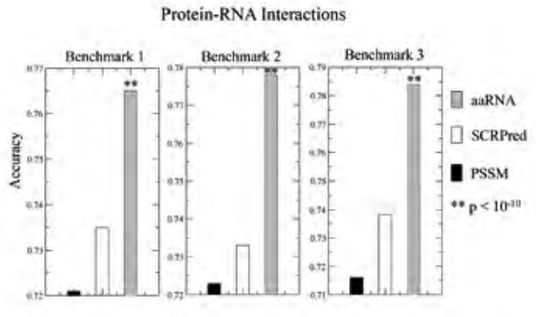

Figure 1. A comparison of aaRNA with two state-of-the-art methods for predicting protein-RNA interactions.

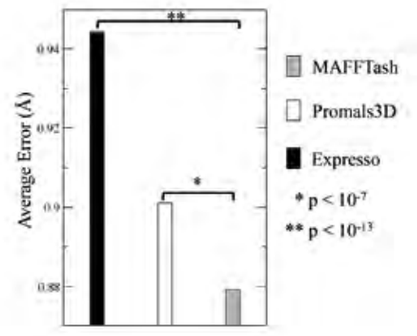

Figure 3. A comparison of MAFFTash and two state-of-the-art multiple sequence alignment methods.

Recent Publications

- Furukawa Y, Teraguchi S, Ikegami T, Dagliyan O, Jin L, Hall D, Dokholyan NV, Namba K, Akira S, Kurosaki T, Baba Y, Standley DM. Intrinsic Disorder Mediates Cooperative Signal Transduction in STIM1. ***J. Mol. Biol.*** 15:2082-97, 2014.
- Uehata T, Iwasaki H, Vandenbon A, Matsushita K, Hernandez-Cuellar E, Kuniyoshi K, Satoh T, Mino T, Suzuki Y, Standley DM, Tsujimura T, Rakugi H, Isaka Y, Takeuchi O, Akira S. Malt1-induced cleavage of regnase-1 in CD4(+) helper T cells regulates immune activation. ***Cell*** 153:1036-49, 2013.
- Shirai H, Ikeda K, Yamashita K, Tsuchiya Y, Mizuguchi K, Higo J - i, Standley DM, Nakamura H. High-resolution structural model building of antibodies by combination of bioinformatics, expert knowledge and molecular simulations. ***Proteins*** doi:10.1002/prot.24591, 2014.
- Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. ***Mol. Biol. Evol.*** 30:772-80, 2013.
- Enokizono Y, Kumeta H, Funami K, Horiuchi M, Sarmiento J, Yamashita K, Standley DM, Matsumoto M, Seya T, Inagaki F. Structures and interface mapping of the TIR domain-containing adaptor molecules involved in interferon signaling. ***Proc. Natl. Acad. Sci. USA.*** 110:19908-13, 2013.

Quantitative Immunology Research

Assistant Professor	**Diego Diez**
	Yutaro Kumagai
	Shunsuke Teraguchi
Postdoctoral Fellow	**1**
Research Assistant	**1**

MINING BIOLOGICAL NETWORKS

The immune system is a complex dynamical network of interconnected, hierarchically organized tissues, cells and molecules, that prevents infection by pathogens and maintains homeostasis. Understanding the whole dynamics of the immune system is critical for the proper development of preventive and curative therapies against diseases. This requires the integration of information at different levels (e.g. tissue, cell and molecule), over time, in a quantitative fashion, and their abstraction into mathematical models that enables predicting future behavior.

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 analyze the immune system through the mining of biological networks, by using 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 a mathematical framework to understand the dynamics of this massive data. These approaches are combined in several projects that aim to gain insight into specific problems 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 will be combined with multiple fluorescent protein knock-in cells to monitor genes induced upon antiviral responses such as IL-6, IL-10.

In spite of the importance of receptor molecule dynamics such as dimerization and clustering with downstream molecules, this process is still poorly understood. 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 dynamics of single immune molecules. We have succeeded in monitoring TLRs and their adaptors, and hope this highly quantitative technique can be used to describe detailed dynamics, possibly leading to rational drug design.

DATA INTEGRATION

The development of high-throughput technologies has brought biology into the *omics* era and the need for "big data" approaches that integrate, summarize and extract the relevant information, while preserving the critical relation between biological components. We make heavy use of experimental approaches to measure transcriptome, cistrome (transcription factor binding locations) and proteome levels over time, and integrate this information with protein-protein interaction data, which allow us to obtain insight into signaling and gene regulatory networks.

For example, we have combined several *omics* datasets to gain insight into hematopoietic differentiation. One of the most important questions in immunology is how immune cells differentiate from the original hematopoietic progenitors into cells capable of eliciting appropriate responses. This process is partly controlled by transcription factors (TFs) forming complexes known as transcriptional regulatory modules (TRMs) that regulate transcriptional programs. We have developed a method called rTRM to identify TRMs from PPI networks (Diez et al. 2014), and applied it to publicly available transcriptional profiles obtained for diverse immune cells (i.e. B-, T-, NK-cells, dendritic cells, monocytes and macrophages) during hematopoietic differentiation. We identified TRMs defining cell type specificity, and predicted transcription factors with critical roles during differentiation of hematopoietic cells.

MATHEMATICAL MODELING

In many other disciplines in science, theoretical framework, typically represented in terms of mathematical language, plays a crucial role. However, advances in theoretical immunology have been hampered by the lack of comprehensive accurate measurements of biological phenomena. The quantification of immunological responses and the integration of massive data open the door to construct such theoretical models in immunology. For this purpose, we are developing novel mathematical frameworks for the description of the immune system.

For example, in one of our collaborative researches, we obtained quantitative data of the cooperative STIM1 dimerization during signal transduction upon activation by decrease in Ca^{2+} concentration. We found that cooperative behavior cannot be described by the conventional biochemical equations due to the disorder nature of the protein, and hence, a suitable theoretical framework was needed for understanding the phenomena. We successfully developed a statistical mechanics-inspired formulation. The model quantitatively agreed with experimental data and enabled us to narrow down the dimerization site of STIM1, which was reinforced by subsequent experiments (Furukawa et al. 2014).

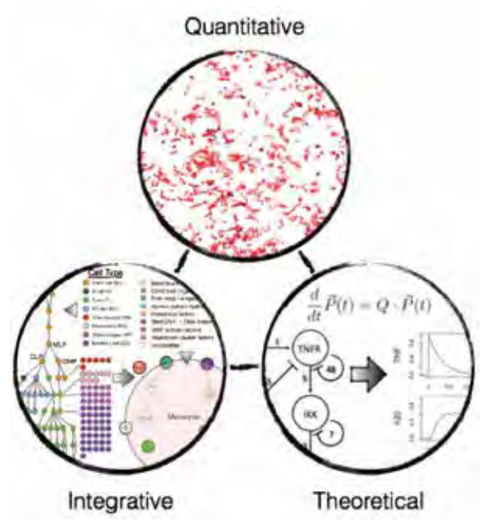

Figure 1. The Quantitative Immunology Research Unit combines quantitative, integrative and theoretical approaches.

We have also developed a mathematical framework (Stochastic Binary Modeling, SBM) to circumvent the necessity of biochemical determination of many parameters, which is a common obstacle for constructing a dynamical model of cellular signaling (Teraguchi et al. 2011). SBM also allows us to represent the stochastic and heterogeneous nature of cell populations. Currently, we are developing a system to automatically identify the structure and parameters of the network of regulatory pathways with the help of data assimilation techniques. We expect that this new system will become a useful tool for mining the immune system's networks.

Recent Publications

- Furukawa Y, Teraguchi S, Ikegami T, Dagliyan O, Jin L, Hall D, Dokholyan NV, Namba K, Akira S, Kurosaki T, Baba Y, Standley DM. Intrinsic Disorder Mediates Cooperative Signal Transduction in STIM1. ***J. Mol. Biol***. 15:2082-97, 2014.
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Next Generation Optical Immune-imaging

Noriko Takegahara, MD/PhD

This unit started on November 1, 2013. Our aim is to understand the complex dynamic mechanisms of cell-cell fusion with novel imaging technologies.

Myeloid cells such as macrophages have a pronounced potential to fuse not only among themselves (homotypic fusion) but also with other cell types (heterotypic fusion). Homotypic cell-cell fusion leads to formation of multinucleated osteoclasts and/or multinucleated giant macrophages. The role of this type of fusion is thought to enhance cellular functions. For example, osteoclasts are specialized multinucleated giant macrophages which resorb bone. Multinucleation allows osteoclasts to resorb larger areas, and mutant osteoclasts, which are not able to fuse, exhibit impaired bone resorption activity. On the other hand, some macrophages have a potential to fuse with malignant tumor cells (heterotypic fusion). In this case, fusion is thought to promote chemo-resistance or metastatic potential of these cells. Macrophages seem to have distinct fusogenic properties which lead to physiological or pathological output. We hope to make advances in understanding the mechanisms and molecules involved in cell-cell fusion. We are mainly focusing on homotypic cell-cell fusion such as osteoclasts and multinucleated giant macrophages.

An approach to find fusion competent cells

Once myeloid precursors receive signals of differentiation factor (RANKL for osteoclasts, IL-4 for multinucleated giant macrophages), they commit to be pre-osteoclasts or pre-giant macrophages and fuse to become mature multinucleated cells (Figure 1). Myeloid precursors need to pass multiple steps to become mature multinucleated cells and one of the steps is to become fusion competent cells. Although it has been reported that cell cycle arrest in osteoclast precursors is important for their differentiation into multinucleated osteoclasts, the biology of cell-cell fusion, especially, how cells commit to become fusion competent status and what molecules are actual fusogens remains to be determined.

To address fusion competent cells, we will focus on the close relationship of cell development to cell cycle. Fucci is a powerful technology developed by Dr. Atsushi Miyawaki to study coordination of cell cycle and cell development. Using Fucci, we can investigate how cell cycle regulates the setting of appropriate timing of generation of different cell types. We apply Fucci technology to find fusion competent cells in osteoclasts- and multinucleated giant macrophage-precursors (Figure 2). We are also trying to identify fusogens which regulate cell-cell fusion.

A challenge of development of a novel imaging technology

Cell-cell fusion is a dynamic process. Various types of molecules change their expression levels and their cellular localization during the process. Recent advances in optical imaging technologies allow us to detect qualitative changes over time. However it is still hard to detect quantitative changes over time. In collaboration with Nikon Instruments Company, we would like to develop a novel technology to quantify changes of molecular levels in in vitro and in vivo images.

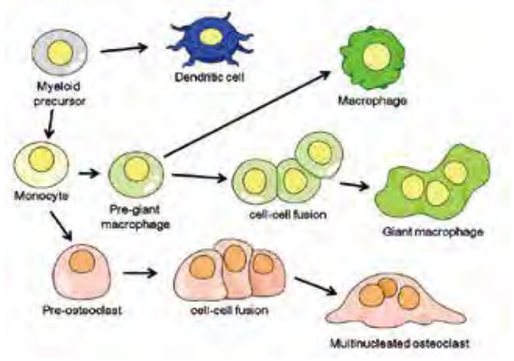

Figure 1. Schematic summary of myeloid cell fusion. Common myeloid lineage precursors commit to becoming fusion competent status and then fuse to become mature multinucleated cells.

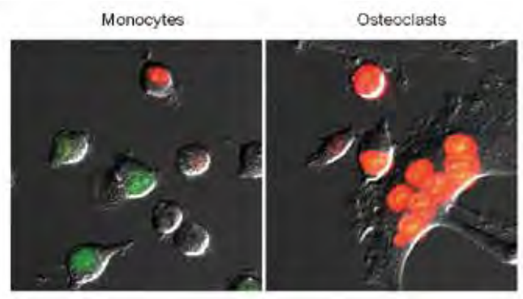

Figure 2. Fluorescence micrographs of Fucci-expressing monocytes and osteoclasts. Cells expressing green fluorescence are in S/G2/M phases while cells expressing red fluorescence are in G1 phase.

Associate Professor **Noriko Takegahara**
Assistant Professor **Kazuaki Tokunaga**
(Visiting Academic Staff)

Recent Publications

- Kim H, Kim T, Jeong BC, Cho IT, Han D, Takegahara N, Negishi-Koga T, Takayanagi H, Lee JH, Sul JY, Prasad V, Lee SH, Choi Y. Tmem64 modulates calcium signaling during RANKL-mediated osteoclast differentiation. ***Cell Metab.*** 17:249-60, 2013.
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Symposia & Seminars

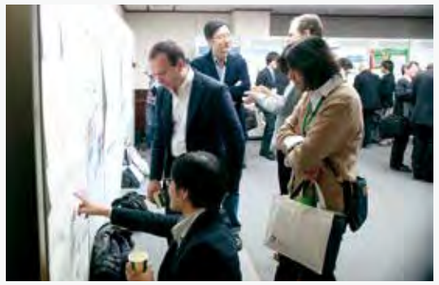
International Symposium TCUID

Date: November 18-20, 2013
Venue: Icho Kaikan & Taniguchi Memorial Hall, Osaka University

The International Symposium "Towards Comprehensive Understanding of Immune Dynamism" (TCUID 2013) was held on the 18th-20th of November at the Icho-Kaikan, Osaka University. This was the third and the final symposium for AKIRA Project, and was supported by the Cabinet Office and the Japan Society for the Promotion of Science with 176 participants in total. Eight speakers from IFReC and AKIRA Project, and nine invited speakers from four different countries including the special lecturer presented their latest research findings. As for the poster session, there were 28 presentations led by young researchers who saw lively questions from the participants. In TCUID 2013, the participants could deepen their understanding of the dynamic mechanisms regulating innate and acquired immunity through presentations and discussions of research findings achieved by using a variety of techniques including imaging and systems biology.

A Young Researchers' Workshop was also held on the first day, where young researchers from various areas of specialization were divided into smaller groups to have group discussions, which promoted exchange among the participants for future interdisciplinary researches.

Speaker	Title
Michael Reth (Keynote Lecturer) (University of Freiburg, Max Planck Institute of Immunobiology and Epigenetics, Germany)	Mapping the Lymphocyte Surface: a Nanoscale Study
Myung-Shik Lee (Sungkyunkwan University School of Medicine, Korea)	The Zinc Finger Domain Containing Nuclease, Regnase-1/Zc3h12a, Controls T Cell Activation
Thirumala-Devi Kanneganti (St. Jude Children's Research Hospital, USA)	Mediators of Inflammatory Responses
Kensuke Miyake (The University of Tokyo, Japan)	Mechanisms Regulating Nucleic Acid Sensing Toll-like Receptors
Masahiro Yamamoto (Osaka University, Japan)	Toxoplasma gondii Polymorphic Dense Granule Protein GRA6 Selectively and Strain-specifically Activates NFAT4
Cevayir Coban (Osaka University, Japan)	A New Gateway for Cerebral Malaria Immunopathology
Kenta Nakai (The University of Tokyo, Japan)	Analyses of Transcriptional Changes over Time in Stimulated Dendritic Cells through Systems Biology
Grégoire Altan-Bonnet (Memorial Sloan-Kettering Cancer Center, USA)	From Local Signals to Global Responses: Self-organization of T- Lymphocyte Activation through Cytokine Communications
Fuyuhiko Inagaki (Hokkaido University, Japan)	Structural Biology of Innate Immunity
Stefan J. Riedl (Sanford-Burnham Medical Research Institute, USA)	Mechanism and Regulation of Cell Signaling by Death Domains
Masaru Ishii (Osaka University, Japan)	Dynamic Behavior of Osteoclasts and Their Precursors Macrophages Visualized by Intravital Microscopy
Joji Fujisaki (Columbia University, USA)	Immune Privilege of the Hematopoietic Stem Cell Niche
Nicholas I. Smith (Osaka University, Japan)	Label Free Measurement of Immune Cells: Inflammasome Induction and RNA Molecular Analysis
Kazuya Kikuchi (Osaka University, Japan)	In Vivo Imaging by Designed MRI Probes with Tunable Chemical Switches
Erik M. Shapiro (Michigan State University, USA)	Monitoring Immune Cell Infiltration Using MRI-based Cell Tracking
Mark S. Sundrud (The Scripps Research Institute, USA)	Dynamics and Molecular Features of Inflammatory T Cell Subsets in Human Autoimmune Disease
Shizuo Akira (Osaka University, Japan)	NLRP3 Inflammasome and Microtubule Acetylation



Immunology Frontier "B to B Seminar"

The Immunology Frontier "B to B (Bench to Bed, Bed to Bench) Seminar" was organized by Prof. Atsushi Kumanogoh (Osaka University School of Medicine, IFReC) and other IFReC PIs. In the meeting, over 150 audience members had the pleasure to listen to "Regulatory T cell and autoimmunity; the basis for cancer immunology" by Shimon Sakaguchi and "The recent breakthroughs in the treatment of rheumatoid arthritis and human immunology" by Kazuhiko Yamamoto.

Date: May 24, 2013
Venue: Icho Kaikan, Osaka University
Speaker: Shimon Sakaguchi (Experimental Immunology, IFReC)
Kazuhiko Yamamoto (Department of Allergy and Rheumatology, The University of Tokyo Hospital)

Mini Symposium on Malaria Immunopathology

Date: January 15, 2014
Venue: Meeting Room #1, IFReC Research Building

Speaker: Laurent Renia (Singapore Network-BMSI-A* STAR)
Breadth of the humoral response and potential vaccine targets following experimental Plasmodium falciparum sporozoite inoculation in humans

Katsuyuki Yui (Nagasaki University)
T cell responses during infection with Plasmodium berghei ANKA

Richard Culleton (Nagasaki University)
Evolutionary origins of P. falciparum and P. vivax as human pathogens

Cevayir Coban (Osaka University)
Imaging cerebral malaria

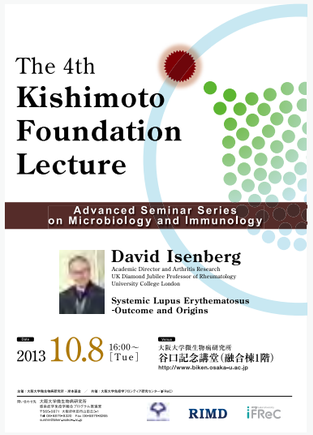
Host: Laboratory of Malaria Immunology, IFReC

Kishimoto Foundation Lecture

Date: October 8, 2013
Venue: Taniguchi Memorial Hall, Osaka University
Speaker: Prof. David Isenberg (Academic Director and Arthritis Research UK Diamond Jubilee Professor of Rheumatology, University College London)

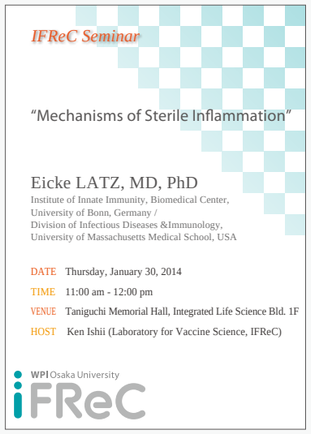
Title: Systemic Lupus Erythematosus -Outcome and Origins

Host: Research Institute for Microbial Diseases & IFReC



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.



FY2013 IFReC Seminar

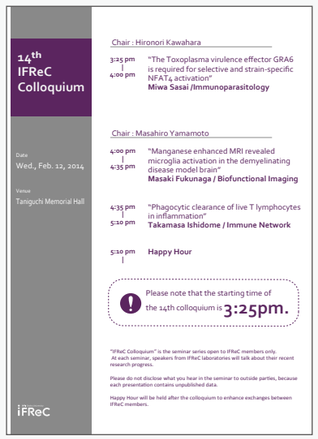
Date	Speaker	Affiliation	Title
2013			
May 9	**Noriko Takegahara**	Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine	Elucidation of osteoclast fusion mechanisms
May 16	**Maho Hamasaki**	Department of Genetics, Graduate School of Medicine, Osaka University	Autophagosome membrane biogenesis
Jun. 4	**Axel Kallies**	Division of Molecular Immunology, Walter and Eliza Hall Institute of Medical Research	IRF4 is a master regulator of T cell differentiation and metabolism
Jun. 17	**Gos Micklem**	Department of Genetics, University of Cambridge	From model organisms to human disease and back again: data warehousing for modern biology
Jul. 18	**Susana Guerra**	Department of Preventive Medicine and Public Health, Universidad Aut.ANsnoma, Madrid.	ISG15 regulates peritoneal macrophages functionality against viral infection
Dec. 9	**Takeshi Egawa**	Pathology and Immunology, Washington University in St. Louise, School of Medicine	Transcription factors regulating duration of T cell immune responses and host protection through metabolic regulation
Dec. 24	**Florent Ginhoux**	Singapore Immunology Network	Dendritic Cells and Macrophages in Health and Disease
2014			
Jan. 15	**Laurent Renia**	Singapore Immunology Network	Breadth of the humoral response and potential vaccine targets following experimental Plasmodium falciparum sporozoite inoculation in humans
Jan. 15	**Katsuyuki Yui**	Graduate School of Biomedical Sciences, Nagasaki University	T cell responses during infection with Plasmodium berghei ANKA
Jan. 15	**Richard Culleton**	Institute of Tropical Medicine, Nagasaki University	Evolutionary origins of P. falciparum and P. vivax as human pathogens
Jan. 15	**Cevayir Coban**	Malaria Immunology, IFReC	Imaging cerebral malaria
Jan. 30	**Eicke Latz**	Institute of Innate Immunity, BiomedicalCenter, University of Bonn Division of Infectious Diseases & Immunology, University of Massachusetts Medical School	Mechanisms of Sterile Inflammation
Feb. 4	**Shan Lu**	Department of Medicine University of Massachusetts Medical School	Heterologous DNA prime-protein boost HIV vaccines

IFReC Colloquia

IFReC started a series of colloquia for IFReC researchers in FY2011. Researchers from all labs are able to present and discuss their current research progress. These events were initiated as a means to make all those present at IFReC aware of the wealth of immunological research that is being conducted, and to promote collaborations between all the immunology, imaging and informatics groups. An average of approximately 100 people attend each time.

Date: **The 9th IFReC Colloquium: April 24, 2013**
The 10th IFReC Colloquium: June 12, 2013
The 11th IFReC Colloquium: August 7, 2013
The 12th IFReC Colloquium: October 16, 2013
The 13th IFReC Colloquium: December 18, 2013
The 14th IFReC Colloquium: February 12, 2014

Venue: **Taniguchi Memorial Hall, Osaka University**



Speaker	Title
Syoji Kobashi (Information Systems)	Soft computing for image processing to quantify in-vivo images
Kei Kuramoto (Information Systems)	Simulation and soft computing for asthmatic attack
Hui Jin (Immunochemistry)	Misfolded ER proteins transported to the cell surface by MHC class II molecules are targeted by autoantibodies
Hiroshi Ike (Cell Signaling)	Fluctuation of DAG in immunological synapse regulates T cell activation
Kouji Kobiyama (Vaccine Science)	Targeting lymph node macrophage and dendritic cells by nano-particulated TLR9-ligand
Hong Zhao (Malaria Immunology)	Immunopathology of cerebral malaria: A new gateway
Tomokazu Ohta (Immune Regulation)	Homeostatic roles of XCR1-expressing dendritic cells in the intestinal immunity
Yoshiko Murakami (Immunoglycobiology)	Genetic and biochemical basis of inherited GPI-anchor deficiencies
Shinichiro Watanabe (Nuclear Medicine)	Whole-body glucose metabolism in identical twin pairs:Effects of genetic and acquired factors on various organs
Yuko Kamikawa (Chemical Imaging Techniques)	Development of PYP-based protein tag system for fluorogenic imaging of cellular proteins
Takeharu Minamitani (Molecular Immunology)	Epstein-Barr Virus LMP2a reduces the stringency of germinal center B cell selection: an implication for persistent virus infection
Yoshihiro Oka (Immuno-pathology)	Cancer antigen WT1-targeting immunotherapy for patients with malignancies
Nam Trung Nguyen (Immune Regulation)	The roles of aryl hydrocarbon receptor in immune responses
Taisuke Nakahama (Immune Regulation)	Aryl hydrocarbon receptor-mediated induction of the microRNA-132/212 cluster promotes TH17 cell differentiation
Masanori Matsumoto (Lymphocyte Differentiation)	Plasmablasts control T-cell autoimmunity through IL-10 production
Tomoyuki Yamaguchi (Single Molecule Imaging)	Models of T cell-mediated immune response
Yoko Yano (BioLegend Japan KK)	BioLegend collaboration strategies in Japan
Takatoku Oida (BioLegend Japan KK)	BioLegend launched Japan R&D lab at Saito
Keizo Nishikawa (Immunology and Cell Biology)	Dnmt3a as a novel nexus coordinating metabolism and epigenetics in osteoclasts
Atsushi Tanaka (Experimental Immunology)	Breakdown of immune tolerance by quantitative and qualitative alterations of TCR signaling through ZAP-70
Shide Liang (Systems Immunology)	Development of knowledge-based energy functions and applications in immunology research
Miwa Sasai (Immunoparasitology)	The Toxoplasma virulence effector GRA6 is required for selective and strain-specific NFAT4 activation
Masaki Fukunaga (Biofunctional Imaging)	Manganese enhanced MRI revealed microglia activation in the demyelinating disease model brain
Takamasa Ishidome (Immune Network)	Phagocytic clearance of live T lymphocytes in inflammation

Live Immuno-Imaging Facility Opening Workshop

IFReC established the “Live Animal Immuno-imaging Facility” in April, 2013. To commemorate its opening, the "Live Immuno-Imaging Facility" Opening Workshop was held. At the beginning, Yoshichika Yoshioka (PI, Biofunctional Imaging Lab, IFReC) gave a lecture titled “Live Immuno-Imaging: From small animal to human.” In the workshop, seven researchers presented their current research progress related to bioimaging technology. Afterwards, a tour to the facility equipped with an 11.7T MRI and a multi-photon fluorescence microscope was arranged for the participants.

Date: **June 19, 2013**
Venue: **Taniguchi Memorial Hall, Osaka University**

<Keynote Lecture>
15:00 -15:30 **Yoshichika Yoshioka** (PI, Biofunctional Imaging Lab, IFReC)
Live Immuno-Imaging: from Small Animal to Human

<Morphological Studies>
15:30 -15:40 **Masaaki Murakami** (Developmental Immunology)
15:40 -15:50 **Yuki Mori** (Biofunctional Imaging)

<Functional Studies>
15:50 -16:00 **Hiroyuki Murota** (Dept. Dermatology, School of Medicine)
16:00 -16:10 **Masaki Fukunaga** (Biofunctional Imaging)

<Cellular-tracking Studies>
16:10 -16:20 **Manabu Kinoshita** (Dept. Neurosurgery, School of Medicine)
16:20 -16:30 **Takashi Jin** (RIKEN QBiC)
16:30 -16:40 **Yoshikazu Tsukasaki** (RIKEN QBiC)

16:40 -17:00 Questions and Answers

Events

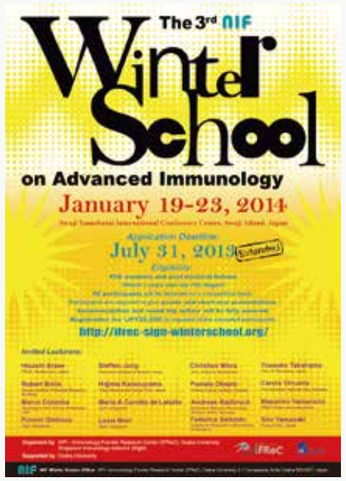
Winter School

The third Winter School on Advanced Immunology was jointly organized with Singapore Immunology Network (SIgN).
Forty eight young researchers, who were competitively selected from 217 applicants, and 15 world leading immunologists got together on Awaji Island in Japan on 19-23 January 2014.

They shared intriguing insights and findings in immunology, discussed new ideas and forged friendships that fueled networking and future collaborations.

The participants enjoyed a one-day tour to IFReC in Suita Campus of Osaka University after the school in Awaji Island.

Date: **January 19-23, 2014**
Venue: **Awaji Yumebutai International Conference Center, Awaji Island, Hyogo**



Lecturer	Title
Hisashi Arase WPI Immunology Frontier Research Center (IFReC), Osaka University, Japan	Regulation of Immune Response by Paired Receptors
Robert Brink Garvan Institute of Medical Research, Australia	Unravelling the Complexities of the Germinal Centre Reaction
Marco Colonna Washington University School of Medicine, USA	Innate Lymphoid Cells in Mucosal Immunity
Florent Ginhoux Singapore Immunology Network	Dendritic Cell and Macrophage Immunobiology
Steffen Jung Weizmann Institute of Science, Israel	Probing Macrophage Functions in Gut and Brain
Hajime Karasuyama Tokyo Medical and Dental University, Japan	Emerging roles for basophils in immunity: a neglected minority gains new respect
Maria A. Curotto de Lafaille Singapore Immunology Network	IgE and the Pathogenesis of Allergic Diseases
Andreas Radbruch German Rheumatism Research Centre (DRFZ)	The Resting and the Restless Immunological Memory
Federica Sallusto Institute for Research in Biomedicine, Switzerland	Effector and memory T cells in humans
Yousuke Takahama University of Tokushima, Japan	T cell repertoire formation in the thymus
Masahiro Yamamoto WPI Immunology Frontier Research Center (IFReC), Osaka University, Japan	A mechanism of IFN-γ-induced cell-autonomous immunity against an intracellular protozoan parasite Toxoplasma gondii
Lucia Mori Singapore Immunology Network (SIgN), Singapore	Lipid Antigen Presentation to T Cells
Sho Yamasaki Kyushu University, Japan	Recognition of pathogens through C-type lectin receptors
Carola Vinuesa The Australian National University	Multiple mechanisms operate to regulate T cell help for B cells
Christian Münz University of Zurich, Switzerland	Macroautophagy in MHC restricted antigen presentation

Staff and Researchers Development Seminars

IFReC offered various Staff and Researcher Development programs in FY2013 to create a motivated, skilled, and well-trained staff.

Berlitz-IFReC Communication Course

The Berlitz-IFReC Communication Course is a new SD/RD program for IFReC members to improve their English skills, communication and practice in the workplace. The 31 participants were selected by interview to join the course.

Distinguished Women Scientist Seminars

One of the goals for IFReC and the university is to attract and recruit the best young researchers. Four distinguished women scientists were invited to IFReC to present about their academic career path and development. Through the series, about 200 participants from both within and outside the university had the privilege to listen the lectures followed by a Question and Answer session.

Date: June 26, 2013
Lecturer: Keiko Nakamura
(Director, JT Biohistory Research Hall)

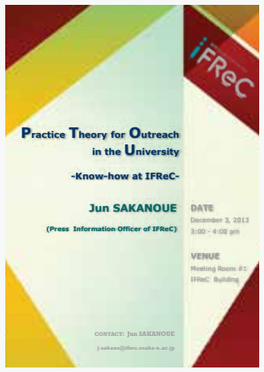
Date: July 3, 2013
Lecturer: Yoshie Harada
(Professor, WPI-iCeMS, Kyoto University)

Date: August 9, 2013
Lecturer: Mitiko Go
(External Executive Director, Research Organization of Information and Systems)

Date: February 5, 2014
Lecturer: Junko Tanaka
(Professor, Graduate School of Biomedical Sciences, Hiroshima University)

Other Seminars for Staff Development

The following two seminars were held for administrative staff to further promote expert knowledge. IFReC plans to continue the "Immunology introductory course series for administrative & technical staff" during and beyond 2014 in collaboration with various scientists.



Date: December 3, 2013
Title: "Practice Theory for Outreach in the University; Know-How at IFReC"
Lecturer: Jun Sakanoue
(Research Planning & Management Office, IFReC)

Date: February 20, 2014
Title: "Immunology Introductory Course Series for Administrative & Technical Staff" Part 1
Lecturer: Jun Sakanoue
(Research Planning & Management Office, IFReC)

Japanese Lessons

We hold Japanese language classes for overseas researchers / students to help reduce the stress and inconvenience that are caused by the language barrier in a research environment as well as in daily life.

We offer "Class A: Absolute beginner class", "Class B: Elementary/Pre-intermediate Class" held in lecture style to learn basic knowledge necessary for conversations, and "Japanese Cafe : Intermediate/advanced class" held in a cafe style to learn more practical phrases and idioms in daily life from a professional Japanese teacher.

This year, interesting themes related to Japan were set in the Japanese Cafe, and interactive lessons were conducted through the themes of Japanese traditional culture and trends. Japanese volunteers joined and helped the lessons to give foreign people an opportunity to practice in Japanese.

August 8, 2013/ February 13, 2014
ORIGAMI

November 14, 2013
Let's make plans for your virtual travel with a Japanese map !

July 18, 2013
Osakan culture "TAKOYAKI"

September 12, 2013
SHODO

January 16, 2014
The first writing of the New Year (KAKIZOME)

December 12, 2013
Let's try to write a New Year's greeting card by using a fude (a brush) !

October 31, 2013
Let's make an ONIGIRI ! (Rice Ball)

March 13, 2014
Let's introduce your country with a world map!

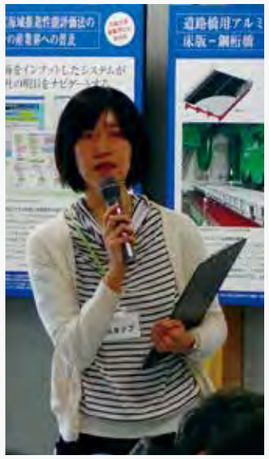
Outreach Activities

Science Café Series “Café on the Edge”

IFReC and FIRST Program AKIRA Project have organized “Café on the Edge” since FY2010. We held “Café on the Edge” two times in FY2013 with the speakers chosen from cutting-edge science fields of IFReC. The number of participants totaled over 150.

Date: May 3, 2013
Venue: Techno Alliance Building, Osaka University
Guest: Yoshichika Yoshioka (Biofunctional Imaging, IFReC)
Title: “Toward the fullest potential of MRI; Neuron activities to immune reactions”

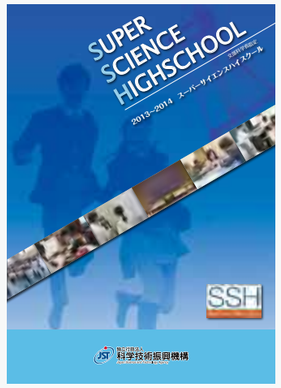
Date: June 12, 2013
Venue: Art Area B1 (Naniwabashi station, Osaka)
Guest: Osamu Takeuchi
(Institute for Virus Research, Kyoto University & IFReC)
Title: “Inflammation; Us-or-them?”



Super Science High School Festa 2013

The Purpose of the project for Super Science High School (SSH) is to promote upper secondary education to foster excellent human resources in science and technology. The SSHs are designated to be developed enriched curricula, teaching methods, and materials for science and mathematics in cooperation with universities and research institutes.

IFReC and other WPI institutes participated in the annual meeting of SSH 2013 in Yokohama. We presented the research fields conducted at IFReC, and hope the information could help students to choose a university in the near future.



Date: August 7 & 8, 2013
Venue: Pacifico Yokohama, Kanagawa
Host: JST, MEXT

Science Agora 2013

"Science Agora" is an event designed based on "Family Science Day" at annual meeting of American Association for the Advancement of Science. In the "Science Agora 2013" in Tokyo, WPI institutes co-organized "Science Talk Live" for kids and general citizens. After the event, we were awarded "Science Agora Prize" for the introduction of the most-advanced science and technology in Japan.

Date: November 9 & 10, 2013
Venue: National Museum of Emerging Science and Innovation, Tokyo
Host: JST


表彰状

サイエンスアゴラ賞

世界トップレベル研究拠点プログラム(WPI) 殿
企画者:WPI Science LIVE!

貴企画はサイエンスアゴラ2013において
科学への興味や理解を深めるとともにこれ
からの豊かな社会をつくる取り組みの見本
として、科学コミュニケーションの推進に
顕著に貢献したと認められました
よってこれを賞します

Science Talk Live 2013 by WPI

This event started in 2011 to encourage a close relationship between WPI scientists and high school students. The third symposium "The perceptive scientists change the World!" was held in Sendai. In the event, the researchers from WPI institutes talked about their cutting-edge researches, and spoke on passion for science. At the end of the event, students and scientists spoke freely with one another and enjoyed experiments at each WPI booth.

Date: December 14, 2013
Venue: Sendai International Center, Miyagi
Host: Advanced Institute for Materials Research, Tohoku University (WPI-AIMR), WPI Institutes
Support: Miyagi Prefecture, MEXT, JSPS

Speaker

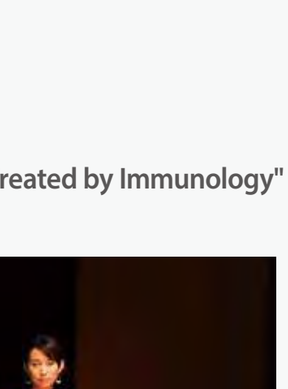
- Kenichiro Itami (Director of WPI-ITbM, Nagoya University)
- Kei Hirose (Director of WPI-ELSI, Tokyo University of Technology)
- Masashi Yanagisawa (Director of WPI-IIIS, University of Tsukuba)
- Alan Greer (Principal Investigator of WPI-AIMR, Tohoku University, Professor of University of Cambridge)
- Akari Takayama (JSPS Postdoctoral Fellow, WPI-AIMR, Tohoku University)

Presentations by Students

Sendai Dai-ichi High School / Sendai Dai-san High School / Furukawa Reimei High School / Eleanor Roosevelt High School, USA

Symposium for General Audience "Future Medical Treatment Created by Immunology"

Date: February 1, 2014
Venue: MIRAICAN Hall, National Museum of Emerging Science and Innovation, Tokyo
Host: IFReC, FIRST Program AKIRA Project, and the Mainichi Newspapers Co.



<Program>
Lecture I "Potential of New Treatments Created by Immunology"
Shizuo Akira (Director of IFReC)

Lecture II "Antibody Drugs- The Potential and Prospect"
Kunihiro Hattori (Chugai Pharmaceutical Co., Ltd.)

Talk Session "Future Medical Treatment Created by Immunology"
- Shizuo Akira (IFReC)
- Kunihiro Hattori (Chugai Pharmaceutical Co., Ltd.)
- Tomohiro Kurosaki (IFReC)
- Junichi Taguchi (Hospital Director, Tokyo Midtown Clinic)
- Yuri Kato (Personality)
- Yukiko Motomura (the Mainichi Newspaper)

AAAS 2014

The American Association for the Advancement of Science (AAAS) is the biggest international non-profit organization for the advancement of science in the world. The main theme for the recent annual meeting of AAAS (AAAS 2014) was "Meeting Global Challenges: Discovery and Innovation".

In the exhibition, as part of the Japan pavilion at AAAS 2014, the Press Information Officers from all the WPI institutes introduced the collaborative researches bridging various research fields in an internationally opened environment of each institute. The Japan pavilion received the greatest number of guests among all the booths. The WPIs and RIKEN co-organized the exhibitor workshop "Build a Career in Japan!" on Feb. 14. In the workshop, we provided an opportunity for discussing Japan's science policy and career path for overseas researchers.

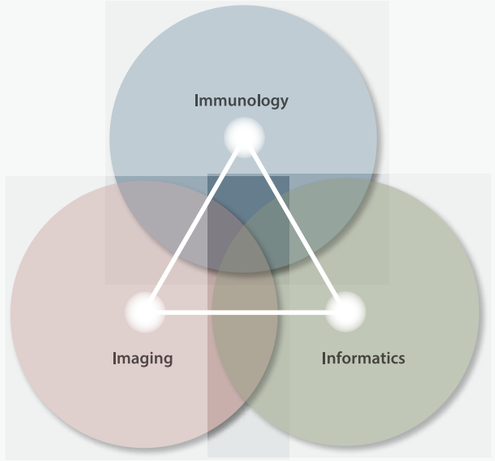
Date: February 13-17, 2014
Venue: Hyatt Regency Chicago, IL, USA

Research Projects

Research Support Program for Fusion of Different Fields

The final goal of the World Premier International Research Center (WPI) Initiative program is to create world leading research centers where top researchers gather, enabling the achievement of high-level research results, and at the same time, providing an excellent research environment. To achieve one of the goals of the WPI program, each center is expected to generate novel research fields through the promotion of combined research projects.

Here at IFReC, we are aiming to create innovative immunology fields by combining with imaging and bioinformatic technologies. In order to support this new challenge, we established the Research Support Program for Combined Research Fields in FY2009 for IFReC members. A total of 10 combined research projects and one Dual Mentor Program are currently underway.



Projects Supported by the Research Support Program for Fusion of Different Fields

Date Started	Principal Researcher	Collaborator	Titles of Proposed Project
Oct. 2012	M Yamamoto	D Standley Eva-Maria Frickel	Trilateral analysis of interferon-γ-mediated cellular innate immunity against Toxoplasma gondii
	F Sugihara	R Hanayama K Kikuchi	*in vivo* Imaging of Germinal Center Development in Mouse Spleen Using MRI
	B Ripley	D Standley G Kurisu	Role of Arid5A in the Selective Control of IL-6 mRNA Stability and Development of TH17 Cells
	M Kohyama	C Coban K Suzuki	Role of tissue macrophage in malaria infection, and their developmental control by parasite metabolite
	T Yamaguchi	H Fujita S Sakaguchi T Watanabe T Jin	Imaging analysis of Imuune activation and regulation
Oct. 2013	Y Kumagai	J Kozuka S Teraguchi N Trost	Visualizing Information Processing of Immune Cells via Combination of Fluorescent Reporter and Single Molecule Imaging
	K Suzuki	Y Baba	Visualizing activation of germinal center B cells using genetically encoded calcium indicators
	N Ohkura	A Vandenbon S Nakamura S Yamazaki S Kato M Hashimoto	Development of an epigenome-based computational classification system for the treatment of autoimmune diseases
	A Vandenbon	S Sakaguchi H Morikawa N Ohkura	Identification of key factors for inducing functionally stable regulatory T cells
	D Diez	R Hanayama	The dynamics of novel signaling networks of macrophages exposed to pathogens

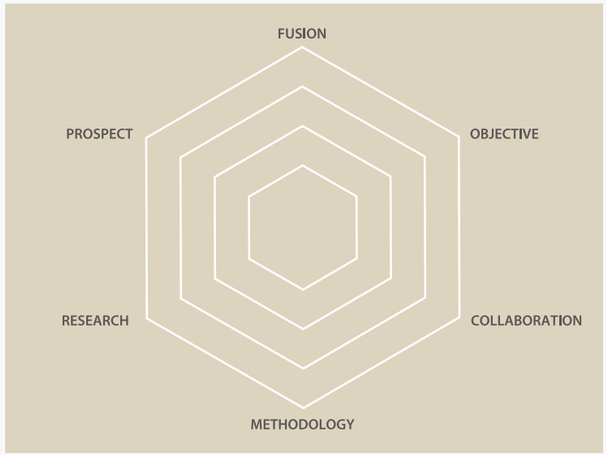
Researchers from the groups of <Immunology>, <Imaging>, <Informatics>, and <Other>

Combined Research Fields Evaluation Workshop

The evaluation workshop was held on September 19, 2013 at Taniguchi Memorial Hall. Both the Combined Research Program and Dual Mentor Program recipients for FY2012 made a research-in-progress presentation for 20 minutes. All IFReC PIs joined the workshop as reviewers. The evaluation workshop provides opportunities for the promotion of interdisciplinary research activities among young researchers, introducing the current research at IFReC. The newly selected recipients of the program for FY2013 gave two-minute presentations to introduce their research.

Date: **September 19, 2013**
Venue: **Taniguchi Memorial Hall**

Evaluation Criteria Chart



Both document and presentation evaluations are made based on each of the elements above by all principal investigators. The results may determine whether the research grant amount will be increased or decreased in the following year.

Dual Mentor Program

The Dual Mentor Program aims to help young researchers engage in inter-disciplinary research by providing them with two advisors and/or mentors with specialization in different research fields. The annual budget for each project is ¥3 million with an internal evaluation once a year. The program was introduced as a platform to further promote interdisciplinary research at IFReC.

Takeshi Yoshida (C) and his two mentors, Rikinari Hanayama (L) and Kazuhiro Suzuki (R).

Recipient	Mentor	Project Title
T YOSHIDA (Immune Network)	R Hanayama (Immune Network) K Suzuki (Immune Response Dynamics)	Visualizing the dynamics of exosomes during various immune responses in vivo

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 towards international collaborative research and to develop their networking skills with researchers overseas. Eleven researchers used this support program in FY2013.

FIRST Program : AKIRA Project

The Funding Program for World-Leading Innovative R&D on Science and Technology, (FIRST Program) AKIRA Project "Comprehensive understanding of immune dynamism: toward manipulation of immune responses" led by core researcher, Shizuo AKIRA and supported by JSPS and the Cabinet Office, Government of Japan, finished successfully at the end of March, 2014. The AKIRA Project produced a lot of fruitful results from the intensive research during the past four years, including interdisciplinary research between immunology, imaging, structural biology and systems biology, and the results were published as approximately 100 papers in journals with high impact factors. During the four years, 13 invention reports were submitted, and one was patented.

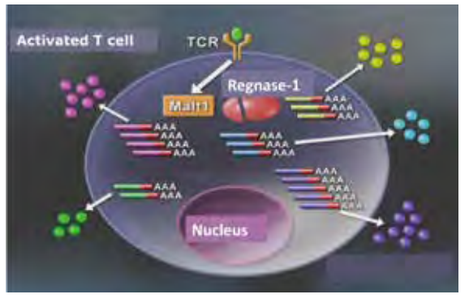
Activities of the AKIRA Project in FY2013 (Except for outreach activities)

Date	Activity
2013	
Apr. 16	The Fourth Patent Committee Meeting of the AKIRA Project
Apr. 24	The Fourth Meeting of 30 Operational Support Institutions in the FIRST Program by Cabinet Office, Government of Japan
May 1	Issue of Leaflet "Immunology On the Edge" (Revised third edition)(in Japanese)
Jul. 16	The Fifth Patent Committee Meeting of the AKIRA Project
Jul. 31	Issue of Leaflet "Immunology On the Edge" (Revised fourth edition) (in Japanese)
Aug. 20	Establishing the Website of Intellectual Property (IP) for AKIRA Project
Sep. 6	The Sixth Patent Committee Meeting of the AKIRA Project
Sep. 27	The Fifth Meeting of 30 Operational Support Institutions in the FIRST Program by Cabinet Office, Government of Japan
Oct. 1	Researchers' Development Seminar "Science Communication Seminar" (in Japanese)
Nov. 18-20	International Symposium TCUID 2013 (including Young Researchers' Workshop)
Nov. 20	The Sixth Steering Committee Meeting of the AKIRA Project, FIRST Program
Nov. 29	Researchers' Development Seminar "Career Path Seminar" (in Japanese)
2014	
Jan. 17	Production of the DVD "Novel Development of Immunology Research" for introducing outcomes of the AKIRA Project (in Japanese)
Mar. 5-6	The AKIRA Project Research Meeting 2014
Mar. 6	The Seventh Steering Committee Meeting of the AKIRA Project, FIRST Program
Mar. 28	Issue of Research Achievement Summary Report of the AKIRA Project, FIRST Program (FY2010-FY2013) (in Japanese)

AKIRA Project made a DVD movie as a final summary of the project and introduced the following four outstanding research results in it. DVDs were distributed at several outreach events and a short version of the movie is planned to be made available on our website.

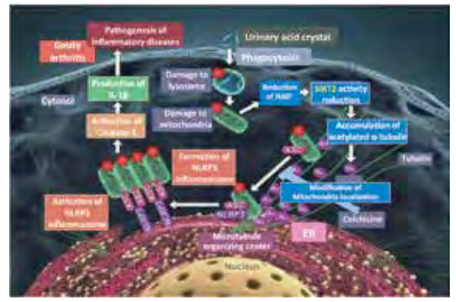
Regulation of inflammation by Regnase-1

Regnase-1 regulates the inflammation by degradation of mRNA. Dynamic control of Regnase-1 expression is critical for controlling activation of innate as well as acquired immune cells.



Formation and activation of the NLRP3 inflammasome

The molecular mechanism of gout in immune cells was elucidated. Microtubule-driven spatial arrangement of mitochondria promotes activation of the NLRP3 inflammasome.

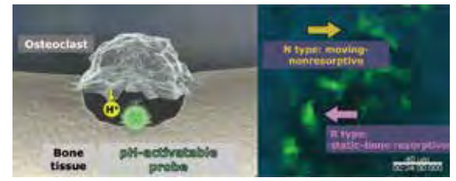


Disease specific M2 Macrophages

S. AKIRA's group made the world-first discovery that macrophages are categorized to disease specific classes, and identified macrophages specific to metabolic syndrome and allergies.

Visualization of the dynamic conversion of osteoclast function

K.KIKUCHI's group and M.ISHII's group have succeeded in visualizing the dynamic conversion of the osteoclast function using 2-photon microscopy and newly developed chemical probes.



Activities

In FY2013, the AKIRA Project actively engaged in more events than ever before. We hosted five outreach activities, and opened booths and/or gave lectures at five other events. In every event, we tried to have better communication with the general public in order to enhance people's understanding of our project and its importance. In the last year of our project, we also held several seminars aimed at researchers' development for their future activities.

Researchers' Development Seminars and Career Path Seminar

AKIRA Project held science communication seminars on Oct 1 (in Japanese) and on November 18 (in English, as young researchers' workshop in TCUID 2013). Guest lecturers from the National Museum of Emerging Science and Innovation (Miraikan) talked about helpful skills to interpret one's research efficiently and effectively. A career path seminar was also held on November 29, in which Masaru Ishii and Joji Fujisaki (Columbia University, USA) talked about their career progression and what were the key points for them.

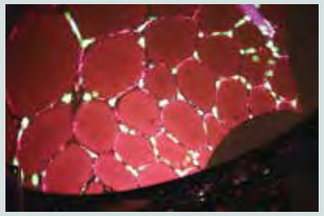
Activities for General Public

Science cafes, lectures and the symposium

Event	Date and Venue	Speaker
Panel exhibition and Science Café On the Edge 12	May2-3 Osaka Univ.	Yoshichika Yoshioka
Science Café On the Edge 13, "Inflammation: Friend or Foe of Our Body?"	June 12 Art Area B1	Osamu Takeuchi
FIRST/WPI Researcher live lectures! Young×Young in the "FY2013 Congress of Super Science High Schools"	August 8 Pacifico Yokohama	Yutaka Suzuki
Lecture for public, "The Milky Way in our body~ the function and the movement of immune cells through two-photon live imaging~"	November 24 'Sophia Sakai,'Kyoikubunka Center (Center for Education & Culture), Sakai City	Masaru Ishii and Joji Fujisaki
Lecture: OSAKA GAKUGEI SECONDARY SCHOOL visit	December 16 Osaka Univ.	Masaru Ishii and Shizuo Akira
Symposium for General Audience, "Future Medical Treatment Created by Immunology"	February 1 MIRAICAN Hall, National Museum of Emerging Science and Innovation	Shizuo Akira,Tomohiro Kurosaki, and Kunihiro Hattori (Chugai Pharmaceutical Co., Ltd.)

Lecture for Public at Planetarium

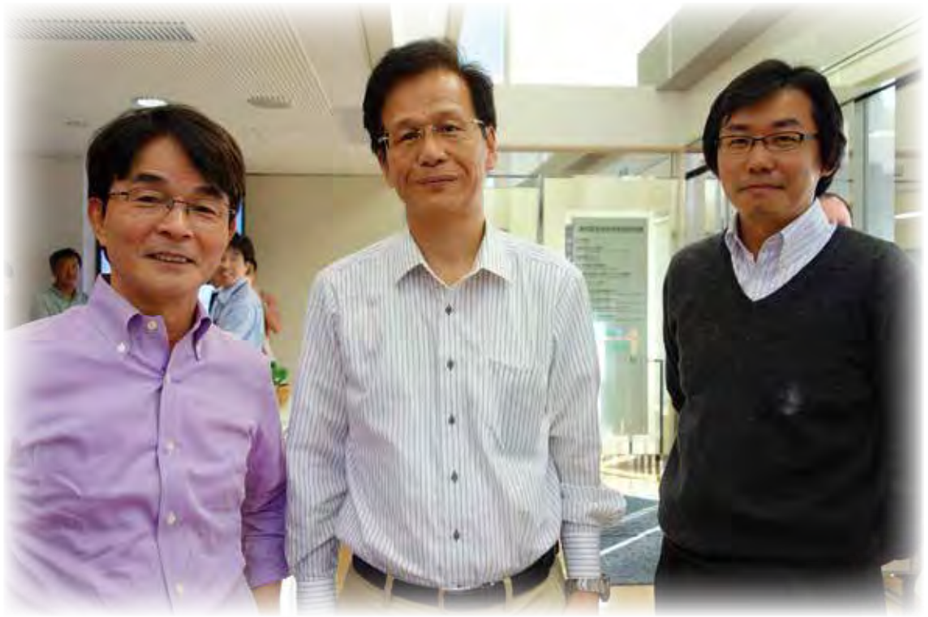
Twinkling cells on planetarium dome - AKIRA Project held a public lecture, in which live cell imaging movies taken by two-photon microscopy were projected on the dome screen of planetarium at 'Sophia Sakai,' on 24 November. Masaru Ishii, one of the Sub-Project Leaders and Joji Fujisaki, an assistant professor of Columbia University, got on stage. Ishii led the event and explained the contents of the movies. They also explained to the participants interesting aspects of conducting researches, as well as what inspired them to become scientists.



Other Open Public Events

We opened a booth at four open public events, "Science Agora 2013 (in Tokyo)", "FIRST Business Matching Symposium (in Sendai and in Kyoto)" and "FIRST EXPO 2014 (in Tokyo)". In addition, Core Researcher Shizuo Akira gave lectures and joined panel sessions at "FIRST Business Matching Symposium in Kyoto" and "FIRST EXPO 2014". All the events gathered thousands of people in total ranging from kids to the elderly.

Data



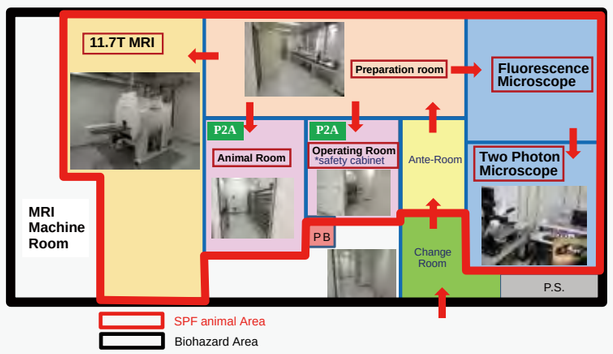
Facilities

IFReC and the Research Institute for Microbial Diseases (RIMD) Research Complex adjoins the Institute for Protein Research and the Graduate School of Engineering. This research complex offers a wealth of facilities to the researchers of IFReC, shared and operated in part with RIMD. Instruments are located in specific laboratories of IFReC or RIMD. Collaboration is key to the goals of IFReC; all facilities are accessible to any researcher, facilitated with help from the core facilities management group.

IFReC–RIMD Research Complex at Suita Campus of Osaka University

Photo: S. Higashiyama

1. IFReC Research Building
2. Integrated Life Science Building
3. Research Institute for Microbial Diseases (RIMD)
4. The Genome Information Research Center, RIMD
5. Cutting-edge Research Building for Infectious Diseases
6. Animal Resource Centers for Infectious Diseases (Building A, B, C)
7. Institute for Protein Research
8. Graduate School for Engineering



The Live Animal Immuno-imaging Facility equipped with an 11.7T MRI and a multi-photon fluorescence microscope, is designed with a clean room adjacent to the laboratory. It is expected that this facility will make great contributions to the development of fusion research between immunology and imaging at IFReC.

Immunology Groups

- DNA sequencers
- Cell sorters and flow cytometers
- Real-time PCRs
- Spectrometers
- Two-photon microscopes
- Super-high resolution microscope
- Basic biological experiment facilities

Imaging Groups

- MRI (11.7T)
- TEM / Cryo TEM
- Raman microscopes
- Two-photon microscopes
- Super-high resolution microscopes
- Total reflective microscopes
- Confocal microscopes
- Cryo-microtomes
- DNA sequencers
- NMR
- Mass spectrometers
- Spectrometers
- Chromatography (LC/HPLC/FPLC)
- Dynamic light scattering analyzer
- Biacores
- Peptide synthesizer

Informatics Groups

- Bioinformatics server

Animal Resource Center (A, B &C)

- Pathogen-free animal facilities
- Embryo freezing and preservation facilities
- Transgenic and knock-out animal generation facilities
- In vivo experiment facilities

Core Facilities with Skilled Technicians

- Animal facility A (for infected animal experiments)
- Animal facilities B&C (specific pathogen-free)
- Radio isotope experiment facilities
- DNA chip facilities
- Next generation DNA sequencer
- DNA sequencers
- Cell sorters and flow cytometers
- TEM
- Mass spectrometers

Technicians of Core Facilities

Facilities available to researchers at IFReC are owned, operated and maintained by the management group for the core facilities, or individual laboratories, to allow researchers to focus more on their work, various kinds of equipment are run and maintained by skilled individuals providing a professional service.

All the staff of Core Facilities outside the RIMD and IFReC buildings

Kishimoto Foundation Fellowships

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 postdoctoral researchers of non-Japanese nationality who seek to collaborate with IFReC researchers. The recipients are provided with a salary and an airfare to Japan.

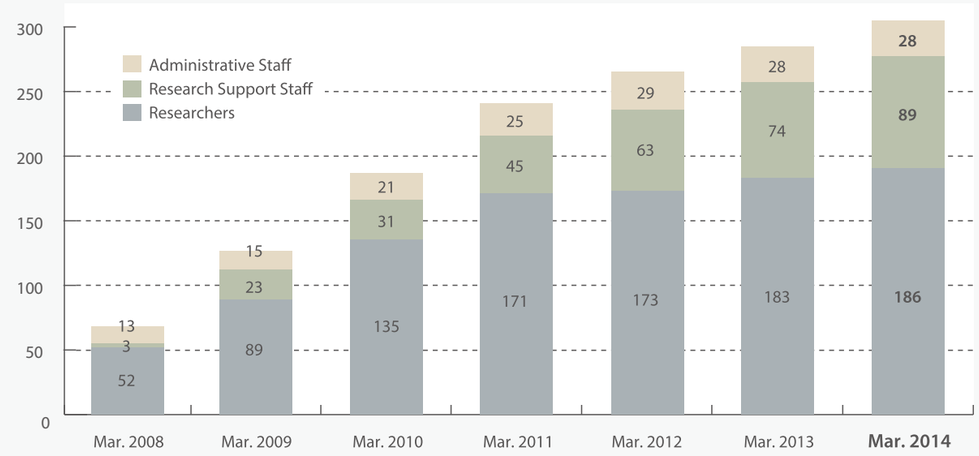
The Kishimoto Foundation was established in 2008 in honor of Tadamitsu Kishimoto, who, during the 1980s and 90s, elucidated the function of interleukin-6 (IL-6), a key molecule for stimulating immune responses. He later developed the anti-IL6 receptor-based therapy, tocilizumab, to treat immune disorders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

FY2013 Kishimoto Fellowship Recipients

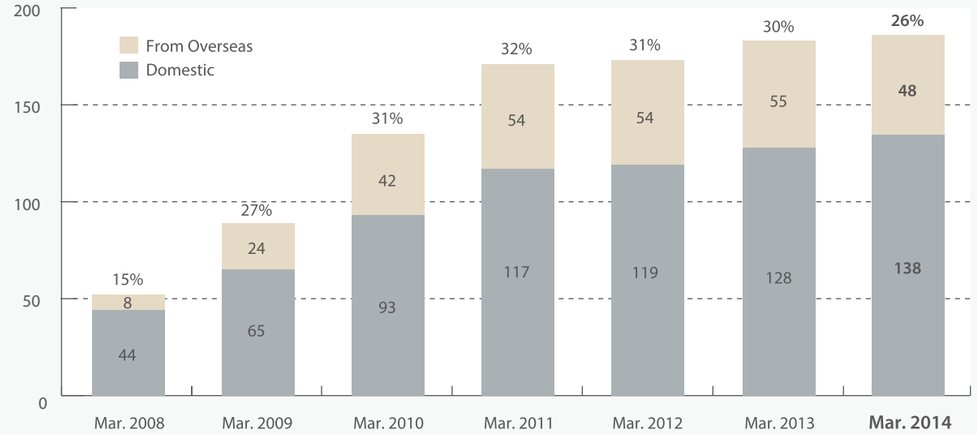
Position of Recipient	Name (initials)	Nationality	Host researcher	Period
Specially Appointed Researcher	H. Z.	China	Coban	Jul. 1, 2010 - Dec. 31, 2013
Specially Appointed Researcher	R. S.	India	Murakami	Aug. 16, 2010 - Aug. 15, 2013
Specially Appointed Researcher	C. T.	Taiwan	Kikutani	Apr. 1, 2011 - Mar. 31, 2014
Specially Appointed Researcher	J. B.	India	Akira	Apr. 1, 2011 - Mar. 31, 2014
Specially Appointed Researcher	S. L.	Korea	Kishimoto	Apr. 16, 2011 - Mar. 31, 2014
Specially Appointed Researcher	W. L.	China	Kishimoto	Sep. 1, 2011 - Jun. 30, 2013
Specially Appointed Researcher	I. B.	Tunisia	Hanayama	Jan. 16, 2012 - Jan. 15, 2015
Specially Appointed Researcher	K. N.	India	Kishimoto	Jun. 1, 2012 - Sep. 15, 2013
Specially Appointed Researcher	P. D.	India	Kishimoto	Nov. 1, 2012 - Oct. 31, 2014
Specially Appointed Researcher	J. H.	China	Sakaguchi	Dec. 1, 2013 - Nov. 30, 2014
Specially Appointed Researcher	D. M.	Britain	Kishimoto	Dec. 1, 2013 - Mar. 31, 2015
Research Fellow	N. N.	Vietnam	Kishimoto	Sep. 21, 2013 - Dec. 17, 2013
Research Fellow	F. G.	France	Kaisho	Dec. 16, 2013 - Jan. 18, 2014
Research Fellow	P. S.	Singapore	Kaisho	Dec. 16, 2013 - Jan. 31, 2014

Members

Number of IFReC Staff



Number of Researchers



Awards

Toshio Yanagida　Person of Cultural Merit

The Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan announced that Toshio Yanagida, Deputy Director of IFReC was named a Person of Cultural Merit on October 25, 2013. MEXT commented that Yanagida was awarded by his outstanding achievements in the studies of fundamental structure of biological system through the developments of single molecular measurements techniques.

This is the second time for a scientist of IFReC to be honored as a Person of Cultural Merit, since Shizuo Akira (Director of IFReC) was awarded in 2009.

Toshio Yanagida　The Honorary Fellow of the Physical Society of Japan (JPS)

The 37 scientists who greatly contributed to the development of physics have been chosen for JPS honorary fellow. The first honoree in 1950 was Hantaro Nagaoka, a former President of Osaka University. The others include, Hideki Yukawa, Shinichiro Tomonaga, Makoto Kobayashi, Toshihide Masukawa, and Yoichiro Nambu (Nobel Prize laureates).

Yanagida was chosen by his outstanding achievements in the studies of fundamental structure of biological system through the developments of single molecular measurements techniques.

Best Contribution Award, The 14th International Symposium on Advanced Intelligent Systems

Yutaka Hata

The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology, Japan

Hisashi Arase

Osaka Science Prize

Kazuya Kikuchi

The Scientific Award, The Japanese Society for Bone and Mineral Research

Masaru Ishii

Young Investigator's Award, The Japanese Medical Association

Masaru Ishii

Bayer International Publication Award

Yuki Mori,
Masaaki Murakami,
Yasunobu Arima,
Dasong Zhu, and Yoshichika Yoshioka

Award for Young Scientists, The Pharmaceutical Society of Japan

Tatsuya Saitoh

Inoue Research Award for Young Scientists

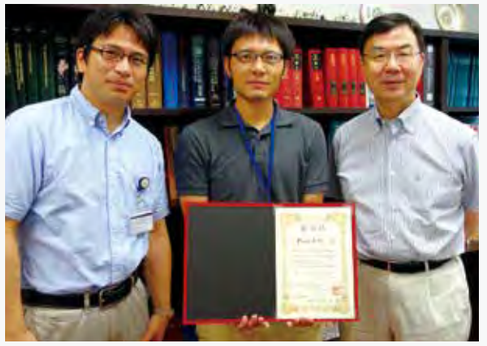
Kenta Maruyama

Koichi Suzuki Memorial Award, The Japanese Biochemical Society

Tetsuya Hirata

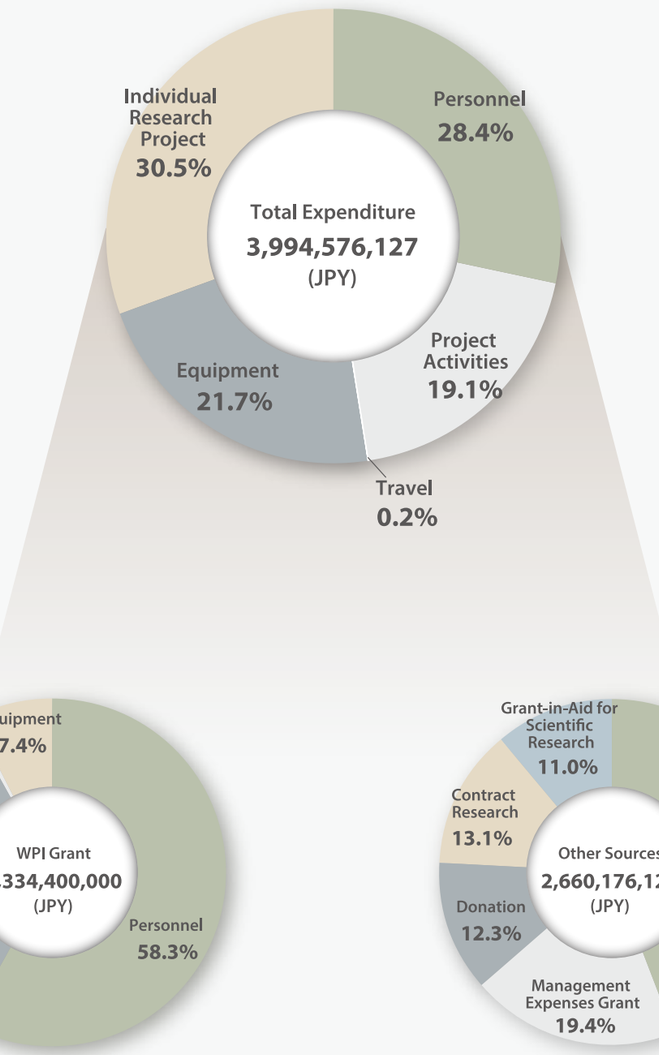
Young Investigator Award, The Japanese Association of Cancer Immunology

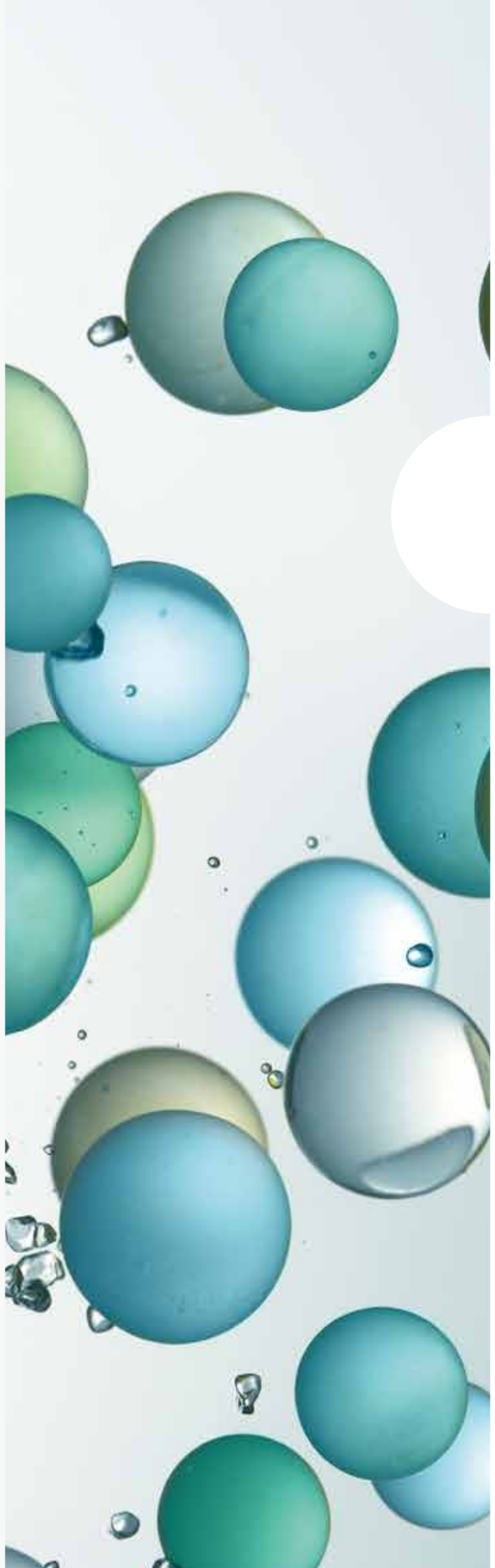
Daisuke Sugiyama



Finance

Break down of total expenditure at IFReC





Research Outputs

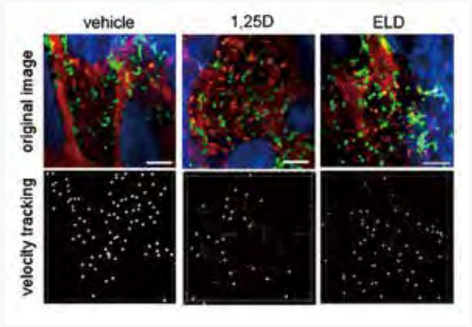
Selected Articles

S1P-mediated osteoclast precursor monocyte migration is a critical point of control in anti-bone-resorptive actions of active vitamin D

Proc Natl Acad Sci USA 110(17):7009-13, 2013.

Junichi Kikuta, Shunsuke Kawamura, Fumie Okiji, Mai Shirazaki, Sadaoki Sakai, Hitoshi Saito, and Masaru Ishii

Masaru Ishii's group show that calcitriol, which is the hormonally active form of vitamin D, and its therapeutically used analog, eldecalcitol, inhibit bone resorption by modulating this mechanism. Vitamin D analogs have been used clinically for treating osteoporosis, although the mode of its pharmacologic action remains to be fully elucidated. In this study, they found that active vitamin D reduced the expression of S1PR2, a chemorepulsive receptor for blood S1P, on circulating osteoclast precursor monocytes both in vitro and in vivo. Calcitriol- or eldecalcitol-treated monocytoid RAW264.7 cells, which display osteoclast precursor-like properties, migrated readily to S1P. Concordantly, the mobility of circulating CX3CR1+ osteoclast precursor monocytes was significantly increased on systemic administration of active vitamin D. These results show a mechanism for active vitamin D in controlling the migratory behavior of circulating osteoclast precursors, and this action should be conducive to limiting osteoclastic bone resorption in vivo.



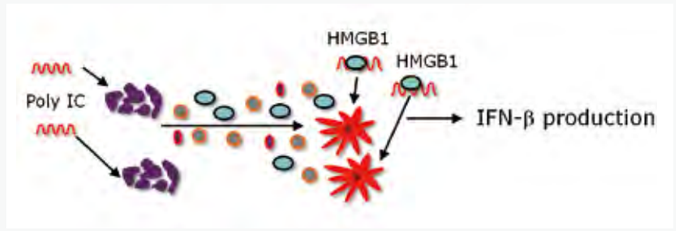
In vivo S1PR2-mediated control of migration of osteoclast precursor monocytes visualized using intravital multiphoton imaging.

Poly IC Triggers a Cathepsin D- and PS-1-Dependent Pathway to Enhance Cytokine Production and Mediate Dendritic Cell Necroptosis

Immunity 38(4):717-28, 2013.

Jian Zou, Taro Kawai, Tetsuo Tsuchida, Tatsuya Kozaki, Hiroki Tanaka, Kyung-Sue Shin, Himanshu Kumar, and Shizuo Akira

RIG-I-like receptors (RLRs) sense virus-derived RNA or polyinosinic-polycytidylic acid (poly IC) to exert antiviral immune responses. Akira's group found the mechanisms underlying the adjuvant effects of poly IC. Poly IC was taken up by dendritic cells (DCs), and it induced lysosomal destabilization, which, in turn, activated an RLR-dependent signaling pathway. Upon poly IC stimulation, cathepsin D was released into the cytoplasm from the lysosome to interact with IPS-1, an adaptor molecule for RLRs. This interaction facilitated cathepsin D cleavage of caspase 8 and the activation of the transcription factor NF-κB, resulting in enhanced cytokine production. Further recruitment of the kinase RIP-1 to this complex initiated the necroptosis of a small number of DCs. HMGB1 released by dying cells enhanced IFN-β production in concert with poly IC. Collectively, these findings suggest that cathepsin D-triggered, IPS-1-dependent necroptosis is a mechanism that propagates the adjuvant efficacy of poly IC.



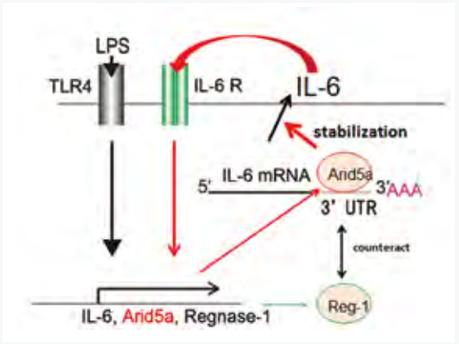
HMGB1 released by dying dendritic cells enhanced IFN-β production in concert with poly IC.

Arid5a controls IL-6 mRNA stability, which contributes to elevation of IL-6 level in vivo

Proc Natl Acad Sci USA 110(23):9409-14, 2013.

Kazuya Masuda, Barry Ripley, Riko Nishimura, Takashi Mino, Osamu Takeuchi, Go Shioi, Hiroshi Kiyonari, and Tadamitsu Kishimoto

Posttranscriptional regulation of IL-6 has been largely uncharacterized, with the exception of the ribonuclease Regnase-1, which prevents autoimmunity by destabilizing IL-6 mRNA. Kishimoto's group identified AT-rich interactive domain-containing protein 5A (Arid5a) as a unique RNA binding protein, which stabilizes IL-6 but not TNF-α mRNA through binding to the 3′ untranslated region of IL-6 mRNA. Arid5a was enhanced in macrophages in response to LPS, IL-1β, and IL-6. Arid5a deficiency inhibited elevation of IL-6 serum level in LPS-treated mice and suppressed IL-6 levels and the development of TH17 cells in experimental autoimmune encephalomyelitis. Importantly, Arid5a inhibited the destabilizing effect of Regnase-1 on IL-6 mRNA. These results indicate Arid5a plays an important role in promotion of inflammatory processes and autoimmune diseases.



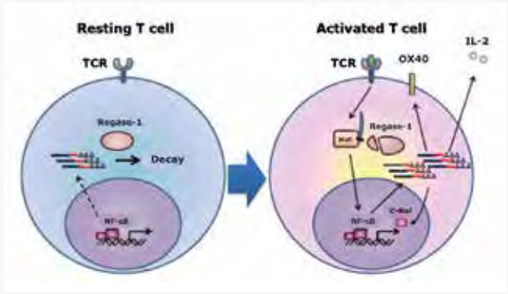
Arid5a stabilizes IL-6 through binding to the 3′ untranslated region of IL-6 mRNA.

Malt-1-induced cleavage of Regnase-1 in CD4+ Helper T cells regulates immune activation

Cell 153(5):1036-49, 2013.

Takuya Uehata, Hidenori Iwasaki, Alexis Vandenbon, Kazufumi Matsushita, Eduardo Hernandez-Cuellar, Kanako Kuniyoshi, Takashi Satoh, Takashi Mino, Yutaka Suzuki, Daron M. Standley, Tohru Tsujimura, Hiromi Rakugi, Yoshitaka Isaka, Osamu Takeuchi, and Shizuo Akira

Although Regnase-1 (also known as Zc3h12a and MCPIP1) inactivation leads to development of an autoimmune disease characterized by T cell activation and hyperimmunoglobulinemia in mice, the mechanism of Regnase-1-mediated immune regulation has remained unclear. Akira's group show that Regnase-1 is essential for preventing aberrant effector CD4+ T cell generation cell autonomously. Moreover, in T cells, Regnase-1 regulates the mRNAs of a set of genes, including c-Rel, Ox40, and Il2, through cleavage of their 30 UTRs. Interestingly, T cell receptor (TCR) stimulation leads to cleavage of Regnase-1 at R111 by Malt1/paracaspase, freeing T cells from Regnase-1-mediated suppression. Furthermore, Malt1 protease activity is critical for controlling the mRNA stability of T cell effector genes. Collectively, these results indicate that dynamic control of Regnase-1 expression in T cells is critical for controlling T cell activation.



TCR signaling leads to Regnase-1 cleavage by Malt1, facilitating T cell activation.

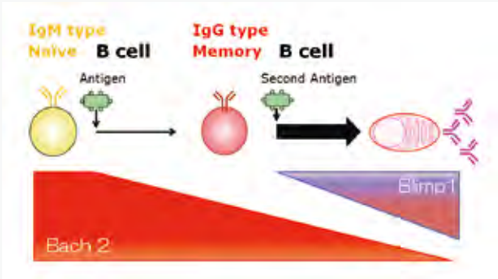
Selected Articles

Repression of the Transcription Factor Bach2 Contributes to Predisposition of IgG1 Memory B Cells toward Plasma Cell Differentiation

Immunity 39(1):136-47, 2013.

Kohei Kometani, Rinako Nakagawa, Ryo Shinnakasu, Tomohiro Kaji, Andrei Rybouchkin, Saya Moriyama, Koji Furukawa, Haruhiko Koseki, Toshitada Takemori, and Tomohiro Kurosaki

Memory B cells are essential for generating rapid and robust secondary antibody responses. It has been thought that the unique cytoplasmic domain of IgG causes the prompt activation of antigen-experienced IgG memory B cells. Kurosaki's group have generated a mouse containing IgG1 B cells that have never encountered antigen. They found that, upon challenge, antigen-experienced IgG1 memory B cells rapidly differentiated into plasma cells, whereas nonexperienced IgG1 B cells did not, suggesting the importance of the stimulation history. In addition, their results suggest that repression of the Bach2 transcription factor, which results from antigen experience, contributes to predisposition of IgG1memoryBcells to differentiate into plasma cells.



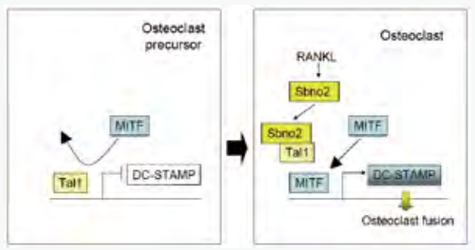
Antigen experience induces repression of Bach2 in IgG1 memory B cells, which in turn contributes to rapid humoral recall responses.

Strawberry notch homologue 2 regulates osteoclast fusion by enhancing the expression of DC-STAMP

J Exp Med. 210(10):1947-60, 2013.

Kenta Maruyama, Satoshi Uematsu, Takeshi Kondo, Osamu Takeuchi, Mikaël M. Martino, Takumi Kawasaki, and Shizuo Akira

Osteoclasts are multinucleated cells formed by fusion of mononuclear precursors in response to receptor activator of nuclear factor κB (NF-κB) ligand (RANKL). Akira's group found that RANKL induced expression of the DExD/H helicase family corepressor strawberry notch homologue 2 (Sbno2). Sbno2-deficient mice exhibited increased bone mass due to impaired osteoclast fusion. Expression of dendritic cell-specific transmembrane protein (DC-STAMP), a critical player in osteoclast fusion, was significantly attenuated, and cell fusion of Sbno2-deficient osteoclasts was rescued by DC-STAMP. Sbno2 directly bound to T cell acute lymphocytic leukemia 1 (Tal1) and attenuated its inhibition of DC-STAMP expression, leading to activation of the DC-STAMP promoter by microphthalmia-associated transcription factor (MITF). Thus, Sbno2 plays a pivotal role in bone homeostasis in vivo by fine-tuning osteoclast fusion.



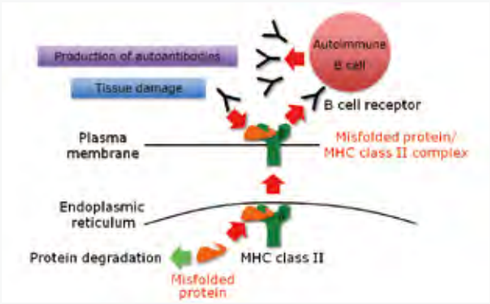
Sbno2 directly bound to Tal1 and attenuated its inhibition of DC-STAMP expression, leading to activation of the DC-STAMP promoter by MITF.

Autoantibodies to IgG/HLA class II complexes are associated with rheumatoid arthritis susceptibility

Proc Natl Acad Sci USA, 2014. in press.

Hui Jin, Noriko Arase, Kouyuki Hirayasu, Masako Kohyama, Tadahiro Suenaga, Fumiji Saito, Kenji Tanimura, Sumiko Matsuoka, Kosuke Ebina, Kenrin Shi, Noriko Toyama-Sorimachi, Shinsuke Yasuda, Tetsuya Horita, Ryosuke Hiwa, Kiyoshi Takasugi, Koichiro Ohmura, Hideki Yoshikawa, Takashi Saito, Tatsuya Atsumi, Takehiko Sasazuki, Ichiro Katayama, Lewis L. Lanier, and Hisashi Arase

Arase's group found a unique function of HLA class II molecules: their ability to aberrantly transport cellular misfolded proteins to the cell surface without processing to peptides. Rheumatoid factor (RF) is an autoantibody that binds to denatured IgG or Fc fragments of IgG and is detected in 70-80% of RA patients but also in patients with other diseases. They report that intact IgG heavy chain (IgGH) is transported to the cell surface by HLA class II via association with the peptide-binding groove and that IgGH/HLA class II complexes are specifically recognized by autoantibodies in RF-positive sera from RA patients. In contrast, autoantibodies in RF-positive sera from non-RA individuals did not bind to IgGH/HLA class II complexes. Of note, a strong correlation between autoantibody binding to IgG complexed with certain HLA-DR alleles and the odds ratio for that allele's association with RA was observed. Their findings suggest that IgGH complexed with certain HLA class II alleles is a target for autoantibodies in RA, which might explain why these HLA class II alleles confer susceptibility to RA.



IgGH/HLA class II complexes are recognized by autoantibodies in RF-positive sera from RA patients.

Articles, Lectures, and Awardees

FY	*Articles*	*Lectures at International Meetings*	*Awardee (International & Domestic)*
2011	153	96	13
2012	179	99	15
2013	**181**	**127**	**11**

Papers on high-impact journals by author(s) affiliated with IFReC

FY	*Cell*	*Immunity*	*JEM*	*Nature*	*Nat Immunol*	*Nat Med*	*Nat Neurosci*	*Science*	*New Eng J Med*	*Nat Rev Immunol*	*High Impact Total*
IF*	*32.4*	*24.2*	*14.8*	*36.0*	*25.7*	*25.4*	*14.2*	*31.4*	*51.7*	*33.1*	-
2011	1	9	3	0	4	2	0	1	0	0	**20**
2012	1	9	3	3	2	1	0	1	0	1	**21**
2013	2	3	1	2	2	0	1	1	1	3	**16**

Data from WEB OF SCIENCE™

*The impact factor means the frequency with which the "average article" in a journal has been cited in a given period of time.

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Lectures by PIs

Lecturers	Meeting	Country	Date
Shimon Sakaguchi	Karolinska Research Lectures at Nobel Forum	Sweden	Apr. 4
Shizuo Akira	Emory Vaccine Center Special Seminar	USA	Apr. 15
Shizuo Akira	Harvard Medical School Committee on Immunology Seminar	USA	Apr. 17
Tadamitsu Kishimoto	Annual Meeting of Japanese Society of Pediatric	Japan	Apr.19
Shimon Sakaguchi	Eberly Distinguished Lectureship in Immunology at Pittsburgh University	USA	Apr. 25
Cevayir Coban	22nd Turkish Immunology Conference	Turkey	Apr. 27
Ken Ishii	22nd Turkish Immunology Conference	Turkey	Apr. 27
Kiyoshi Takeda	American Association of Immunologists Annual Meeting 2013	USA	May 3
Shimon Sakaguchi	American Association of Immunologists Annual Meeting 2013	USA	May 3
Takashi Saito	American Association of Immunologists Annual Meeting 2013	USA	May 3
Cevayir Coban	10th Prof. Dr. Fethi Tezok Microbiology and Infectious Diseases Symposium	Turkey	May 3
Kiyoshi Takeda	33rd Korean College of Rheumatology Annual Scientific Meeting	Korea	May 10
Kazuya Kikuchi	Advanced Practical Course in Chemical Biology	Germany	May 16
Ken Ishii	21st International Symposium of Macrophage Molecular and Cellular Biology	Japan	May 20
Tsuneyasu Kaisho	Joint International Meeting of the 78th Meeting of the Japanese Society of Interferon and Cytokine Research and the 21st International Symposium on Molecular Cell	Japan	May 20
Tsuneyasu Kaisho	Biology of Macrophage (JSICR-MMCB 2013)	Japan	May 20
Jun Hatazawa	BRAIN & Brain PET 2013	Chaina	May 20
Yutaka Hata	IEEE 43rd International Symposium on Multiple-Valued Logic	Japan	May 22
Tomohiro Kurosaki	Okinawa Institute of Science and Technology Seminar	Japan	May 24
Toshio Yanagida	8th Symposium of Asian Biophysics Association	Korea	May 26
Masaru Ishii	10th Anniversary Symposium of the Hwason Optical Imaging Workshop & Symposium (Plenary)	Korea	May 28
Shizuo Akira	78th Cold Spring Harbor Symposium on Quantitative Biology "Immunity & Tolerance"	USA	May 30
Shimon Sakaguchi	40th IMSUT (The Institute of Medical Science, The University of Tokyo) Founding Commemorative Symposium	Japan	May 31
Shimon Sakaguchi	6th International Workshop of Kyoto T Cell Conference	Japan	Jun. 3
Tadamitsu Kishimoto	Charles A. Janeway Jr. MD Memorial Symposium	USA	Jun. 4
Tsuneyasu Kaisho	France-Japan Joint Forum "Frontiers in Innate Immunity"	France	Jun. 5
Shimon Sakaguchi	Immunology Interest Group Seminar Series in NIH	USA	Jun. 12
Shimon Sakaguchi	Program in Immunology Seminar Series University of Alabama at Birmingham	USA	Jun. 13
Toshio Yanagida	CREST & PRESTO Joint Symposium for Photonics	Japan	Jun. 20
Shizuo Akira	13th Annual Meeting of the Federation of Clinical Immunology Societies (FOCIS2013)	USA	Jun. 28
Toshio Yanagida	2nd HDP International Symposium on Multi-Level Systems Biology	Japan	Jun. 28
Kazuya Kikuchi	7th International Conference on Materials for Advanced Technologies (ICMAT 2013)	Singapore	Jun. 30
Shimon Sakaguchi	34th Japanese Society of Inflammation and Regeneration Annual Meeting	Japan	Jul. 2
Taroh Kinoshita	3rd Austria/Japan Seminar on Comparative and Developmental Glycobiology	Japan	Jul. 3
Taroh Kinoshita	FASEB SRC-Protein Lipidation, Signaling, and Membrane Domains	USA	Jul. 15
Taroh Kinoshita	Seminar at Department of Microbiology, University of Massachusetts	USA	Jul. 18
Kazuya Kikuchi	27th Annual Symposium of The Protein Society	USA	Jul. 20
Masahiro Yamamoto	XIVth International Congress of Protistology	Canada	Jul. 28
Takashi Saito	Frontiers in Immunology Conference	Japan	Jul. 29
Tomohiro Kurosaki	JSI Immunology Summer School	Japan	Jul. 31
Hisashi Arase	15th JSI Immunology Summer School	Japan	Aug. 1
Masaru Ishii	FASEB Summer Research Conference	Japan	Aug. 5
Kazuya Kikuchi	15th Asian Chemical Congress (15 ACC)	Singapore	Aug.19
Tomohiro Kurosaki	5th International Conference on B cell and Autoimmunity	Italy	Aug. 20
Masaru Ishii	Cold Spring Harbor Conference Asia	China	Aug. 20
Tadamitsu Kishimoto	15th International Congress of Immunology	Italy	Aug. 22
Shimon Sakaguchi	15th International Congress of Immunology	Italy	Aug. 22
Shizuo Akira	15th International Congress of Immunology	Italy	Aug. 23
Hisashi Arase	67th Annual Meeting of Tohoku Society of Microbiology	Japan	Aug. 30
Tomohiro Kurosaki	Immune-related Pathologies: Understanding Leukocyte Signaling and Emerging Therapies – IMPULSE 2013	Hungary	Sep. 2
Nicholas Isaac Smith	EMSG Ardgour Symposium, Scotland	Scotland	Sep. 3
Rikinari Hanayama	86th Annual Meeting of the Japanese Biochemical Society	Japan	Sep. 12
Shizuo Akira	Annual SAB Meeting Dallas PO1 Genetic Analysis of Resistance to Viral Infection	USA	Sep. 16
Ken Ishii	5th International Conference on Crossroads between Innate and Adaptibe Immunity	Greece	Sep. 21
Jun Hatazawa	5th Asia-Pacific Symposium on Radiochemistry '13	Japan	Sep. 25
Shimon Sakaguchi	4th International GK Symposium Regulators of Adaptive Immunity	Germany	Sep. 27
Takashi Saito	4th International GK Symposium Regulators of Adaptive Immunity	Germany	Sep. 27
Tadamitsu Kishimoto	International Cytokine Meeting	USA	Sep. 29
Shimon Sakaguchi	4th International Workshop on Humanized Mice	Korea	Sep. 30
Tadamitsu Kishimoto	Genentech Institute Seminar	USA	Oct. 1
Masaru Ishii	72nd Annual Meeting of the Japanese Cancer Association	Japan	Oct. 3
Hisashi Arase	13th Kyoto Clinical Immunology Seminar	Japan	Oct. 5
Toshio Yanagida	7th GREEN Symposium	Japan	Oct. 9
Jun Hatazawa	Japan-Russia International Symposium for Medical Exchange	Japan	Oct. 10
Shimon Sakaguchi	75th Japan Society for Hematology Annual Meeting	Japan	Oct. 11
Kazuya Kikuchi	9th AFMC International Medicinal Chemistry Symposium (AIMECS 2013)	Taiwan	Oct. 15
Taroh Kinoshita	Seminar at School of Pharmaceutical Science, Busan National University	Korea	Oct. 16
Taroh Kinoshita	Fall International Convention of the Pharmaceutical Society of Korea	Korea	Oct. 17
Kiyoshi Takeda	FIMSA International Symposium on Autoimmune Diseases	China	Oct. 17
Ken Ishii	7th Annual Vaccine Renaissance Conference	USA	Oct. 18
Toshio Yanagida	Joint Weizmann-MBI Mechanobiology Conference "Dynamic Architecture of Cell and Tissues"	Singapore	Oct. 21
Toshio Yanagida	51st Annual Meeting of the Biophysical Society of Japan	Japan	Oct. 27
Ken Ishii	7th Vaccine & ISV Congress	Spain	Oct. 28
Atsushi Kumanogoh	EMBO Workshop	France	Oct. 31
Toshio Yanagida	Workshop on Modeling Biomolecular Systems in Cellular Environments	Japan	Nov. 1
Tadamitsu Kishimoto	100th Anniversary of Kitasato Institute	Japan	Nov. 5
Tsuneyasu Kaisho	2013 Agricultural Biotechnology Symposium "Immune Modulation and Bioconvergence"	Korea	Nov. 6
Kiyoshi Takeda	2013 Fall Conference of the Korean Association of Immunologists	Korea	Nov. 7
Tsuneyasu Kaisho	2013 Fall Conference of the Korean Association of Immunologists	Korea	Nov. 7
Shimon Sakaguchi	12th International Xenotransplantation Association Congress 2013	Japan	Nov. 10
Ken Ishii	2013 Symposium on Complex Biodynamics and Networks	Japan	Nov. 12
Tomohiro Kurosaki	2013 Oversea Scholar Seminar, Tsinghua University	China	Nov. 14
Jun Hatazawa	CJK2013 The 6th CJK Conference on Nuclear Medicine	Korea	Nov. 15
Cevayir Coban	International Symposium TCUID 2013	Japan	Nov. 18
Kazuya Kikuchi	International Symposium TCUID 2013	Japan	Nov. 18
Jun Hatazawa	ICRT2013 8th International Conference on Radiopharmaceutical Therapy	Philippines	Nov. 20
Kazuya Kikuchi	2nd Japan-France Coordination Chemistry Symposium	Japan	Nov. 24
Shimon Sakaguchi	41st Japan Society for Clinical Immunology Meeting	Japan	Nov. 27
Shimon Sakaguchi	67th Japanese Society of Allergology Meeting	Japan	Nov. 28
Takashi Saito	43rd Annual Scientific Meeting Australasian Society for Immunology	New Zealand	Dec. 2
Ken Ishii	Key Lab Medical Molecular Virology Shanghai Medical College, Fudan University	China	Dec. 2
Tadamitsu Kishimoto	Annual Meeting of Japanese Society of Molecular Biology	Japan	Dec. 3
Hisashi Arase	Germany-Japan Immunology Seminar 2013	Japan	Dec. 5
Tadamitsu Kishimoto	Germany-Japan Immunology Seminar 2013	Japan	Dec. 5
Kiyoshi Takeda	Germany-Japan Immunology Seminar 2013	Japan	Dec. 5
Shimon Sakaguchi	Germany-Japan Immunology Seminar 2013	Japan	Dec. 5
Takashi Saito	Germany-Japan Immunology Seminar 2013	Japan	Dec. 5
Kazuya Kikuchi	International Conference on Emerging Trends in Chemical Science (IETC2013)	India	Dec. 5
Shimon Sakaguchi	42th Japanese Society for Immunology Annual Meeting	Japan	Dec. 11
Tsuneyasu Kaisho	42th Japanese Society for Immunology Annual Meeting	Japan	Dec. 11
Masaru Ishii	42th Japanese Society for Immunology Annual Meeting	Japan	Dec. 11
Jun Hatazawa	ARCCNM2013 The 12th Annual General Meeting of the ARCCNM	India	Dec. 12
Tomohiro Kurosaki	36th Annual Meeting of the Molecular Biology Society of Japan	Japan	Dec. 13
Tomohiro Kurosaki	Memorial Symposium for Professor Tatsushi Muta	Japan	Dec. 21
Toshio Yanagida	25th Rheumatism Seminar, Nakanoshima	Japan	Dec. 21
Masaru Ishii	Gordon Research Conferences	USA	Jan. 12
Daron M. Standley	Antibody Design, Modeling, and Applications	Japan	Jan. 14
Toshio Yanagida	German-Japanese Symposium 2014	Germany	Jan. 16
Tadamitsu Kishimoto	Keystone Symposia	Canada	Jan. 17
Shimon Sakaguchi	Keystone Symposia	Canada	Jan.17
Nicholas Isaac Smith	Annual Meeting, The Physical Society of Republic of China 2014	Taiwan	Jan. 22
Taroh Kinoshita	IUBMB 10th International Symposium on Roles of Eukaryotic Cell Surface Macromolecules	India	Jan. 24
Tomohiro Kurosaki	World-leading Immunology Workshop at Institute of Development, Aging, and Cancer, Tohoku University	Japan	Jan. 29
Tadamitsu Kishimoto	Senri Life Science International Symposium "Innate Immunity, Cytokines, and Immune Regulation"	Japan	Jan. 31
Shimon Sakaguchi	Senri Life Science International Symposium "Innate Immunity, Cytokines, and Immune Regulation"	Japan	Jan. 31
Tomohiro Kurosaki	Keystone Symposia: Biolgy of B Cell Reponses	USA	Feb. 11
Rikinari Hanayama	10th Miyazaki Science Camp	Japan	Feb. 14
Toshio Yanagida	GCOE-CNR Workshop: Past and Future Directions of Cognitive Neuroscience Robotics	Japan	Feb. 24
Nicholas Isaac Smith	Japan Taiwan Bilateral Conference on Biomedical and Plasmonic Imaging	Taiwan	Feb. 26
Shimon Sakaguchi	13th Japanese Society for Regenerative Medicine Meeting	Japan	Mar. 4
Shimon Sakaguchi	10th German Society of Immunology Spring School on Immunology	Germany	Mar. 9
Shizuo Akira	8th World Immune Regulation Meeting-VIII2014	Switzerland	Mar. 19
Ken Ishii	World Vaccine Congress & Expo	USA	Mar. 24
Tadamitsu Kishimoto	Conference on Advances in Targeted Therapies	Greece	Mar. 26
Jun Hatazawa	1st KURRI International Workshop on Neutron Capture Therapy	Japan	Mar. 27

Access Map

SUITA CAMPUS Osaka University

Access from the nearest station

By Train

15 min. walk east from Kita-Senri Station on the Hankyu Senri Line.

By Monorail

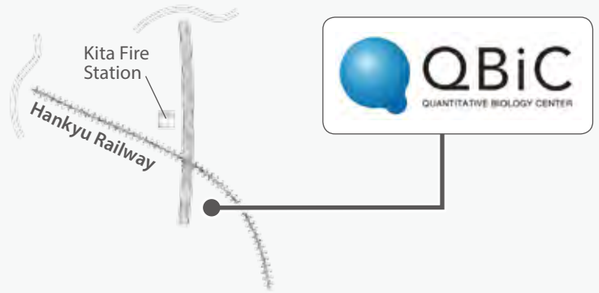
25 min. walk from Handai Byoin Mae Station on the Osaka Monorail.

By Hankyu Bus

Option1:
Get on the bus bound for "Handai Honbu Mae" or "Ibaraki Mihogaoka" from Senri-Chuo Station.Get off at Handai Honbu Mae, walk 15 min.
Option2:
Get on the bus bound for "Onohara Higashi, Fuji Kasai" from Senri-Chuo tation. Get off at Handai-guchi, walk 5 min.

By Kintetsu Bus

Get on the bus bound for "Handai Honbu Mae" or "Ibaraki Mihogaoka" from Hankyu Ibarakishi Station (via JR Ibaraki Station). Get off at Handai Honbu Mae, walk 15 min.



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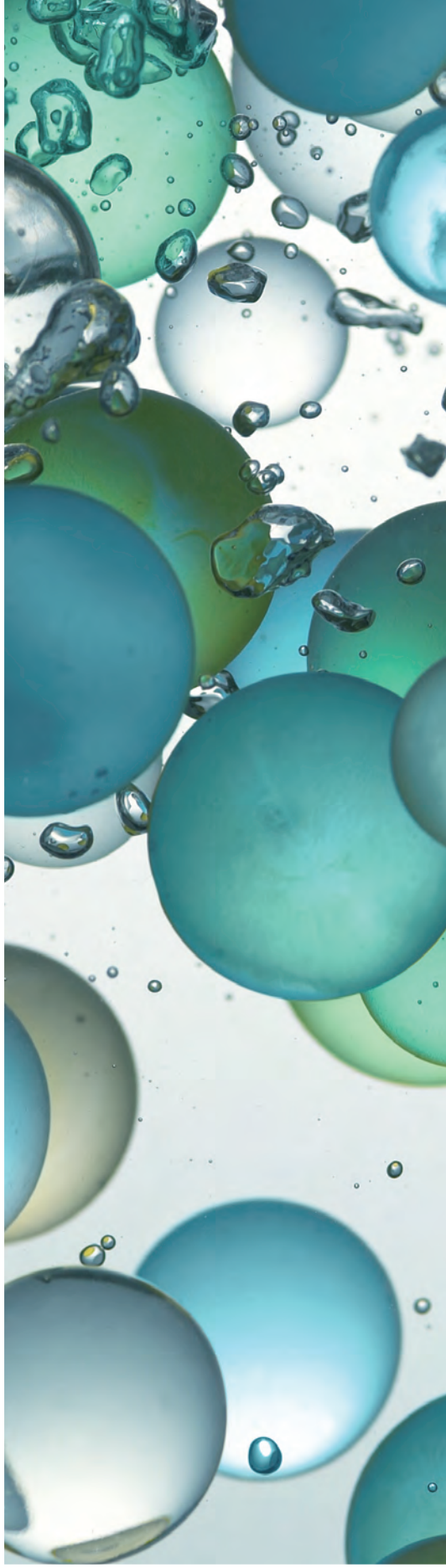

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