World Premier International
Research Center

Osaka University

Immunology Frontier Research Center

Annual Report of IFReC FY 2012

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World Premier International
Research Center Initiative

WPI Osaka University
iFReC

• Contents

- **Message from the Director** 1
- **Organization Chart** 2
- **Committee and Advisory Board for IFReC** 4
- **Administrative Office of IFReC** 6
- **Laboratories** 7
  - Immunology Group 8
  - Imaging Group 40
  - Bioinformatics Group 54
- **Symposia & Seminars** 61
  - International Symposium “Dynamism of Immune Reactions & Regulation” 62
  - “LICHT Leica Center” Opening Seminar 64
  - Kishimoto Foundation Lecture 65
  - French-Japanese Immunology Meeting 66
  - IFReC Colloquia 67
  - IFReC Seminars 68
- **Events** 71
  - IFReC Retreat 2012 72
  - NIF Winter School on Advanced Immunology 2013 74
  - Explanatory Meetings for Members 76
  - Extra-Curricular Activities by Liaison Office Japanese Lessons / Happy Hour 77
- **Outreach Activities** 79
  - WPI Joint Outreach Symposium 80
  - AAAS 2013 in Boston 81
  - Science and Technology Festa in Kyoto 82
  - Science Café Series “Café on the Edge” 83
  - Lectures for Career Development 84
  - Lectures at High Schools 84
- **Research Projects** 85
  - Research Support Program for Combined Research Fields 86
  - Funds for Young Researchers 88
  - Dual Mentor Program / IFReC Young Scientist Support Program for Research Abroad
  - FIRST Program : AKIRA Project 89
- **Data** 93
  - Facilities 94
  - LICHT Leica Center 95
  - Kishimoto Foundation Fellowship 96
  - Members 97
  - Visitors 98
  - Awards 100
  - Finance 102
- **Research Outputs** 103
  - Selected Articles 104
  - Publications 108
  - Lectures by PIs 116
- **Access Map** 118

Message from the Director

As the Director of Immunology Frontier Research Center (WPI-IFReC) of Osaka University, I am very pleased to provide the IFReC annual report for the fiscal year 2012.

Since the start of IFReC in 2007, we have been aiming for the establishment of a "Visible International Research Center of Immunology," incorporating various suggestions from the WPI Program Director, the Program Officer, and the WPI Management Committee. The ratio of IFReC researchers from overseas has already reached 30%, and the total number of IFReC members has exceeded 240 persons, achieving the target level of the WPI program.

In the WPI interim evaluation report compiled by the committee at the end of fiscal year 2011, IFReC was awarded a score of “A” for its implementation of the WPI program, which means “It should be possible for the center to achieve its initial goals by continuing its current efforts.” We take this evaluation result as an objective indication that IFReC should be preserved as one of the institutes belonging to the University after the ten-year WPI grant is concluded. In alignment with this prospective view, the operating officers at IFReC, Osaka University, and the Ministry of Education, Culture, Sports, Science & Technology in Japan are plotting the future concept of IFReC at Osaka University.

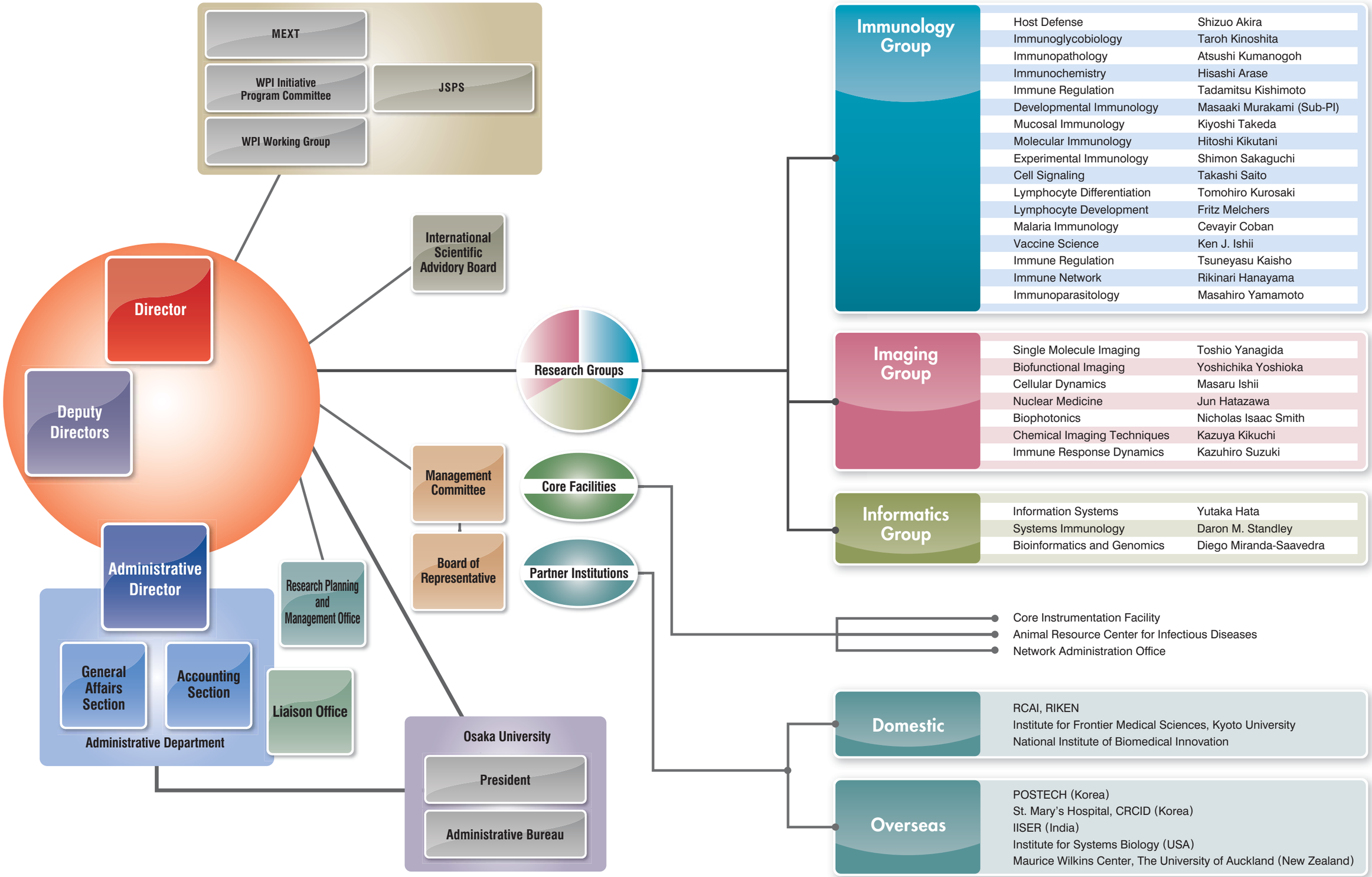
In 2013, a new facility at the IFReC Research Building, the “Live Immuno-imaging Facility,” will go into full-scale operation. I firmly believe that this facility will provide further contributions to the advancement of the studies underway in combined research fields at IFReC, especially immunology and imaging. Naturally, the activity of a research institute cannot be estimated only by the number of persons involved in the research, or by its facilities, no matter how gorgeous they may be. Lively discussions and viable communication between researchers are essential for development at IFReC, both now and in the future. To that purpose, we have established and developed an original grant, the "Research Support Program for Combined Research Fields" to promote collaborative studies at IFReC. In addition, we founded the “Dual Mentor Program” for young researchers, so that they might be trained by a number of instructors in different research fields. Furthermore, IFReC organized the “First IFReC Retreat,” as an opportunity for all of the staff of IFReC to share the concept of IFReC, and communicate with each other in order to promote interdisciplinary research.

As one of the international events conducted in fiscal year 2012, IFReC held the international symposium "Dynamism of Immune Reactions & Regulation" on May 22nd and 23rd, 2012. This symposium was originally scheduled in 2011 and cancelled due to the Great East Japan Earthquake. On this occasion, we invited top-level scientists from all around the world as speakers, and welcomed many participants to Osaka. In January, 2013, IFReC jointly hosted the “NIF (Network of Immunology Frontier) Winter School” with the Singapore Immunology Network. IFReC sent several lecturers and students to this educational training camp for young immunologists.

All of these activities will amount to far less than their inherent worth if the researchers working at IFReC do not continue to produce premier, top-level research results. In this respect, we have produced satisfactory results in FY 2012, and we fully intend to contribute much more to scientific progress through immunology research and education, aiming for a “World-class, Well-known Visible International Research Center of Immunology.”

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

Organization Chart



Committee and Advisory Board for IFReC

World Premier International Research Center Initiative (WPI)

Program Committee Members List

As of March, 2013

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

Program Director (PD)

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

Working Group Leaders and Assigned Members

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	Professor, Department of Immunology and Cell Biology, Graduate School of Medicine, Kyoto University
Kazuhiko Yamamoto	Professor and Chair, Department of Allergy and Rheumatology, Graduate School of Medicine, The University of Tokyo
Günter J. Hämmerling	Director of the Institute for Immunology and Genetics, German Cancer Research Center in Heidelberg
Hisataka Kobayashi	Associate Scientist, Molecular Imaging Program, National Cancer Institute, National Institutes of Health
Philippe Kourilsky	Professor, College de France
Diane Mathis	Chief, Division of Immunology, Department of Microbiology and Immunobiology, Harvard Medical School

International Advisory Board Members of IFReC

As of April, 2013

Jeffrey Ravetch	Laboratory of Molecular Genetics and Immunology, The Rockefeller University	Immunology
Chris Goodnow	John Curtin School of Medical Research and Australian Phenomics Facility, The Australian National University	
Richard Locksley	Departments of Medicine and Microbiology/Immunology, University of California, San Francisco	
Anne O'Garra	Division of Immunoregulation, The National Institute for Medical Research	
Lewis Lanier	Department of Microbiology & Immunology, University of California, San Francisco	
Kiyoshi Takatsu	Toyama Prefectual Institute for Pharmaceutical Research	
Kayo Inaba	Graduate School of Biostudies, Kyoto University	
Yasuyoshi Watanabe	Center for Molecular Imaging Science, RIKEN Kobe Institute	Imaging
Masamitsu Iino	Graduate School of Medicine, The University of Tokyo	
Yale Goldman	Pennsylvania Muscle Institute, University of Pennsylvania	
Akinori Kidera	Graduate School of Integrated Science, Yokohama City University	Informatics
Hiroyuki Toh	The Computational Biology Research Center, The National Institute of Advanced Industrial Science and Technology	
David Westhead	School of Biochemistry and Molecular Biology, Leeds University	
Vladimir Brusic	Dana-Farber Cancer Institute, Harvard Medical School	
Mo Jamshidi	Department of Electrical and Computer Engineering, University of Texas San Antonio	
Philip Chen	Faculty of Science and Technology, University of Macau	
Takeshi Yamakawa	Fuzzy Logic Systems Institute	

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

- IFReC Advisory Board Members evaluate PI’s scientific achievements.

Administrative Office of IFReC

General Affairs Section

- Support for foreign researchers
- Procedure of employment & acceptance of staff
- Social insurance / Employment insurance
- Public dormitory for Osaka University Workers
- Procedure related to international students
- Management of work hours
- Procedure related to patents
- Issuing various certifications

Accounting Section

- Budget drafting / implementation / management
- Procedure for purchasing
- Acceptance and implementation of third-party funding
- Payment of payroll, travel expense and honorarium
- Procedure for health insurance
- Management of buildings and assets
- Procedure related to RI

Research Planning & Management Office

- Research promotion & support
  (Consultation for grants & patents, Combined research program, *etc.*)
- Establishing research environments
  (Facility & Safety management, Research agreement, *etc.*)
- Fostering young scientists
  (Winter school, Faculty retreat, Dual mentor program, *etc.*)
- Organizing scientific events
  (Symposia, Seminars, *etc.*)
- Public relations
  (Publishing, Website, Outreach to citizens, *etc.*)
- WPI evaluation issues
  (Progress report, Advisory board meeting, *etc.*)

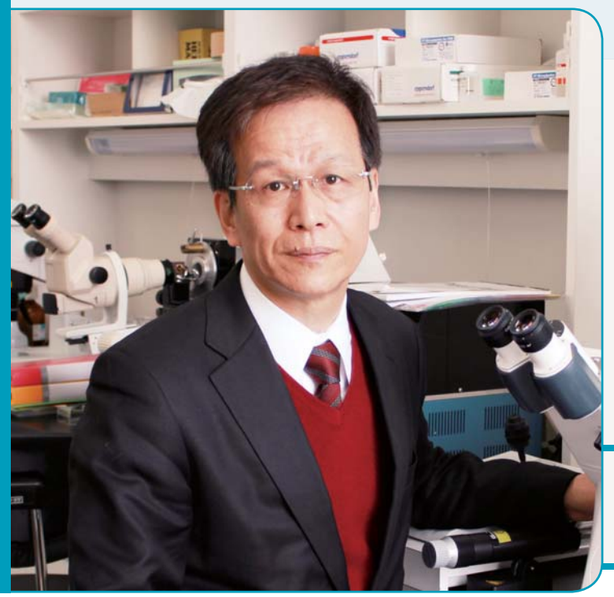
Liaison Office

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

Laboratories

Immunology Frontier Research Center

Host Defense



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Shizuo Akira, MD/PhD

RESEARCH REPORT

We have studied the innate immune system, which is an evolutionally conserved host defense mechanism against microbes. Pattern-recognition receptors (PRRs), which recognize microbial components, are critically involved in induction of innate immunity. PRRs sense microorganisms ranging from bacteria to fungi, protozoa and viruses, and play a major role in induction of host defense response. However, PRRs also sense environmental irritants and host-derived stimulatory factors, such as asbestos and uric acid crystals, and their activation causes development of inflammatory diseases. Thus, innate immune system can be a double-edged sword for hosts. To gain a deeper understanding of innate immune system, we have addressed following issues; a mechanism of PRR-mediated elimination of microbes, a mechanism of regulation of PRR signaling pathway and a novel function of innate immune cells.

1 TLR-mediated elimination of HIV-1 by neutrophil extracellular traps

Neutrophils mediate host defense response by producing neutrophil extracellular traps (NETs), which are genomic DNA-based net-like structures that capture bacteria and fungi. Although NETs also express antiviral factors, such as myeloperoxidase and α-defensin, the involvement of NETs in antiviral responses remained unclear. We found that NETs capture human immunodeficiency virus (HIV)-1 and promote HIV-1 elimination through myeloperoxidase and α-defensin (Saitoh T et al, *Cell Host Microbe*, 2012). Neutrophils detect HIV-1 by Toll-like receptors (TLRs) TLR7 and TLR8, which recognize viral nucleic acids. Engagement of TLR7 and TLR8 induces the generation of reactive oxygen species that trigger NET formation, leading to NET-dependent HIV-1 elimination. However, HIV-1 counteracts this response by inducing C-type lectin CD209-dependent production of interleukin (IL)-10 by dendritic cells to inhibit NET formation. Therefore, NET formation is an antiviral response that is counteracted by HIV-1.

2 Microtubule-dependent activation of the NLRP3-inflamamsome activation

NLRP3 forms an inflammasome with its adaptor ASC, and its excessive activation can cause inflammatory diseases. However, little was known about the mechanisms that control assembly of the inflammasome complex. We found that microtubules mediated assembly of the NLRP3 inflammasome (Misawa T et al., *Nat Immunol*., 2013). Inducers of the NLRP3 inflammasome caused aberrant mitochondrial homeostasis to diminish the concentration of the coenzyme NAD, which in turn inactivated the NAD-dependent α-tubulin deacetylase sirtuin 2; this resulted in the accumulation of acetylated α-tubulin. Acetylated α-tubulin mediated the dynein-dependent transport of mitochondria and subsequent apposition of ASC on mitochondria to NLRP3 on the endoplasmic reticulum. Therefore, in addition to direct activation of NLRP3, the creation of optimal sites for signal transduction by microtubules is required for activation of the entire NLRP3 inflammasome (Figure 1).

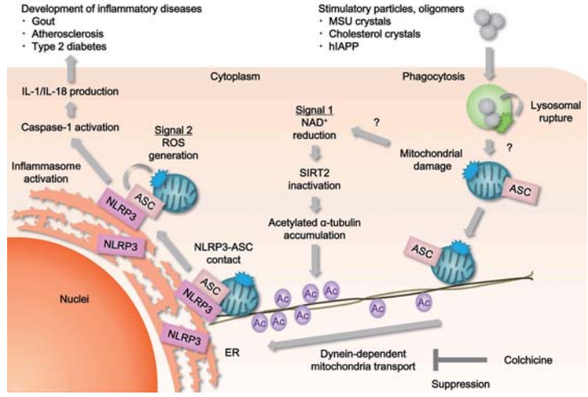

Figure 1. Microtubules mediate movement of mitochondria to promote activation of the NLRP3 inflammasome.

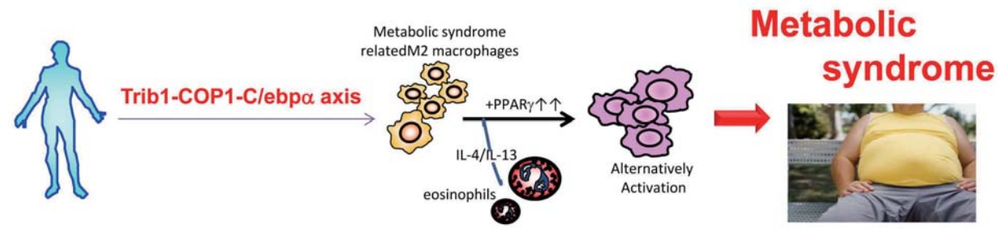

Figure 2. Trib1 is indispensable for differentiation of tissue-resident M2-like macrophages.

3 A novel role of macrophages in maintenance of adipose tissues

Macrophages consist of at least two subgroups, M1 and M2. Whereas M1 macrophages are pro-inflammatory and have a central role in host defense, M2 macrophages are associated with responses to anti-inflammatory reactions, and tissue remodeling. Genome-wide association studies in humans have implicated TRIB1 in lipid metabolism. We found that Trib1 is critical for the differentiation of tissue-resident macrophages—that share characteristics with M2 macrophages (which we term M2-like macrophages) (Satoh T et al., *Nature*, 2013). Trib1 deficiency results in a severe reduction of M2-like macrophages in various organs, including bone marrow, spleen and adipose tissues. Aberrant expression of C/EBPα in Trib1-deficient bone marrow cells is responsible for the defects in macrophage differentiation. Mice lacking Trib1 in haematopoietic cells show diminished adipose tissue mass accompanied by evidence of increased lipolysis. In response to a high-fat diet, mice lacking Trib1 in haematopoietic cells develop hypertriglyceridaemia and insulin resistance. Therefore, Trib1 is critical for adipose tissue maintenance and suppression of metabolic disorders by controlling the differentiation of tissue-resident M2-like macrophages (Figure 2).

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

Recent Publications

- Satoh T, Kidoya H, Naito H, Yamamoto M, Takemura N, Nakagawa K, Yoshioka Y, Morii E, Takakura N, Takeuchi O, Akira S. Critical role of Trib1 in differentiation of tissue-resident M2-like macrophages. ***Nature*** 495:524-8, 2013.
- Misawa T, Takahama M, Kozaki T, Lee H, Zou J, Saitoh T, Akira S. Microtubule-driven spatial arrangement of mitochondria promotes activation of the NLRP3 inflammasome. ***Nat. Immunol.*** 14:454-60, 2013.
- Kondo T, Kobayashi J, Saitoh T, Maruyama K, Ishii KJ, Barber GN, Komatsu K, Akira S, Kawai T. DNA damage sensor MRE11 recognizes cytosolic double-stranded DNA and induces type I interferon by regulating STING trafficking. ***Proc. Natl. Acad. Sci. USA.*** 110:2969-74, 2013.
- Saitoh T, Komano J, Saitoh Y, Misawa T, Takahama M, Kozaki T, Uehata T, Iwasaki H, Omori H, Yamaoka S, Yamamoto N, Akira S. Neutrophil extracellular traps mediate a host defense response to human immunodeficiency virus-1. ***Cell Host Microbe.*** 12:109-16, 2012.
- Maruyama K, Fukasaka M, Vandenbon A, Saitoh T, Kawasaki T, Kondo T, Yokoyama KK, Kidoya H, Takakura N, Standley D, Takeuchi O, Akira S. The transcription factor Jdp2 controls bone homeostasis and antibacterial immunity by regulating osteoclast and neutrophil differentiation. ***Immunity*** 37:1024-36, 2012.

Immunoglycobiology

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Taroh Kinoshita, PhD

RESEARCH REPORT

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

1 Roles of lipid moiety of GPI-APs

The primary function of GPI is to act as membrane-anchor of many cell surface proteins that directs those proteins to specific membrane domains. GPI-APs are associated with cholesterol- and sphingolipid-rich lipid microdomains or lipid rafts. For lipid raft association, lipid moiety of GPI bearing two saturated fatty-chains is critical. We previously reported (1) that nascent GPI-APs generated in the endoplasmic reticulum (ER) have a polyunsaturated fatty acid at the sn2 position, (2) that after transport to the Golgi apparatus the sn2 chain is exchanged with stearic acid (a saturated chain), and (3) that PGAP3 and PGAP2 are required for the first and the second steps, respectively, of this fatty-acid exchange. Therefore, raft compatible lipid moiety of GPI-AP is elaborated by fatty-acid remodeling in the Golgi. Using PGAP3-defective CHO mutant cells and MEF derived from PGAP3-knockout mouse, we found an unusual phenomenon that fatty-acid un-remodeled GPI-APs in general are not efficiently detected by western blotting (Figure 1) and demonstrated that fatty-acid remodeled GPI-APs have ability to homodimerize even on the PVDF membrane whereas the un-remodeled GPI-APs failed to do so. Anti-GPI-AP antibodies are usually of low affinity and efficient binding occurs to homodimerized GPI-APs but not monomeric GPI-APs. The homo-dimerizing characteristic of GPI-APs may be relevant to their biological functions (Seong J et al, ***J. Lipid Res.***, 54:1077, 2013).

We generated a systemic PGAP3 knockout mouse ($PGAP3^{-/-}$) and found that a significant number of aged $PGAP3^{-/-}$ mice developed autoimmune-like symptoms, such as increased anti-DNA antibodies, spontaneous germinal center formation and enlarged renal glomeruli with deposition of immune complexes. A possible cause for this was the impaired engulfment of apoptotic cells by resident peritoneal macrophages in $PGAP3^{-/-}$ mice. Mice with conditional targeting of PGAP3 in either B or T cells did not develop such autoimmune-like symptoms. Moreover, $PGAP3^{-/-}$ mice exhibited Th2 polarization. These data demonstrate that PGAP3-dependent fatty acid remodeling of GPI-APs has a significant role in the control of autoimmunity, possibly by the regulation of apoptotic cell clearance and Th1/Th2 balance (Wang Y et al, manuscript submitted).

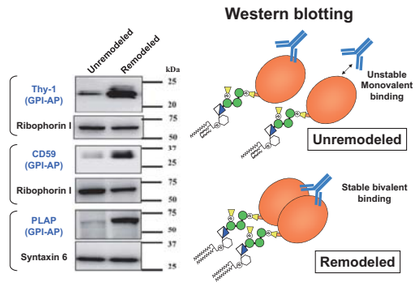

Figure 1. Homo-dimerizing nature of GPI-AP is dependent upon fatty acid remodeling.

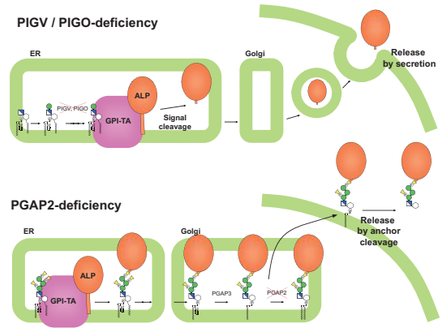

Figure 2. Different mechanisms of alkalinephosphatase release in PIGV/PIGO- and PGAP2-deficiencies.

2 Molecular pathogenesis of GPI-anchor deficiencies.

Hyperphosphatasia mental retardation syndrome (HPMRS) is a recently identified inherited GPI-anchor deficiency caused by mutation in *PIGV*, which is involved in transfer of the second mannose in GPI. We collaborated with Dr. Krawitz and his colleagues in Germany and reported that mutations in *PIGO*, which is involved in transfer of ethanolamine phosphate to the third mannose, is the second gene responsible for HPMRS (Krawitz P et al, ***Am. J. Hum. Genet.***, 91:146, 2012).

We also found the first Japanese patient with inherited GPI-deficiency caused by mutation in *PIGO*. The patient has HPMRS sufferring from mental retardation with multiple anomalies and intractable seizures. The seizure was ameliorated by vitamin B6, most likely because administration of membrane-permeable form vitamin B6 improved inefficient transmembrane incorporation of vitamin B6 due to a loss of membrane-anchored alkaline phosphatase and subsequent decrease in GABA synthesis by vitamin B6-dependent glutamate decarboxylase (Kuki I et al, ***Neurology***, in press).

In collaboration with groups in Germany and Denmark, we studied mutations in *PGAP2* gene found in several patients with HPMRS or non-syndromic intellectual disability. All mutations caused partial loss of function of PGAP2 resulting in inefficient reacylation during fatty acid remodeling and release of GPI-APs. These results established that molecular mechanisms of release of alkaline phosphatase in PIGV/PIGO-HPMRS and PGAP2-HPMRS are different and also indicated that phenotypic spectrum of GPI-deficiency is much wider including non-syndromic intellectual disability (Hansen L et al, ***Am. J. Hum. Genet.***, 92:575, 2013; Krawitz et al, ***Am. J. Hum. Genet.***, 92:584, 2013).

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.

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.
- Fukuda T, Matsumura T, Ato M, Hamasaki M, Nishiuchi Y, Murakami Y, Maeda Y, Yoshimori T, Matsumoto S, Kobayashi K, Kinoshita T, Morita YS. Critical roles for lipomannan and lipoarabinomannan in cell wall integrity of mycobacteria and pathogenesis of tuberculosis. ***mBio*** 4:e00472-12, 2013.

Immunopathology

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| Support Staff 1

Atsushi Kumanogoh, MD/PhD

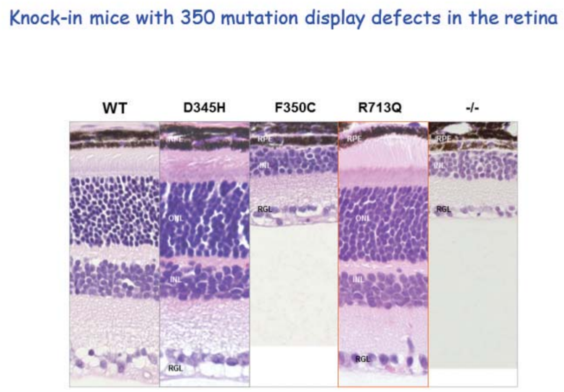

Figure 1. A point mutation in Semaphorin 4A associates with defective endosomal sorting and causes retinal degeneration.

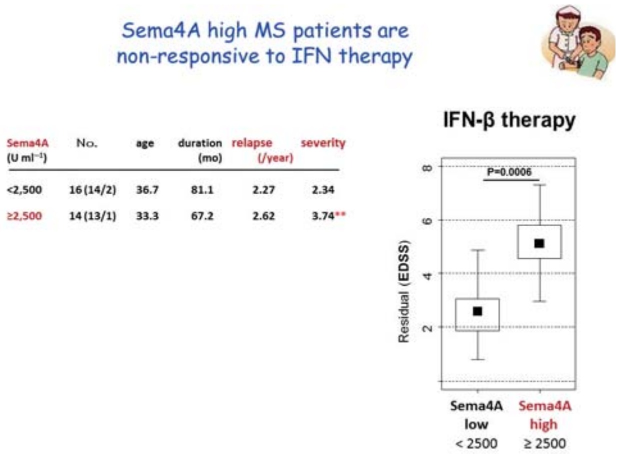

Figure 2. Sema4A is a prognostic and therapeutic marker for MS.

RESEARCH REPORT

The main subject of our proposed study is the regulation of immune cell motility and migratory behavior *in vivo* by soluble and membrane-bound 'immune guidance molecules' such as semaphorins and their receptors. Semaphorins were originally identified as axonal guidance cues that determine the direction and migration of neurons during neuronal development. In the last couple of years, we have determined that they also function as immune-regulatory molecules. Beyond such basic implications, we are trying to apply the findings from this proposed study into the other research fields and diagnosis/therapy for human diseases such as autoimmunity, allergy, immune deficiency, cancer/metastasis, and neurodegenerative diseases.

1 Sema4A and retinitis pigmentosa.

Semaphorin 4A (Sema4A) plays an essential role in photoreceptor survival. In humans, mutations in *Sema4A* are thought to contribute to retinal degenerative diseases. Here, we generated a series of knock-in mouse lines with corresponding mutations (D345H, F350C, or R713Q) in the *Sema4A* gene and found that Sema4A^{F350C} caused retinal degeneration phenotypes. The F350C mutation resulted in abnormal localization of the Sema4A protein, leading to impaired endosomal sorting of molecules indispensable for photoreceptor survival. Additionally, protein structural modeling revealed that the side chain of the 350th amino acid is critical to retain the proper protein conformation. Furthermore, *Sema4A* gene transfer successfully prevented photoreceptor degeneration in *Sema4A*$^{F350C/F350C}$ and Sema4A$^{-/-}$ mice. Thus, our findings not only indicate the importance of the Sema4A protein conformation in human and mouse retina homeostasis but also identify a novel therapeutic target for retinal degenerative diseases.

2 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, Ag presentation and subsequent myelin-reactive CD4(+) T cell activation/differentiation are essential for the pathogenesis of MS. Although semaphorins were initially identified as axon guidance cues during neural development, several semaphorins are crucially involved in various phases of immune responses. Sema4A is one of the membrane-type class IV semaphorins, which we originally identified from the cDNA library of dendritic cell (DC). Sema4A plays critical roles in T cell activation and Th1 differentiation during the course of experimental autoimmune encephalomyelitis, an animal model of MS; however, its pathological involvement in human MS has not been determined. In this study, we report that Sema4A is increased in the sera of patients with MS. The expression of Sema4A is increased on DCs in MS patients and shed from these cells in a metalloproteinase-dependent manner. DC-derived Sema4A is not only critical for Th1 but also for Th17 cell differentiation, and MS patients with high Sema4A levels exhibit Th17 skewing. Furthermore, patients with high Sema4A levels have more severe disabilities and are unresponsive to IFN-β treatment. Taken together, our results suggest that Sema4A is involved in the pathogenesis of MS by promoting Th17 skewing.

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.
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Immunochemistry

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RESEARCH REPORT

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 working extensively the interactions between pathogens and various paired receptors. These studies would be important to understand not only host-pathogen interactions but also mechanism of immune regulation.

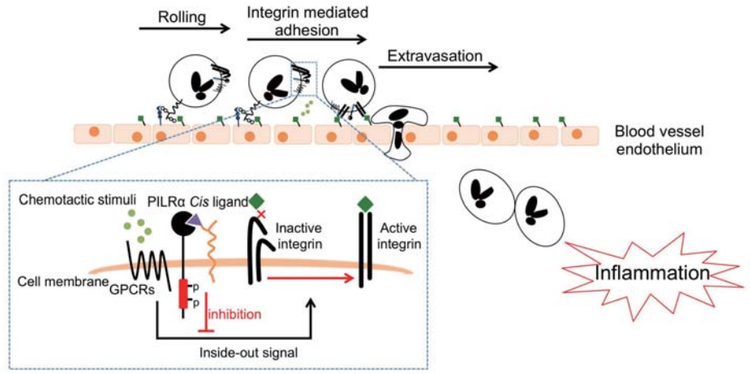
A Interaction between PILR and herpes simplex virus (HSV)

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 gB and the interaction between PILRα and gB is involved in HSV-1 infection. Furthermore, HSV-1 infection of PILRα expressing cells was blocked by anti-PILRα mAb or PILRα-Ig fusion protein. In addition, we found that interaction between PILRα and gB is involved in membrane fusion during HSV-1 infection by using cell-cell fusion assay. These data suggested that interaction between PILRα and gB plays and important role in HSV-1 infection. Especially, we showed that immune inhibitory receptors can be utilized 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, indicating an important role of gB receptors in HSV-1 infection (Arii et al. ***Nature*** 2010).

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

VZV belongs to α-herpesvirus 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).



Spatiotemporal regulation of integrin activation by PILRα

Figure. 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.

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 inhibitory 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. These findings indicated that PILRα plays an important role in the regulation of inflammation by regulating integrin function (Wang et al. ***Nat. Immunol.*** 2012).

D Presentation of ER misfolded proteins by MHC class II molecules

During the analyses of KIR recognition of MHC class I, we found that MHC class II induce cell surface expression of misfolded β2-microglobulin-free MHC class I. Further analyses revealed that a linear epitope of misfolded MHC class I associates with peptide binding groove of MHC class II molecules and misfolded MHC class I is transported to the cell surface by MHC class II without processing to peptides. Similar results were obtained using misfolded hen egg lysozyme (HEL). More interestingly, misfolded proteins presented on MHC class II stimulated antigen-specific B cells. These findings suggested that MHC class II molecules could be directly involved in antigen specific B cell response (Jiang et al. ***Int. Immunol.*** 2013).

Recent Publications

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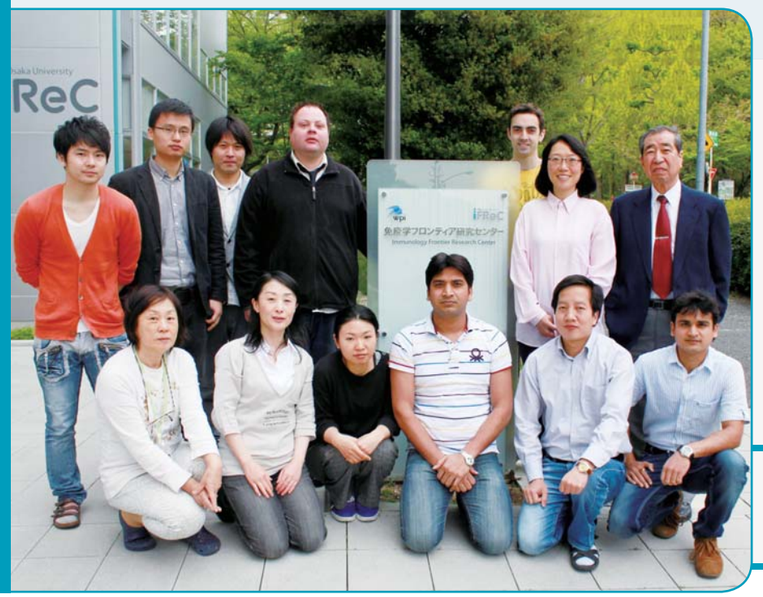
Immune Regulation



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Tadamitsu Kishimoto MD/PhD

RESEARCH REPORT

Introduction to our Laboratory.

The discovery of interleukin 6 (IL-6) and elucidation of its associated receptor signaling pathway, highlighted the important role of cytokines in regulating immune cells. When cytokines become aberrantly regulated, this leads to the inappropriate activation of immune cells. In some cases, this is an important step in the development of autoimmune diseases, such as rheumatoid arthritis (activation of IL-6 signaling) and systemic lupus erythematosus (activation of type-I interferon signaling). The current challenges, and principal aims of our research, are to make advances in our understanding of the mechanisms of autoimmune disease and related cytokine signaling pathways, to ultimately improve health care- thus "from bench to bedside".

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 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 potential target of miR-132/212 cluster. We investigated the roles of miR-132/212 cluster in experimental autoimmune encephalomyelitis (EAE), a murine model of MS. miR-132/212 cluster deficient mice showed resistance to the development of EAE and decreased frequency of both T_H1 and T_H17 cells in draining lymph nodes. Our findings indicate the novel mechanism of AHR-dependent T_H17 cell differentiation *via* miR-132/212 cluster.

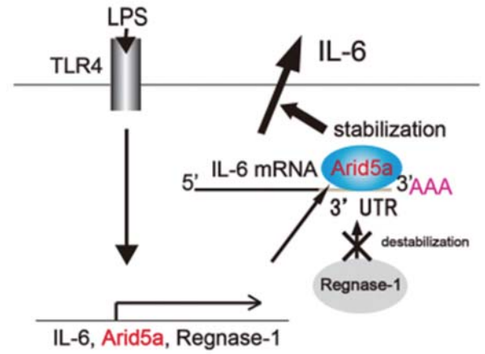

Figure. Arid5a may play an important role in autoimmune disease through control of IL-6 levels *in vivo*.

3 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. Thus all aspects of the type-I IFN system, its production in innate and adaptive immunity and associated immunopathology, are self-controlled through a two-step process of (i) type-I IFN-induced PPAR-γ expression and (ii) formation of an inhibitory PPAR-γ/IRF7 complex.

4 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.

(iii) 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.

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Developmental Immunology

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RESEARCH REPORT

The IL-6-triggered positive-feedback-loop for NFκB-signaling (or the Inflammation-amplifier) was originally discovered as a synergistic-activation signal that follows IL-17A/IL-6 stimulation in type 1 collagen+ non-immune cells including fibroblasts, endothelial cells, astrocytes, and epithelial cells. Subsequent results from animal models have shown the Inflammation amplifier is activated by a simultaneous stimulation of NFκB and STAT3 and induces chemokines followed by inflammation via an NFκB loop. However, its role in human diseases was unclear. This year, we combined two mouse genome-wide screens with SNP-based disease association studies, revealing 1,700 genes related to the Inflammation-amplifier, with 202 showing 492 indications of association with ailments beyond autoimmune diseases. We followed up on ErbB1 from our list. Blocking ErbB1 signaling suppressed the Inflammation-amplifier, while the expression of epiregulin, an ErbB1-ligand, was higher in patients with inflammatory-diseases such as rheumatoid arthritis, multiple sclerosis, and atherosclerosis. These results indicate that the Inflammation-amplifier is indeed associated with human diseases and disorders, and that the identified genes here may make for potential therapeutic targets (Fig.1).

We also showed that Inflammation-amplifier activation in grafts plays important roles in allogenic graft-rejection by using a tracheal heterotopic transplantation model that includes bronchiolitis obliterans (BO), a pathological marker for chronic rejection in this year. IL-6, EGF, and IFNγ all stimulate the Inflammation-amplifier activation, while CCL2, a chemotactic factor for Th1 cells, was one of the amplifier's main targets. Interestingly, IFNγ hyper-induced CCL2 in type 1 collagen+ cells via the Inflammation-amplifier activation at least in vitro. Additionally, we detected IL-6, CCL2, phosphorylated-Erbb1, -STAT3, and -NFκB in epithelial type 1 collagen+ cells of allogeneic tracheal grafts. These results show that the Inflammation-amplifier activation in grafts plays a critical role for graft rejection responses after allogenic transplantation including chronic rejection (Fig. 2). From these results, we consider whether the Inflammation-amplifier in grafts might be a valuable therapeutic target for the prevention of transplant rejection including chronic rejection.

We then investigated whether the Inflammation amplifier is activated in BO regions of a human lung graft after allogeneic transplantation. NFκB, STAT3, and Erbb1 molecules were indeed phosphorylated in the epithelial regions of bronchi that localized in the BO regions (Fig. 3, red arrows). Additionally, CCL2 and CD4+ T cells and macrophages increased in these regions. Furthermore, human lung epithelial cells expressed CCL2 by IFNγ in the presence of IL-6 and EGF via enhanced STAT3 signaling, which parallels behavior seen in the above mouse model. Thus, our results suggest that the Inflammation-amplifier in the epithelial cells of grafts is involved in chronic rejection after lung transplantation, suggesting the Inflammation amplifier may be a valuable therapeutic target to prevent chronic rejections after lung transplantation even in the clinical cases.

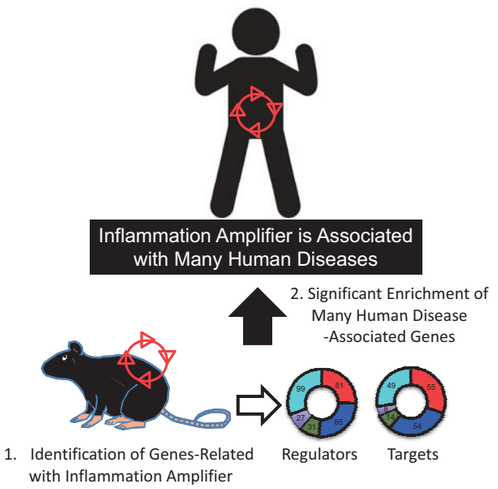

Figure 1. Reverse direction method shows that the Inflammation-amplifier activation is associated with various human diseases and disorders. (Adapted from Cell Reports 3:946-959)

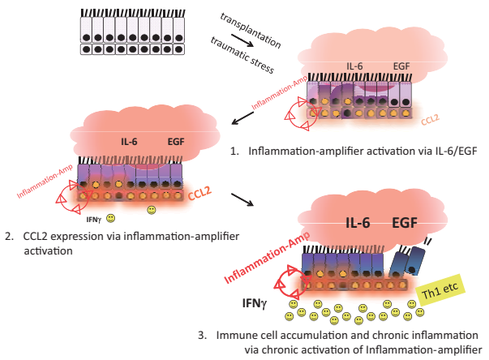

Figure 2. The Inflammation-amplifier activation is critical for transplantation rejection responses in a mouse model. (Adapted from J. Immunol.189:1928-1936)

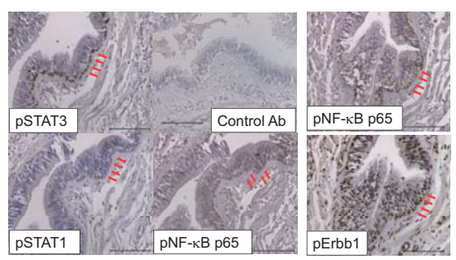

Figure 3. The Inflammation-amplifier is activated in a lung graft with chronic rejection responses after allogenetic lung transplantation. (Adapted from Int. Immunol. 25:319-332)

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Mucosal Immunology

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RESEARCH REPORT

The gastrointestinal tract is a unique site, covered by a single layer of epithelial cells that are exposed to a variety of intestinal environmental factors such as microbiota and dietary compounds. The intestine is also a main target for entry of pathogenic microorganisms into the host tissues. Therefore, the intestinal immune system is unique in that it maintains homeostasis by discriminating between invasive pathogens and non-pathogenic environmental factors. Recently, intestinal microbiota has been shown to regulate development of mucosal immune responses. However, it is still elusive how microbiota contributes to the maintenance of gut homeostasis. We analyzed the effect of intestinal environmental factors on the maintenance of intestinal homeostasis.

ATP-dependent regulation of gut homeostasis

We have previously identified a unique subset of intestinal dendritic cells (DCs) characterized by $CD70^{high}$ $CD11b^{+}$ $CD11c^{+}$, which highly induce Th17 cell development. We further demonstrated that these DCs are activated by extracellular (luminal) adenosine 5'-triphosphate (ATP). Based on these findings, we became interested in how luminal ATP is controlled. The level of extracellular ATP is finely regulated by ATP hydrolyzing enzymes, such as ecto-nucleoside triphosphate diphosphohydrolases (ENTPDases). ENTPDase1/CD39, which is expressed in immune cells, has been shown to regulate immune responses by down-regulating the ATP level. We analyzed the immuno-modulatory function of ENTPDase7, which is preferentially expressed in epithelial cells in the small intestine. The targeted deletion of *Entpd7* encoding ENTPDase7 in mice resulted in increased ATP level in the small intestinal lumen. The number of Th17 cells was selectively increased in the small intestinal lamina propria in *Entpd7*$^{-/-}$ mice. Th17 cells were decreased by oral administration of antibiotics or the ATP antagonist in *Entpd7*$^{-/-}$ mice, indicating that commensal microbiota-dependent ATP release mediates the enhanced Th17 cell development in the small intestinal lamina propria of *Entpd7*$^{-/-}$ mice. In accordance with the increased number of small intestinal Th17 cells, *Entpd7*$^{-/-}$ mice were resistant to oral infection with *Citrobacter rodentium*. *Entpd7*$^{-/-}$ mice suffered from severe experimental autoimmune encephalomyelitis, which was associated with increased numbers of $CD4^{+}$ T cells producing both IL-17 and IFN-γ. Taken together, these findings demonstrate that ENTPDase7 controls the luminal ATP level and thereby regulates Th17 cell development in the small intestine.

Folic acid-dependent regulation of gut homeostasis

We extended our analysis on intestinal environmental factors that are potentially involved in the maintenance of gut homeostasis to dietary compounds. Dietary compounds are metabolized by commensal microbiota and these metabolites are important as nutrients for our health. In addition, several dietary compounds and metabolites are implicated in regulation of mucosal barrier functions.

Among these dietary compounds, we analyzed the effect of vitamin B9 (folic acid: FA or folate) on the gut homeostasis. Mice fed with FA-free diet were analyzed for Th1, Th17 and Treg cells in several organs, such as spleen, mesenteric lymph nodes, small intestinal lamina propria and colonic lamina propria. The number of Th1 and Th17 cells was not altered in any organs analyzed. However, deficiency of FA in the diet resulted in marked reduction of $Foxp3^{+}$ Treg cells in the colon. Since the number of $Foxp3^{+}$ Treg cells was not altered in spleen, mesenteric lymph nodes, small intestinal lamina propria, the reduction is specific to the colon. Since $Foxp3^{+}$ Treg cells express a folate receptor 4 (FR4), we analyzed whether the folate/FR4 pathway is involved in the maintenance of $Foxp3^{+}$ Treg cells in the colon. Blockade of FR4 by Fab fragment of anti-FR4-neutralizing Ab decreased colonic $Foxp3^{+}$ Treg cells. IL-2 is known to be essential for the maintenance of $Foxp3^{+}$ Treg cells in the periphery. Indeed, a general reduction of splenic and colonic Treg cells was induced by a neutralizing antibody against IL-2, but a further decrease by additional FA deficiency was observed exclusively in the colon. Compared with splenic Treg cells, colonic Treg cells were more activated to vigorously proliferate and highly sensitive to apoptosis. In colonic Treg cells derived from mice fed with a FA-deficient diet, expression of anti-apoptotic molecules, Bcl-2 and Bcl-xL, was severely decreased. Mice fed with a FA-deficient diet exhibited higher susceptibility to intestinal inflammation induced by TNBS. These findings reveal the previously unappreciated role of dietary FA for promoting survival of $Foxp3^{+}$ Treg cells in the colon. When compared with splenic T reg cells, colonic Treg cells are highly activated possibly due to continuous exposure to intestinal environmental factors, and are highly sensitive to apoptosis. Dietary FA acts on these highly activated colonic Treg cells to prevent apoptosis and thereby contributes to the maintenance of gut homeostasis.

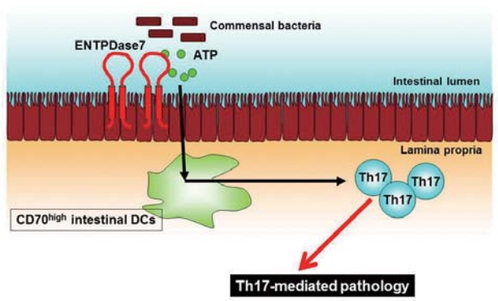

Figure 1. ENTPDase7 controls luminal ATP levels and regulates Th17 responses in the small intestine.

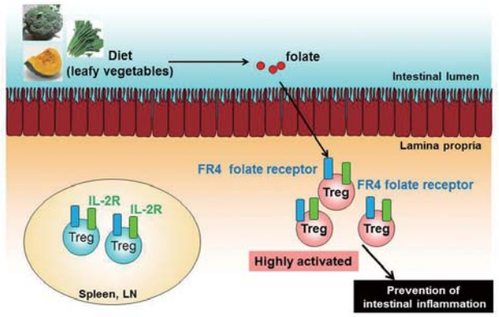

Figure 2. Folic acid is responsible for the maintenance of Foxp3+ Treg cells in the colon.

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Molecular Immunology

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Shuhei Sakakibara

Postdoctoral Fellow 2

Research Assistant 1

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RESEARCH REPORT

Role of Protein Kinase N1 (PKN1), an inhibitor of Akt for quality control of germinal center formation

PKN1 is a serine/threonine kinase that is structurally related to members of the protein kinase C (PKC) family. We observed that PKN1 interacted with Akt in anti-IgM stimulated B cells. Constitutive active PKN1 interacted strongly with Akt and inhibited not only its kinase activity but also its transforming activity, demonstrating that PKN1 is a cellular inhibitor of Akt. To determine functions of PKN1 in vivo, $PKN1^{-/-}$ mice were generated. $PKN1^{-/-}$ mice showed spontaneous formation of germinal centers (GC) even without immunization or infection and autoantibody production. B cells but not T cells of $PKN1^{-/-}$ mice were hyperreactive to antigen receptor stimulation. $PKN1^{-/-}$ B cells had higher levels of Akt phosphorylation after BCR stimulation. Although $PKN1^{-/-}$ mice developed much larger GCs than WT mice after immunization with NP-CGG, the frequency of NP-binding GC B cells was significantly lower in $PKN1^{-/-}$ mice compared to WT mice after immunization. The frequency of B cells expressing the $V_H186.2$ gene with more than 4 mutations was significantly lower in $Pkn1^{-/-}$ mice than in the control. $PKN1^{-/-}$ mice also had a lower frequency of B cells carrying the tryptophan to leucine mutation at position 33 of the $V_H186.2$ gene, a signature of high-affinity anti-NP antibodies, indicating that low affinity B cells were selected in GC of PKN1-/- mice. These findings clearly demonstrate that PKN1-mediated regulation of Akt activity is crucial in quality control of GC formation, particularly selection of high affinity B cells.

An impact of Epstein-Barr virus (EBV) encoded Latent membrane 2a (LMP2a) on differentiation and selection of GC B cells

EBV infects GC B cells and persists in memory B cells. EBV-infected GC B cells express LMP2a, which is known to mimic B-cell antigen receptor (BCR) signals. However, it remains unclear how LMP2a affects B cell differentiation in GC or contributes to latent infection of EBV in B cells. To solve this question, we generated knock-in mice expressing LMP2a in GC B cells ($LMP2a^{GCB}$). After immunization with NP-CGG, immunohistochemical and flow cytometric analyses showed normal GC formation and increased number of $CD138^+$ plasma cells in spleen of $LMP2a^{GCB}$ mice. However, $LMP2a^{GCB}$ mice had markedly reduced number of NP-specific B cells and significantly lower serum levels of NP-specific IgG, particularly high affinity antibodies than control mice. Sequencing analysis of Ig heavy chain revealed that the frequency of $V_H186.2$ gene from the NP-specific B cells was more than 2-fold lower in $LMP2a^{GCB}$ mice than in control mice, but the frequency of somatic hypermutation in $LMP2a^{GCB}$mice was similar to that in control mice. LMP2a-expressing B cells showed rather enhanced CD40-dependent IgM and IgG production *in vitro*, suggesting that LMP2a might not inhibit Ig production and Ig class switching. In addition, serum level of autoantibodies was significantly elevated in $LMP2a^{GCB}$ mice. These results suggest that LMP2a contributes to the establishment of EBV-latent infection in GC and memory B cells by reducing antigen-dependency during GC selection and also raise the possibility that EBV-infected B cells could produce autoantibodies in human.

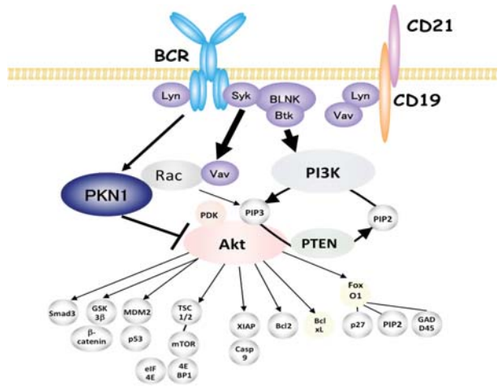

Figure 1. BCR-dependent PKN1 activation for modulating Akt activity.

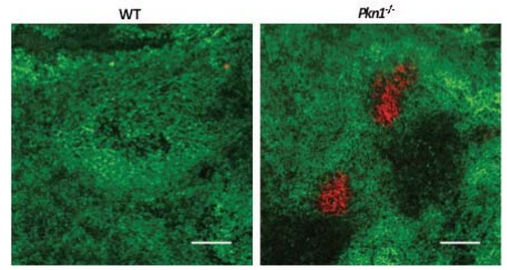

Figure 2. Spontaneous formation of GC in PKN1-deficient mice.

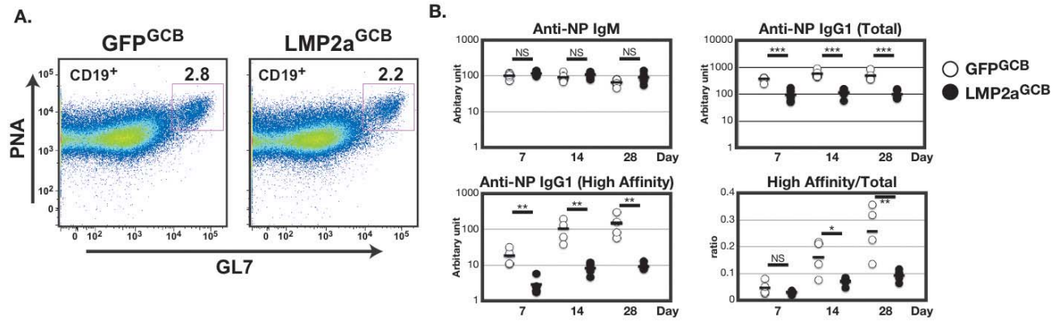

Figure 3. GC-specific LMP2a expression results in reduced antigen-specific antibody production accompanied with normal GC formation.

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- Morihana T, Goya S, Mizui M, Yasui T, Prasad DV, Kumanogoh A, Tamura M, Shikina T, Maeda Y, Iwamoto Y, Inohara H, Kikutani H. An inhibitory role for Sema4A in antigen-specific allergic asthma. ***J. Clin. Immunol.*** 33:200-209, 2013.
- Yasui T, Sakakibara-Yada K, Nishimura T, Morita K, Tada S, Mosialos G, Kieff E, Kikutani H. Protein kinase N1, a cell inhibitor of Akt kinase, has a central role in quality control of germinal center formation. ***Proc. Natl. Acad. Sci. USA.*** 109:21022–27, 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.
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- Toyofuku T, Yoshida J, Sugimoto T, Yamamoto M, Makino N, Takamatsu H, Takegahara N, Suto F, Hori M, Fujisawa H, Kumanogoh A, Kikutani H. Repulsive and attractive semaphorins cooperate to direct the navigation of cardiac neural crest cells. ***Dev. Biol.*** 321:251-62, 2008.

Experimental Immunology

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Shimon Sakaguchi, MD/PhD

RESEARCH REPORT

This year we have continued the study on the molecular basis of the development and function of naturally occurring $CD25^+CD4^+$ regulatory T (Treg) cells, which specifically express the transcription factor Foxp3. We have previously demonstrated that proper development of Treg cells requires the establishment of Treg-specific DNA hypomethylation pattern. The process is independent of Foxp3 expression and necessary for $Foxp3^+$ T cells to acquire Foxp3-independent gene expression, lineage stability, and full suppressive activity. It remains elusive, however, how the two events, Foxp3 expression and epigenetic modification, contribute to Treg-specific gene expression. This has led us to study the effects of Treg-specific DNA hypomethylation on Treg-type transcriptional regulation, and also analyze possible differences between epigenome-dependent transcriptional regulation and Foxp3-dependent one. By transcriptional start site (TSS) cluster analysis, we found that Treg-specific DNA hypomethylated regions were closely associated with Treg-up-regulated TSS clusters, whereas Foxp3-binding regions had no significant correlation with either up- or down-regulated clusters, in non-activated Treg cells. On the other hand, in activated Treg cells, Foxp3-binding regions showed a strong correlation with down-regulated clusters. In accord with these findings, the above two features of activation-dependent gene regulation in Treg cells tend to occur at different locations in the genome. The results collectively indicate that Treg-specific DNA hypomethylation is instrumental in gene up-regulation in steady state Treg cells, whereas Foxp3 down-regulates the expression of its target genes in activated Treg cells. Thus, the two events appear to play distinct but complementary roles in Treg-specific gene expression. These findings contribute to our understanding of the molecular mechanisms by which specific transcriptional networks are established in natural Treg cells to determine and maintain their functions.

Regarding Treg suppressive function, natural $Foxp3^+CD4^+$ Treg cells are known to scarcely produce interleukin-2 (IL-2), constitutively express cytotoxic lymphocyte antigen-4 (CTLA-4), and bear a self-reactive T cell receptor (TCR) repertoire. We have examined how Treg suppressive activity and self-skewed TCR repertoire can be genetically constructed in conventional T cells, without Foxp3. We show that a combination of IL-2 non-production and transgenic CTLA-4 expression in T cells, but not either one alone, was able to prevent autoimmunity/immunopathology in Treg-deficient mice, and that IL-2-nonproducing and CTLA-4-expressing T cells exerted potent *in vitro* and *in vivo* suppressive activity when they are preactivated by antigenic stimulation. In addition, in the thymus, constitutive CTLA-4 expression skewed the TCR repertoire of developing $Foxp3^-$ conventional T cells towards higher self-reactivity. The extracellular portion of CTLA-4 was sufficient for the suppression and repertoire shifting. It interfered with CD28 signaling to responder T cells via out-competing CD28 for binding to CD80 and CD86 or modulating CD80/CD86 expression on thymic or peripheral antigen-presenting cells. Thus, a triad of IL-2 repression, CTLA-4 expression, and antigenic stimulation is a minimalistic requirement for conferring Treg-like suppressive activity on conventional T cells. Moreover, CTLA-4 expression is required for the formation of a self-reactive TCR repertoire in developing T cells. These findings provide molecular clues to the development and function of $Foxp3^+$ natural Treg cells, and are instrumental for controlling immune responses by targeting IL-2 and CTLA-4 in Treg and conventional T cells.

We have also continued to study human $Foxp3^+$ Tregs under a variety of disease states, in particular in tumor immunity, and plan to initiate this year immunotherapy of cancer by targeting Tregs.

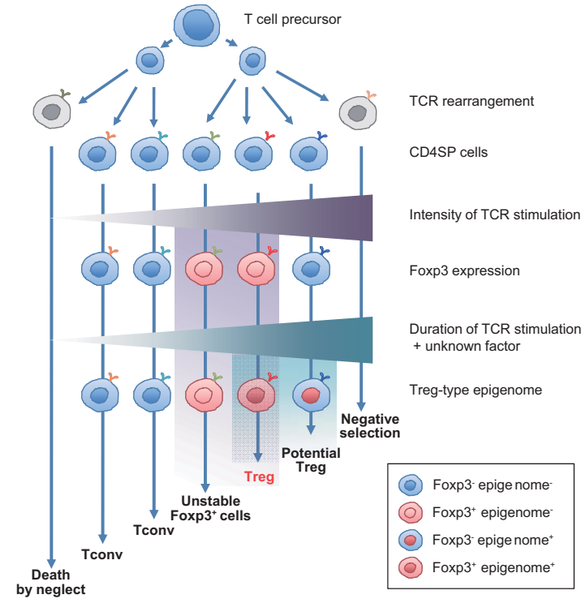

Figure. A Model for Treg Cell Development in the Thymus.
In developing T cells in the thymus, TCR gene rearrangement generates diverse TCRs that recognize self-ligands at various intensities and durations (shown as gradients). TCR stimulation with relatively higher intensities (but below the threshold required to induce apoptosis) induces Foxp3 expression, whereas TCR stimulation for an appropriate length of time produces the Treg-cell type DNA hypomethylation pattern. Developing T cells that happen to have both events ($Foxp3^+$ $epigenome^+$ T cells) are driven to a stable Treg cell lineage. $Foxp3^+$ T cells without the accompanying Treg-cell-type epigenome ($Foxp3^+$ $epigenome^-$) are unstable and might lose Foxp3 expression, whereas T cells with the Treg-cell-type epigenome but without Foxp3 expression ($Foxp3^-$ $epigenome^+$) are ready to express Foxp3 and are capable of differentiating into functional Treg cells. T cells that recognize self-ligands too strongly are negatively selected by apoptosis, whereas those that recognize self-ligands too weakly fail in positive selection (death by neglect).

Recent Publications

- Ohkura N, Hamaguchi M, Morikawa H, Sugimura K, Tanaka A, Ito Y, Osaki M, Tanaka Y, Yamashita R, Nakano N, Huehn J, Fehling HJ, Sparwasser T, Nakai K, Sakaguchi S. T cell receptor stimulation-induced epigenetic changes and foxp3 expression are independent and complementary events required for Treg cell development. ***Immunity*** 37:785-99, 2012.

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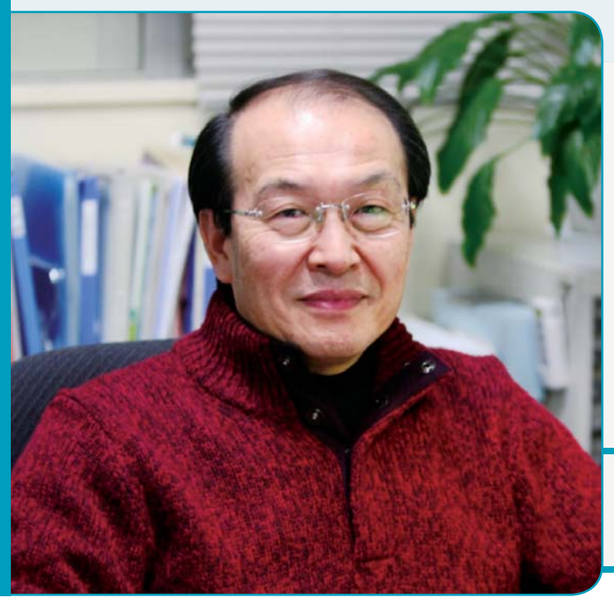
Cell Signaling



Professor
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Postdoctoral Fellow
Hiroshi Ike

Takashi Saito, PhD

RESEARCH REPORT

We have analyzed the molecular mechanisms of activation, differentiation and homeostasis of T cells from the signal transduction perspective. Particularly in studies of T cell activation, we has been using real-time imaging analysis to elucidate the spatiotemporal dynamic regulation of the TCR signaling complex and signal transduction of related downstream pathways upon antigen-recognition using imaging and biochemical analysis. Using a planar bilayer system and TIRF microscopy, we previously identified TCR microcluster as the signaling complex responsible for transducing activation signals. TCR microclusters are generated at the interface between T cell and antigen-presenting cells prior to the formation of Immunological synapse. These studies also include the roles of positive/negative co-stimulation signals, innate-related signals and cytoskeletal regulation as well as transcriptional regulation in T cell activation and functional differentiation.

Dynamic regulation of T cell activation by co-stimulation

We then had analyzed the dynamic features of co-stimulation signals by the positive co-stimulatory receptor CD28 and their relationship with TCR-MCs. We found that CD28-induced co-stimulation for T cell activation is also mediated through TCR-CD28 microclusters. CD28 was accumulated in the unique region of the central supre-molecular activation cluster (cSMAC) as "signaling" cSMAC. CD28 recruited PKCθ and CARMA1 into this signaling cSMAC to mediate sustained co-stimulation signals. CTLA-4 also accumulates in the same signaling cSMAC, where it inhibits activation by competing with CD28 for ligand binding.

We now analyzed the dynamic regulation of another inhibitory co-stimulation receptor PD-1 (Figure 1). Unlike CTLA-4 accumulation in the cSMAC, PD-1 initially accumulates in the TCR-MCs and then in the signaling cSMAC (Figure 1 Top). PD-1 microclusters specifically recruit the phosphatase SHP-2 that mediates inhibition of TCR activation by de-phoephorylating TCR proximal signals within TCR-MCs such as CD3ζ and ZAP70 (Figure 1 Bottom). Analyzing the function of the PD-1 mutant possessing various sizes of extracellular domain of PD-1, it became clear that PD-1 has to be co-localized in the same TCR-MC in order to inhibit T cell activation. Such PD-1-mediated inhibition of T cell activation was observed in antigen peptide-repeatedly immunized normal T cells expressing PD-1, which exhibited anergic status, and the anergy was restored by blocking the PD-1/PD-L1 interaction. Thus, we have clarified the spatio-temporal dynamic regulation of positive/negative co-stimulation and how these signals exhibit quantitatively fine-tuned regulation.

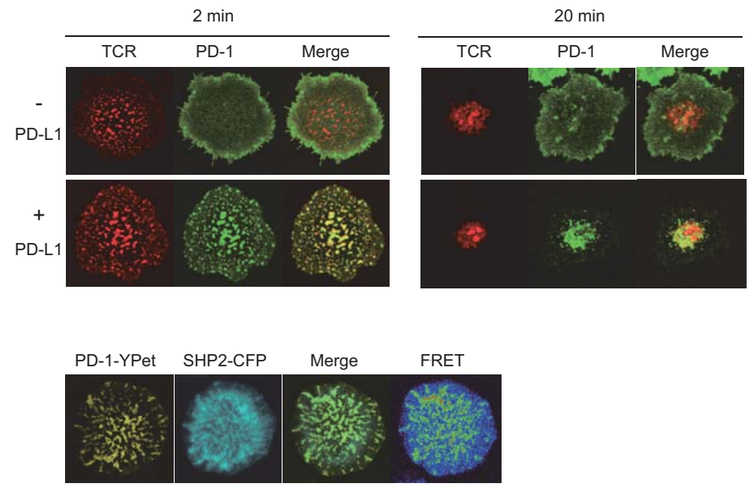

Figure. (Top) AND-TCR transgenic T cells expressing PD-1-GFP and pre-stained DyLight-anti-TCRβ Fab were stimulated on a planar bilayer containing PD-L1. Real time imaging analysis revealed that PD-1 (green) was initially (2 min) co-localized with TCR-microclusters (red), and move to cSMAC at 20 min where PD-1 accumulates in the TCR^{lo} area which CD28 and CTLA4 also accumulate. For -1-mediated suppression, it is critical to be within the same TCR-microclusters.
Figure. (Bottom) T cells expressing both PD-1-YPet and SHP2-CFP were stimulated on the planar membrane with specific peptide-MHC, and analyzed for specific FRET between PD-1 and SHP2. SHP2 was transiently recruited to TCR-microclusters upon stimulation in PD-L1-dependent manner and dephosphorylates upstream signaling molecules.

Regulation of T cell activation and differentiation

We have analyzed genes, which are up-regulated during T cell development and/or activation. To this end, we identified the transcription factor Bach2 is well expressed even in T cells and up-regulated during T cell differentiation although it has been thought to be predominantly expressed in B cells. Bach2 deficiency resulted in the reduction of naïve T cells and up-regulation of gene expression in naïve T cells which are related to effector-memory T cells, particularly to Th2-type cells. Bach2-deficient T cells induce rapid secretion of Th2 cytokines and memory-effector related molecules. We identified the direct target genes of Bach2, which are also regulated in effector-memory cells. Collectively, Bach2 is a critical repressor to maintain the naïve state of T cells and regulate memory T cell development by suppressing effector-memory related genes.

Recent Publications

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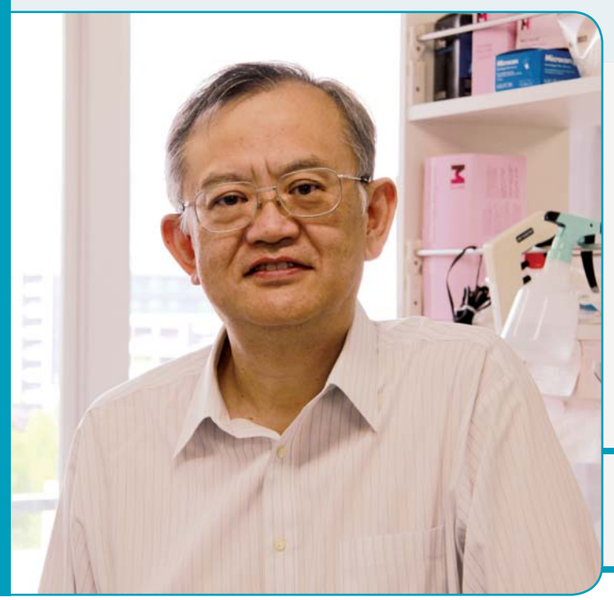
Lymphocyte Differentiation



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Tomohiro Kurosaki, MD/PhD

RESEARCH REPORT

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. When B cells recognize the same antigen the 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. To understand the unique traits of the memory antibody responses, previous studies have focused on B cell intrinsic differences between naïve and memory B cells, for instance, gene profiling differences. However, recent advances in identifying a special subset of $CD4^+$ helper T cell (called T_{fh}) for helping primary B cell activation provoked the idea that T_{fh} type T cells might enter the memory pool, thereby stimulating memory B cells. Our laboratory has now focused on characterizing the unique aspects of memory T cells as well as memory B cells in terms of inducing robust memory antibody responses.

Regulatory mechanisms of the transcription factor Bach2

In regard to explaining the robust antibody responses of IgG type memory B cells, two-non-mutually exclusive models (BCR-intrinsic and BCR-extrinsic) have been long debated. By establishing a mouse model in which antigen-non-experienced B cells express an IgG type BCR, we previously showed that the BCR-intrinsic model alone is not sufficient to explain the heightened differentiation activity of IgG type memory B cells. Instead, we showed that the decreased expression level of the transcription factor Bach2 in IgG memory B cells is involved in manifesting the heightened differentiation activity.

Then, the question arises about how Bach2 is down-regulated during primary responses, resulting in its low level in IgG memory B cells. To address this question, we focused upon the early regulatory events initiated by BCR stimulation. Among various inhibitors which are well known to modulate the transcription processes in B cells, rapamycin and an Akt inhibitor significantly inhibited the BCR-mediated Bach2 repression. This inhibition was also recapitulated in vivo contexts, too. Thus, our data suggest that the PI3K-AKT-mTOR pathway could be a potential regulator for Bach2 repression (Fig. 1).

Involvement of memory T_{fh} cells in memory antibody responses

Our previous data demonstrated the existence of $CXCR5^+$ T cells located in the close proximity to IgG type memory B cells at the memory state. This observation allowed us to take the following hypothesis. The $CXCR5^+$ T cells might be memory type T_{fh} cells, because one of the hallmarks of T_{fh} cells is the expression of $CXCR5^+$. If so, because memory T_{fh} cells and memory IgG cells are in a closed proximity with each other, these two types of cells are allowed to interact quickly. This quick interaction might contribute to mutual stimulation, which, in turn, could lead to activation of IgG type memory B cells.

Thus, to examine our hypothesis, we used T cells harboring transgenic TCR together with Bcl6-YFP reporter and set up the experiments. Our data showed that $CXCR5^+$ T cells at the memory state are indeed memory type T_{fh} cells and that this type of memory T cells is responsible for activation of IgG memory B cells after antigen re-encountering. Furthermore, we demonstrated that after antigen re-encountering, Bcl6 in memory T_{fh} cells is quickly reactivated by the help of memory IgG B cells, but not dendric cells (DCs). Hence, our data strongly suggest that memory IgG B cells function as an antigen-presenting cell, thereby allowing memory T_{fh} cells to be quickly differentiated into effector cells (Fig. 2).

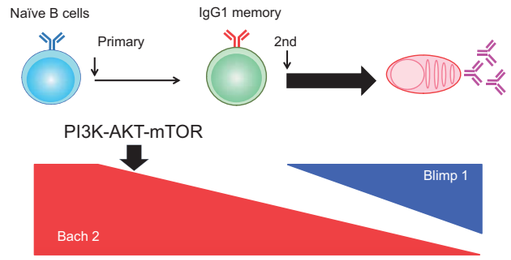

Figure 1. Repression of Bach2 through PI3K-AKT-mTOR pathway predisposes igG1 memory B cells towards plasma cell differentiation
IgG memory B cells express less Bach2 by virtue of PI3K-AKT-mTOR pathway. Bach2 suppresses Blimp1 expression. Thus, because of the low level of Bach2 in IgG memory B cells, they are predisposed to be differentiated toward plasma cells after secondary antigen stimulation.

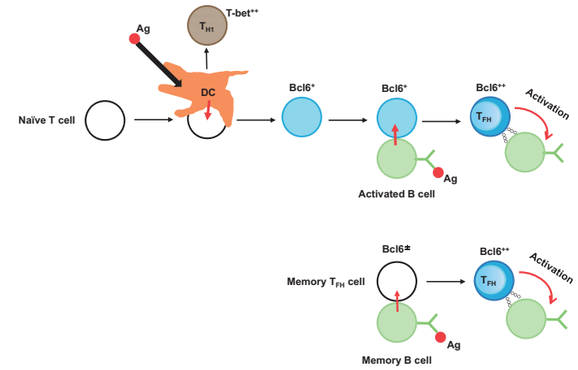

Figure 2. Differences in T cell activation modes between primary and secondary antibody responses.
In primary responses (above), naïve T cells are activated by two steps; first by DC cells, second by antigen-activated B cells. Through such consecutive two interactions, Bcl6 in T cells is at maximum level activated, thereby activating antigen-activated B cells. In the case of secondary responses (below), Bcl6 in memory T_{fh} cells is activated by antigen-presentation on memory B cells, which appears to be sufficient for activating memory T_{fh}cells.

Recent Publications

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Malaria Immunology

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Postdoctoral Fellow 1
Research Assistant 2
Support Staff 1

Cevayir Coban MD/PhD

RESEARCH REPORT

In general, research in our laboratory focuses on understanding how immune system functions against infectious organisms. We are particularly interested in *Plasmodium* parasites, the causative agent of malaria, because malaria still kills more than 1 million people every year, mostly children and pregnant women. Our work attempts to understand host-parasite interactions both in animal models and humans, and translate our understanding into safe interventions such as vaccines and drugs.

Immune system-iron homeostasis interactions during malaria infection

We've recently identified a role for immune system on the iron homeostasis during malaria infection (*Zhao et al, Cell Host Microbe, 2012*). The immune system's response to infectious diseases in the context of iron status is complex and poorly understood. Given that iron is an essential nutrient required by all microbial organisms for growth, many pathogenic microorganisms, including *Plasmodium* parasites, possess the ability to manipulate their host's iron metabolism in order to acquire sufficient quantities for growth. In fact, *Plasmodium* parasites grow and multiply within erythrocytes (an abundant source of iron-containing hemoglobin), little has been known about the interactions between malaria parasites and host iron status during malaria infection. Our recent research has identified that Lipocalin 2 (Lcn2), a known anti-bacterial siderocalin and a component of the innate immune system, is abundantly secreted into serum during malaria infection and has a pivotal role in controlling parasite levels and in the successful resolution of the infection. During infection, Lcn2 bolsters both host macrophage function and granulocyte recruitment and limits reticulocytosis. If Lcn2 is absent, a chronic iron imbalance occurs and results in impaired adaptive immune responses against Plasmodium parasites (Figure). We believe our findings have many important implications for chronic disease conditions such as malaria infection as well as their treatment.

New microbe/microbe-related product detection modalities

We also work on the development of new detection modalities for the diagnosis of microbes and/or their related pathologies. Our studies are well-encouraged by the WPI program and their support for combined research between immunology, imaging and informatics disciplines. With an international and/or interdisciplinary collaboration, we detected hemozoin crystals, a malaria parasite metabolite, by using a new detection system called Whispering Gallery Mode microtoroidal optical resonator (WGM resonator) (*Kim et al., Optics Express, 2012*). This mode splitting technique has been proposed and used as a highly sensitive and robust platform to detect individual nanoscale materials and well established by Physics Group (Prof. L. Yang and Dr.S. K. Ozdemir) at Washington University in St. Louis. For the first time, we measured the detection and size measurement of individual synthetic hemozoin nanoparticles in an aquatic environment using WGM resonator and as a platform for label-free detection.

With a different collaboration at IFReC (Prof. NI Smith), we performed Raman spectroscopy to monitor the changes in erythrocytes and plasma during *Plasmodium* infection in mice (*Hobro et al., Analyst, 2013*). In plasma samples, due to low heme background, heme-based changes in the Raman spectra could be detected in the very early stages of infection, as little as one day after *Plasmodium* infection suggesting that plasma analysis has significant potential for early, quantitative and automated detection of malaria, and to quantify heme levels in serum which modulate malarial effects on the immune system.

In future, our aim is to use more imaging technologies to diagnose malaria infection as well as its related pathologies.

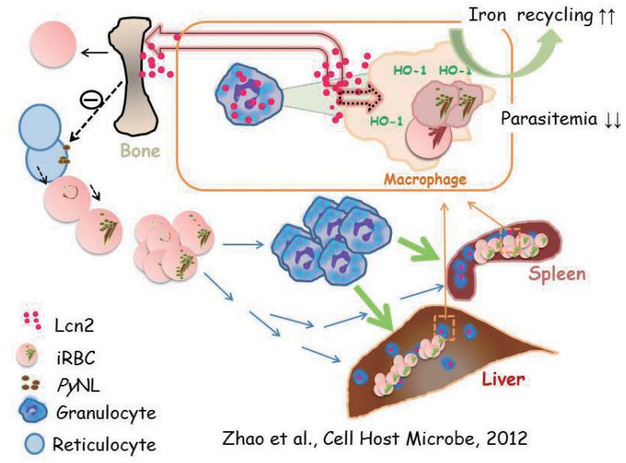

Figure. Lipocalin 2 has multiple tasks in immunity against malaria. Young erythrocytes (reticulocytes) are the preferred target cell of *P. vivax* (in humans) and *P. yoelii*NL (in mouse) parasites. As soon as host is infected by these parasites, Lcn2 is abundantly secreted into serum mainly from activated granulocytes and is essential to control parasitemia, anemia and host survival. Secreted Lcn2 functions as bolstering power for host macrophage function and granulocyte recruitment and also limits reticulocytosis via controlling iron-recycling. These events are eventually needed for potent adaptive immune responses against Plasmodium.

Recent Publications

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- Coban C, Igari Y, Yagi M, Reimer T, Koyama S, Aoshi T, Ohata K, Tsukui T, Takeshita F, Sakurai K, Ikegami T, Nakagawa A, Horii T, Nuñez G, Ishii KJ, Akira S. Immunogenicity of Whole Parasite Vaccines Against Plasmodium falciparum Involves Malarial Hemozoin and Host TLR9. ***Cell Host Microbe.*** 7:50-61, 2010.

Vaccine Science

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Ken J. Ishii, MD/PhD

RESEARCH REPORT

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

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). Innate immune receptors including TLR and RLRs can detect such DNA or RNA, and the resultant immune activation is uniquely regulated by intra- and inter-cellular signaling pathways, which are indispensable for the ensuing vaccine immunogenicity (Ishii KJ et al *Cell Host Microbe* 2008, Aoshi T et *al Curr. Op. Virol* 2011).
- **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 propose that nucleic acid-sensing mechanisms (Desmet C and Ishii KJ *Nat Rev Immunol* 2012) (FIGURE), 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) not only have revealed their critical role in driving the responses mediated by many current vaccines, but are also revealing how they could be harnessed 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 are 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. As results, we obtained approval from the IRB of Osaka university hospital and PMDA (Japanese regulatory agency) to initiate investigator driven GCP Phase-I clinical trial during 2013 in Osaka university hospital.
- **Development of the second-generation adjuvants** are in the pre-clinical stage, whose mechanism of action are being analyzed; a β-glucan-CpG (SPG-CpG) complex, a novel dectin-1-assisted TLR9 agonist, showed promising results as an adjuvant for Flu vaccine as well as immunotherapeutic agents for cancer. We have several other adjuvant candidates for vaccines against influenza and cancer which are under pre-clinical evaluation including a novel STING ligand DMXAA that are being developed as an anti-cancer drug (Tang CK et al *PLoS One* 2013), a beta-cyclodextrin (HP-β-CD); used as an approved excipient for many drugs, and several other particulate bio-degradable adjuvants and small compound-based adjuvants.
- **Clinical studies on seeking bio-marker(s) for safety as well as efficacy of adjuvanted vaccines** are 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.

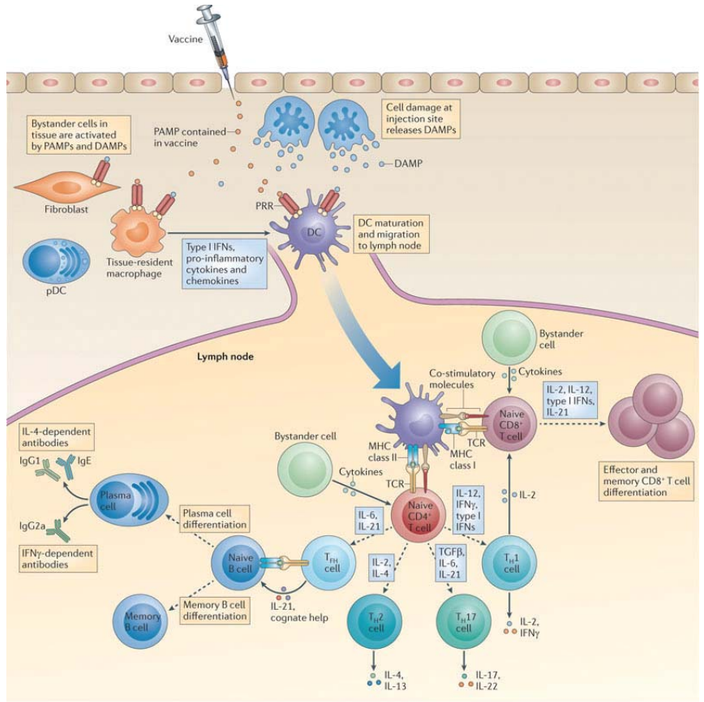

Figure. Vaccines may contain pathogen-associated molecular patterns (PAMPs) or may induce the local release of damage-associated molecular patterns (DAMPs). These PAMPs and DAMPs are detected directly by pattern-recognition receptors (PRRs) expressed by dendritic cells (DCs), leading to DC activation, maturation and migration to the lymph nodes. Alternatively, PRR-mediated recognition of PAMPs and DAMPs by bystander cells may induce the release of tissue-derived factors, such as cytokines, that may cooperate in the activation and orientation of the DC response. In the lymph nodes, the activated DCs may present antigens to T cells, provide them with co-stimulatory signals and stimulate their differentiation by providing a favourable cytokine milieu.(Desmet C and Ishii KJ *Nat. Rev. Immunol.* 2012)

Recent Publications

- Tang CK et al The chemotherapeutic agent DMXAA as a unique IRF3-dependent type-2 vaccine adjuvant. ***PLoS One*** 8:e60038, 2013.
- Kuroda E, Coban C, Ishii KJ. Particulate adjuvant and innate immunity: past achievements, present findings, and future prospects. ***Int. Rev. Immunol.*** 32:209-20, 2013.
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- Tougan T et al TLR9 adjuvants enhance immunogenicity and protective efficacy of the SE36/AHG malaria vaccine in nonhuman primate models. ***Hum. Vaccin. Immunother.*** 9(2), 2012.
- Desmet C and Ishii KJ Nucleic acid sensing at the interface between innate and adaptive immunity in vaccination. ***Nat. Rev. Immunol.*** 12:479-91, 2012.

Immune Regulation

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RESEARCH REPORT

Dendritic cells (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 various autoimmune and inflammatory disorders.

Dendritic cells 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.

pDC can sense host- or microorganism-derived nucleic acids through TLR7 and TLR9 and produce vast amounts of type I IFNs. pDC is featured by this ability, which contributes to both protective immunity against viral infection and pathogenesis of certain autoimmune disorders such as SLE. It is still largely unknown how the pDC-specific functions are regulated. We have focused on an Ets family of transcription factor, Spi-B, which is expressed abundantly in pDC and demonstrated that Spi-B is critical for pDC function (Blood 2012). 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 interacted 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. Notably, Spi-B-deficient pDC also showed defects in TLR7/9-induced proinflammatory cytokine production. This is based on the ability of Spi-B to transactivate the promoters of proinflammatory cytokines in synergy with NF-κB p65 subunit.

Spi-B was also highly expressed in the intestinal microfold cells (M cells), which are a 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 (Nat. Immunol. 2012).

Among murine cDCs, splenic CD8α$^+$ DC (CD8α$^+$ cDC) is a DC subset featured by its high ability to ingest dead cells and crosspresent antigens to generate CD8 T cell responses. This activity is important for protective cytotoxic responses against viral infection and tumors. CD8α$^+$ cDC also shows an increased ability to produce proinflammatory cytokines in response to various TLR signals. Furthermore, in the steady state, CD8α$^+$ cDC can be involved in maintaining the immune tolerance. However, it remains unknown whether or how CD8α$^+$ cDC is involved in developing various inflammatory diseases or maintaining the immune homeostasis.

In order to address this issue, the system for tracking or ablating CD8α$^+$ cDC is necessary. A chemokine receptor, XCR1, is exclusively expressed in CD8α$^+$ cDC (BBRC 397:756, 2010). By knocking the genes for a fluorescent protein, venus, and for the fusion protein of diphtheria toxin receptor (DTR) and venus into the XCR1 gene locus, we have generated the XCR1-venus and XCR1-DTRvenus mice, respectively. In the spleen of XCR1-venus mice, venus expression was detected only in CD8α$^+$ cDC. In the XCR1-DTRvenus mice, the XCR1-expressing DC was efficiently ablated after injection of DT. By analyzing these mice, we have found that the XCR1-expressing DC is critical for double-stranded RNA (dsRNA)-induced CD8 T cell responses (crosspresentation) of protein, cell-associated and bacteria-derived Ags (C. Yamazaki et al. J. Immunol. 2013). These mutant mice should be useful for elucidating the functions and behavior of the XCR1-expressing DC.

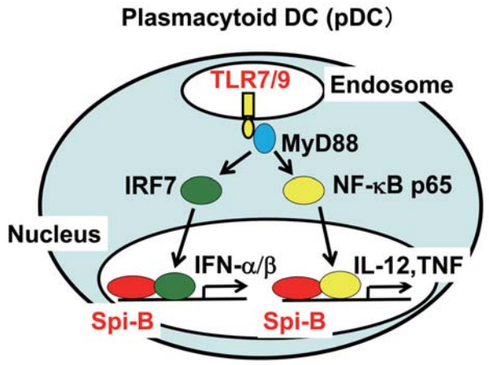

Figure 1. Molecular mechanisms for TLR7/9-induced type I IFN production in pDC. An Ets family transcription factor, Spi-B, is involved in TLR7/9-induced type I IFN and proinflammatory cytokine gene expression in synergy with IRF-7 and NF-κB p65, respectively.

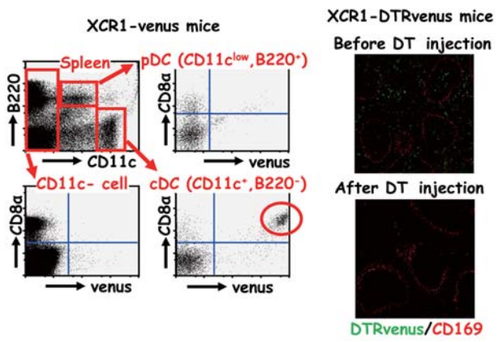

Figure 2. Characterization of XCR1-venus and XCR1-DTRvenus mice. A chemokine receptor, XCR1, is expressed highly in splenic CD8α+ cDC. We have generated XCR1-venus and XCR1-DTRvenus mice in which venus and the fusion protein of DTR and venus are expressed selectively in splenic CD8α+ cDC, respectively. In the XCR1-DTRvenus mice, venus expressing cells can be ablated after injection of DT.

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- Sasaki I, Hoshino K, Sugiyama T, Yamazaki C, Yano T, Iizuka A, Hemmi H, Tanaka T, Saito M, Sugiyama M, Fukuda Y, Ohta T, Sato K, Ainai A, Suzuki T, Hasegawa H, Toyama-Sorimachi N, Kohara H, Nagasawa T, Kaisho T. Spi-B is critical for plasmacytoid dendritic cell function and development. ***Blood*** 120:4733-43, 2012.
- Kanaya T, Hase K, Takahashi D, Fukuda S, Hoshino K, Sasaki I, Hemmi H, Knoop K.A, Kumar N, Sato M, Katsuno T, Yokosuka O, Toyooka K, Nakai K, Sakamoto A, Kitahara Y, Jinnohara T, McSorley S.J, Kaisho T, Williams I.R, Ohno H. The Ets transcription factor Spi-B is essential for the differentiation of intestinal microfold cells. ***Nat. Immunol.*** 13:729-36, 2012.
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Immune Network

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| Postdoctoral Fellow 3
| Research Assistant 1

Rikinari Hanayama, MD/PhD

RESEARCH REPORT

Before joining IFReC, we had been working on the molecular mechanisms 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.

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 (Fig 1.). 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. 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 an shRNA-based high throughput screening system to sort out the cells with impaired exosome secretion. Using this assay system, we recently identified several candidate genes that might be the critical regulators for exosome secretion. We obtained some knockout mouse lines of these candidate genes to examine whether exosome secretion is impaired in these mice and also to investigate the physiological functions of exosomes. To clarify how exosomes travel in vivo, we generated a mouse model to visualize exosomes. Since 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 cell population. This mouse line will provide a good model system to detect exosomes in vivo.

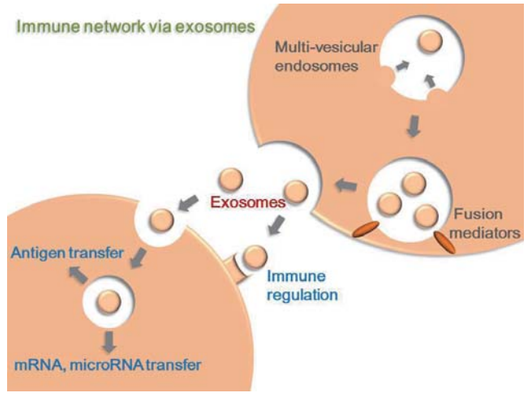

Figure 1. Exosomes: novel signaling entities that regulate complex immune system.

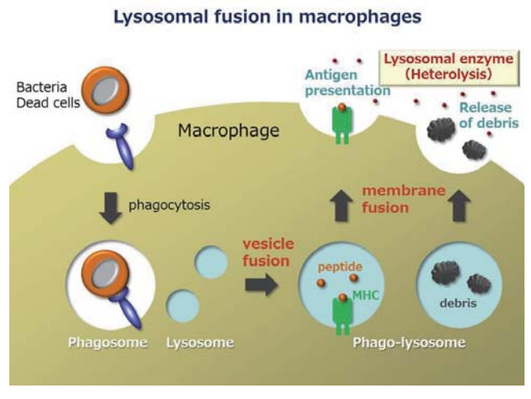

Figure 2. Lysosomal fusion events in macrophages.

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 (Fig 2.). This process is called heterolysis, but its molecular mechanisms as well as its relevance to the development of chronic inflammation have been unclear.

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 C2 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 mediates lysosomal fusion upon calcium stimuli, raising the possibility that it mediates fusion between lysosomes and endosomes, phagosomes or autophagosomes.

In addition, we found that it is also expressed at the cell plasma membrane and mediates fusion between the plasma membrane and lysosomes upon calcium stimuli, causing the release of undigested debris and the secretion of lysosomal enzymes. Our findings would help to elucidate the molecular mechanisms of heterolysis that can be a critical process for the development of chronic inflammation.

Recent Publications

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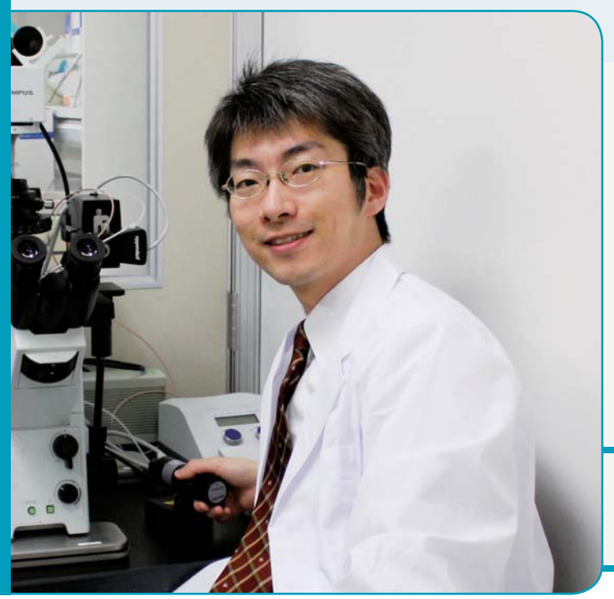
- Nagata S, Hanayama R and Kawane K. Autoimmunity and the clearance of dead cells. ***Cell*** 140:619-30, 2010.
- Hanayama R, Miyanishi M, Yamaguchi H, Suzuki J, Nagata S. Engulfment of apoptotic cells and its physiological roles. ***Encyclopedia of Life Sciences***, Melino G and Vaux D, eds. (John Wiley and Sons) 165-75, 2009.

Immunoparasitology



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Masahiro Yamamoto, PhD

RESEARCH REPORT

Interferon-γ (IFN-γ) is an important Th1 cytokine that strongly suppresses the growth and survival of intracellular pathogens. Stimulation of innate immune cells such as macrophages and dendritic cells by IFN-γ results in robust gene expression of a number of effector molecules. These include immunity-related GTPases such as the Mx proteins, immunity-related p47 GTPases (IRGs), and p65 guanylate-binding proteins (GBPs). Mx proteins have been shown to participate in host defense against RNA viruses such as influenza and vesicular stomatitis virus. Among the IRGs, mice deficient in Irgm1 (LRG-47) are highly susceptible to *Listeria, Salmonella,* and mycobacteria. Furthermore, GBPs have recently been shown to induce antibacterial responses involving phagocytic oxidases, autophagic effectors, and inflammasome. Thus, IFN-γ-inducible immunity-related GTPases play pivotal roles in anti-viral and anti-bacterial immune systems.

Toxoplasma gondii is an obligatory intracellular protozoan parasite that infects virtually all warm-blooded vertebrates including human and mouse. Infection of immunocompromised individuals such as those suffering from AIDS or being treated with chemotherapy often leads to fatal toxoplasmosis encephalitis. Innate immune cells, which recognize microbial components mainly *via* Toll-like receptors (TLRs) and CCR5, are essential in controlling *T. gondii* infection *via* the production of proinflammatory cytokines such as interleukin-12 (IL-12). IL-12 potentiates polarization of naïve T cells to Th1 cells, from which IFN-γ is produced in an antigen-dependent fashion. IFN-γ-inducible GTPases are also important for the inhibition of *T. gondii* growth by IFN-γ. Mice lacking Irgm1, Irgd (IRG-47), Irgm3 (IGTP), or Irga6 (IIGP1) are susceptible to acute and chronic infection. IRGs are recruited to the parasitophorous vacuole (PV), a membrane formed during invasion that is maintained to surround the intracellular replicating parasites. Accumulation of IRGs eventually leads to disruption of the integrity of the PV membranes.

Not only IRGs but also GBPs are known to accumulate around the PV shortly after *T. gondii* invasion. Moreover, since virulent strains of *T. gondii* inhibit the recruitment of GBPs around the PV, GBPs are considered anti-*T. gondii* defensive factors. Among GBPs, Gbp1 and Gbp2 are reported to modulate cellular proliferation. In addition, Gbp1 is involved in the regulation of matrix metalloproteinase 1 in cancer cell lines. Although *in vitro* studies have been reported, the physiological protective role of GBPs against *T. gondii* remains uncertain. The mouse genome carries 13 GBP genes (11 active members and two pseudogenes) that are organized in clusters and share a high degree of homology. Six and seven family members are tandemly aligned on chromosomes 3 and 5, respectively. Such a complex configuration has hampered *in vivo* investigation of the GBP genes through genetic approaches.

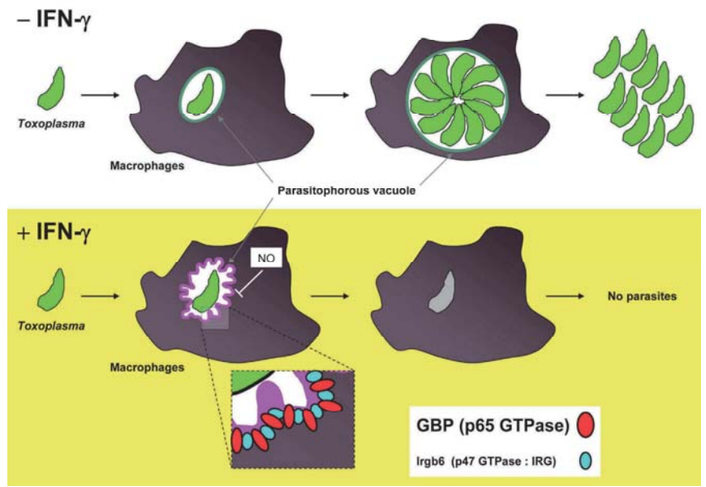

Figure. GBPs are critically involved in the IFN-γ-mediated cell autonomous immunity against Toxoplasma gondii (Yamamoto M, et al. Immunity 2012).

To elucidate the *in vivo* functional contribution of the GBPs to host defense against *T. gondii*, we have generated mice lacking the entire cluster of GBPs on chromosome 3 (Gbp^{chr3}) by Cre-loxP-based chromosome engineering. Gbp^{chr3}-deleted mice were highly susceptible to *T. gondii* infection with a considerably increased parasite burden in tissues. Furthermore, Gbp^{chr3}-deleted macrophages showed defective suppression of parasite growth in response to IFN-γ. Although parasite infection-induced production of oxidants and proinflammatory cytokines as well as Atg4b recruitment to the parasites were normal, IFN-γ-induced disruption of the PV membrane and localization of Irgb6 and Irgb10 to the PV were compromised in Gbp^{chr3}-deleted cells. Moreover, endogenous GBPs co-localized and interacted with Irgb6. The re-introduction of Gbp1, Gbp5, or Gbp7 into Gbp^{chr3}-deleted cells restored the IFN-γ-dependent anti-*T. gondii* response. Taken together, these results demonstrate that this cluster of redundant GBPs has a defensive function by positively regulating IFN-γ-induced Irgb6-dependent cellular innate immunity against *T. gondii*.

Recent Publications

- Yamamoto M, Okuyama M, Ma JS, Kimura T, Kamiyama N, Saiga H, Ohshima J, Sasai M, Kayama H, Okamoto T, Huang DS, Soldati-Favre D, Horie K, Takeda J, Takeda K. A cluster of interferon-γ-inducible p65 GTPases plays a critical role in host defense against Toxoplasma gondii. ***Immunity*** 37:302-13, 2012.
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- Yamamoto M, Standley DM, Takashima S, Saiga H, Okuyama M, Kayama H, Kubo E, Ito H, Takaura M, Matsuda T, Soldati-Favre D, Takeda K. A single polymorphic amino acid on Toxoplasma gondii kinase ROP16 determines the direct and strain-specific activation of Stat3 ***J. Exp. Med.*** 206:2747-60, 2009.

Single Molecule Imaging

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RESEARCH REPORT

Secretomics of single T- and B-cells

Secretomics is the study of substances secreted by a cell and can be used to distinguish cell types. It can be very useful when studying immune cells, since T-cells, B-cells, and other immunological cells often secrete distinct molecular species. However, secretomics techniques are not sufficiently sensitive for single cell analysis, which is needed if seeking understanding of how cells of the same type behave differently.

We have developed a technique that introduces mass spectroscopy (MS) into secretomics, enabling single-cell secretomics. Single cells were trapped in a small microwell so that releasates could be trapped. MS peaks were detected from single T- or B-cells including 157 metabolite candidates according to KEGG database matching. These candidates are potential markers for individual cells from a homogeneous population. Although individual cells from T- or B-cell populations show similar characteristics, we can distinguish them using our technique by the quality and/or quantity of the molecules they secrete.

Raman microscopy of T- and B-cell

Although a cell state can be determined by profiling the protein expression or by characterizing the epigenetic status, these data are difficult to obtain without compromising the cell. Raman microscopy takes advantage of Raman scattering, which describes molecular vibrations that can act as markers for identifying a chemical species, composition, or quantity. These data can be used to measure the quantities of various cellular components like proteins, lipids, DNA, and RNA. These quantities can then be used to determine cell state transitions like cell apoptosis, differentiation, and division.

We have successfully visualized T- and B-cells using Raman microscopy, finding the two cell types can be distinguished by their Raman spectra. We also found large heterogeneity among the T-cells based on their Raman spectrum even when they had common surface markers.

Chronic in vivo imaging in the mouse lymph node using an implanted chamber

To enhance the fusion between imaging and immunology, more accessible procedure for optical microscopy is required. Currently, the only available imaging modality for the study of cell dynamics in living animals is two-photon microscopy. However this technique requires multiple invasive surgeries making it difficult to observe the same animal several days and evaluate disease progression. We have therefore developed an implantable skin window that allows for chronic in vivo imaging of live animals without the need for repeat surgical procedures.

In collaboration with Developmental immunology Team, we used our system to observe T-cells during homeostatic proliferation. The proliferation was induced by transfusing Fucci-T cells into irradiated wild type mice. All mice survived for more than two weeks except for those that died within a day of window implantation. We could successfully image T-cell migration, proliferation and transmigration during a homeostatic proliferation.

Our implantable inguinal skin window system is the only one that can continuously observe immunological events in the lymph node for more than two weeks and can potentially be used to observe similar events in the lymph node follicles. We have already expanded our collaborations with immunology researchers to investigate T- and B-cell dynamics induced by foreign antigen. Preliminary results are showing behavior at the very early phase of the response that could not be observed with conventional methods.

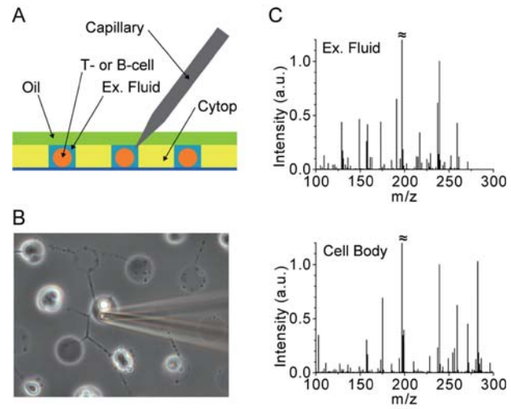

Figure 1. (A) Schematic illustration of the experimental setup. T- or B-cells was seeded on a microwell array made of cytop and covered with oil. The buffer surrounding the cell was collected with a microneedle and analyzed with MS to identify secreted molecules. (B) Phase contrast image of a T-cell in a micro well. The glass microneedle is also seen. (C) Examples of mass spectrum from buffer surrounding a cell (upper panel) and single cell body (lower panel).

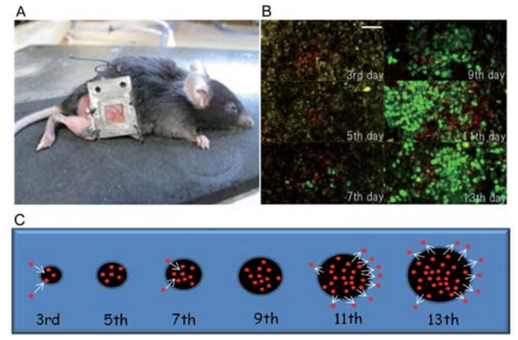

Figure 2. (A) An implanted observation window. (B) Fucci red and green cells imaged with two-photon microscopy for 2 weeks at the same position (C) A mathematical model with recorded images; a large number of proliferated T-cells left lymph node follicles around 9th day.

Recent Publications

- Bai Z, Cai L, Umemoto E, Takeda A, Tohya K, Komai Y, Veeraveedu PT, Hata E, Sugiura Y, Kubo A, Suematsu M, Hayasaka H, Okudaira S, Aoki J, Tanaka T, Albers HM, Ovaa H, Miyasaka M. Constitutive lymphocyte transmigration across the basal lamina of high endothelial venules is regulated by the autotaxin/lysophosphatidic acid axis. ***J. Immunol.*** 190:2036-48, 2013.
- Ikezaki K, Komori T, Yanagida T. Spontaneous Detachment of the Leading Head Contributes to Myosin VI Backward Steps. ***PLoS One*** 8:e58912, 2013.
- Watanabe TM, Tsukasaki Y, Fujita H, Ichimura T, Saitoh T, Akira S, Yanagida T. Distinct modulated pupil function system for real-time imaging of living cells. ***PLoS One*** 7:e44028, 2012.
- Ichimura T, Fujita H, Yoshizawa K, Watanabe TM. Engineering strain-sensitive yellow fluorescent protein. ***Chem. Commun. (Camb)*** 48:7871-73, 2012.
- Fujita K, Iwaki M, Iwane AH, Marcucci L, Yanagida T. Switching of myosin-V motion between the lever-arm swing and brownian search-and-catch. ***Nat. Commun.*** 3:956, 2012.

Biofunctional Imaging

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RESEARCH REPORT

This group has developed new high sensitive and specific in vivo visualization techniques with magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) to contribute to a more comprehensive understanding of the dynamics of the immune system. The group applied 2 patents on MRI visualization techniques in this year (on the high sensitive MRI coil: PCT/JP2012/63325, on the new MRI contrast probe: 2012-246538). Figure 1 shows 11.7T MRI of mouse popliteal lymph nodes using one of the new high sensitive coils; migration and localization of labeled immune cells in the mouse lymph nodes could be visualized in vivo at normal and also at pathological conditions.

MRI using contrast agents like superparamagnetic iron oxide particle (SPIO) is an extremely versatile technique to traffic immune cells, diagnose diseases, assess inflammations, and monitor therapeutic treatments. This group tested the relative importance of particle size and surface coating for the optimization of MRI contrast and labeling efficiency of macrophages migrating to remote inflammation sites. Four SPIOs such as alkali-treated dextran magnetite (ATDM) with particle sizes of 28 and 74 nm, and carboxymethyl dextran magnetite (CMDM) with particle sizes of 28 and 72 nm were tested. This study suggests that coating is more critical than size to optimize the SPIO labeling of macrophages. Among the formulations tested in this study, the best MRI contrast and labeling efficiency are expected with ATDM-coated 74 nm nanoparticles (*Intnational J Nanomed* 7: 5415-5421, 2012).

Immune systems play an important role in inflammatory diseases of the central nervous system (CNS). Although immune cells may help to maintain a neural environment, the visualization of immune cell dynamics in the CNS at normal as well as pathological conditions is not easy with in vivo imaging techniques. The visualization of immune cell behaviors in the CNS in vivo before and after inflammation would improve our understanding of the mechanisms for the CNS pathology and repair. The group has succeeded in the visualization of the recruitment of peripheral endogenous immune cells into the CNS in vivo. Figure 2 shows the recruitment of labeled immune cells into the injured mouse brain. SPIO-labeled endogenous macrophages produce distinct and robust dark spots on T2* weighted MRI. Labeled macrophages located at the margin of the injured brain region. The recruited labeled macrophages were confirmed histologically. The group also found the recruitment of immune cells into the brain tissues at normal as well as pathological conditions. MRI in Figure 3 shows the recruitment of endogenously labeled immune cells into the mouse brain. The endogenously labeled cells were detected 1day after labeling even in control mouse brain tissues. The labeled cells disappeared from brain tissues by 1 week after labeling. The existence of labeled macrophages in the brain parenchyma was confirmed histologically with brain tissues fixed just after MRI experiments.

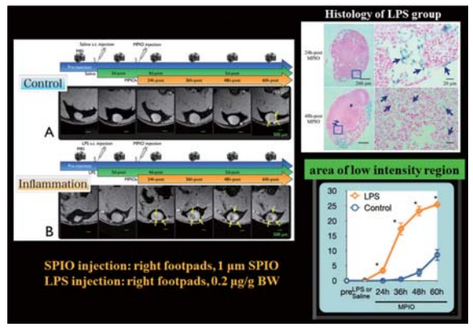

Figure 1. In vivo visualization of immune cells in mice lymph nodes by MRI. The migration speed and localization of immune cells could be assessed at several conditions such as inflammations.

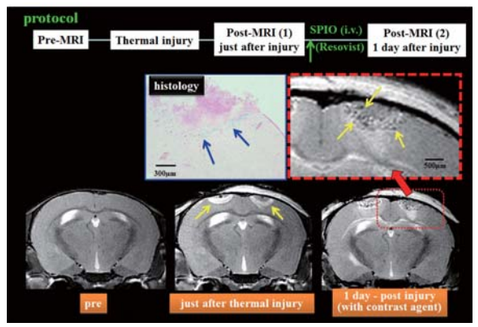

Figure 2. In vivo visualization of labeled immune cells in mouse brain. The mouse brain was partially injured by an electrosurgical knife. Immune cells recruited into brain tissues produce distinct and robust dark spots on in vivo MRI.

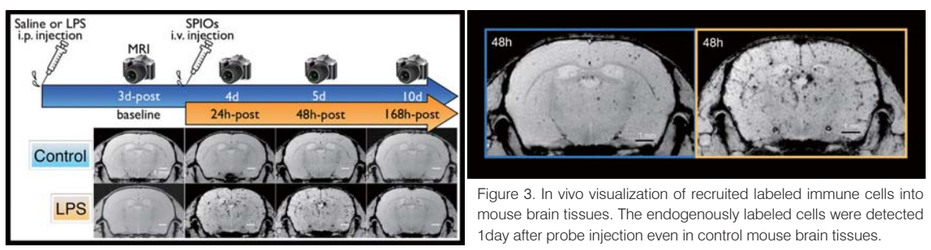

Figure 3. In vivo visualization of recruited labeled immune cells into mouse brain tissues. The endogenously labeled cells were detected 1day after probe injection even in control mouse brain tissues.

The tissue temperatures are determined by the balance between heat produced by tissue energy turnover, which is identical to tissue metabolism, and heat that is removed, primarily by tissue blood flow. Although the tissue temperatures change on the inflammations, it is not easy to measure temperatures at deep regions noninvasively. This group has tried to measure brain temperatures noninvasively to monitor inflammations by MRS. The group investigated whether brain temperature measured by MRS can detect cerebral hemodynamic impairment in patients. Cerebral blood flow and cerebrovascular reactivity were measured using SPECT. A significant correlation was observed between the brain temperature ratio (pathological side/contralateral side) and the cerebrovascular reactivity ratio (pathological side/contralateral side). Brain temperatures measured by MRS can detect the cerebral hemodynamic impairment in patients (*Clinical Neurology Neurosurgery* 115: 445-449, 2013).

Imaging and spectroscopic techniques of this group are non-invasive and will enable direct visualization of the immune responses in order to clarify how the immune system actually works in the body and how immune cells behave under pathological conditions in vivo.

Recent Publications

- Satoh T, Kidoya H, Naito H, Yamamoto M, Takemura N, Nakagawa K, Yoshioka Y, Morii E, Takakura N, Takeuchi O, Akira S. Critical role of Trib1 in differentiation of tissue-resident M2-like macrophages. ***Nature*** 495:524-28, 2013.

- Ma Q, Nakane Y, Mori Y, Hasegawa M, Yoshioka Y, Watanabe T, Gonda K, Ohuchi N, Jin T. Multilayered, core/shell nanoprobes based on magnetic ferric oxide particles and quantum dots for multimodality imaging of breast cancer tumors. ***Biomaterials*** 33:8486-94, 2012.

- Saito S, Tsugeno M, Koto D, Mori Y, Yoshioka Y, Nohara S, Murase K. Impact of Surface Coating and Particle Size on the Uptake of Small and Ultrasmall Superparamagnetic Iron Oxide Nanoparticles by Macrophages. ***Internat. J. Nanomed.*** 7:5415-21, 2012.

- Mori Y, Umeda M, Fukunaga M, Ogasawara K, Yoshioka Y. MR contrast in mouse lymph nodes with subcutaneous administration of iron oxide particles: size dependency. ***Magn. Reson. Med. Sci.*** 10:219-27, 2011.

- Kinoshita M, Yoshioka Y, Okita Y, Hashimoto N, Yoshimine T. MR molecular imaging of HER-2 in a murine tumor xenograft by SPIO labeling of anti-HER-2 Affibody. ***Contrast Media Mol. Imaging*** 5:18-22, 2010.

Cellular Dynamics

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Masaru Ishii, MD/PhD

RESEARCH REPORT

The main subject of our laboratory is to reveal the cellular dynamics in various kinds of tissues and organs in vivo, by using advanced imaging techniques, especially focusing on the dynamic phenomenon within bone marrow cavity. Bone marrow is a mysterious tissue where various immune and hematopoietic cells are originated and undergo differentiation. However, conventional methods have limited studies to static histological specimens. Recent advances in optical imaging technology, especially using two-photon excitation microscopy, have made possible visualization of living cells in intact organs and evaluation of their movements and interactions with high spatiotemporal resolution. However, the penetration depths of two-photon excitation lasers depend on the contents of the tissues and organs to be imaged. For example, soft, homogenous tissues, chiefly brain cortex, can be penetrated to as deep as 800–1000 μm, whereas calcium phosphate crystals in scaffolds of collagen fiber networks, the main structural component of the bone matrix, scatter laser beams so that the penetration depth within bone tissues is limited to 200 μm. This has hindered the visualization of intact bone.

By improving the microscopy systems, we originally developed an intravital bone imaging system that enabled visualization of the movements of the diverse cell type resident in the bone marrow cavity (Nature 2009; Nature Protocol 2009, etc). The dynamic nature of different bone cell types can be visualized in a time-dependent manner, in addition to the spatial and structural information generated by conventional histomorphometric analyses, has resulted in a paradigm shift in research on bone biology and immunology.

1 Migration and positioning of cells—dynamic observation with intravital bone marrow imaging.

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 has been 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.

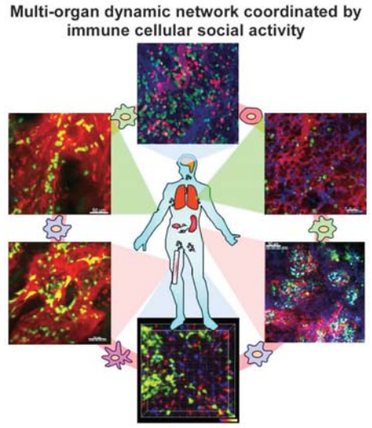

Figure.
Multi-organ dynamic network coordinated by immune cell activity. Immune cells are high dynamic and interconnecting various tissues and organs, by forming a 'soft-wired' network. We are elucidating the basic principle controlling the dynamic nature of immune cells by visualizing their in situ behaviors with advanced imaging techniques.

2 Function of bone-resorbing cells in vivo—two distinct functional states of differentiated osteoclasts.

Osteoclasts are a characteristic macrophage that forms large polykaryons for resorbing hard bone materials. Histological sections have identified TRAP-positive multinucleated cells lining the erosive surface of bone trabeculae, although they are static and their dynamic nature cannot be analyzed. 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)—indispensable machinery for acidic bone resorption—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.

3 Establishment of novel imaging technology for revealing various immune systems

The intravital imaging method can also be adopted on the examination of various kinds of tissues and organs beside bone marrows, and our lab is now developing methods such as for spleen, small intestine, liver, kidney, skin, lung, etc, and we are now elucidating the dynamic systems in immunology as well as biological science such as hematopoiesis and cell lineage commitment and other 'niches', cell dynamics during inflammation in skin, lung and intestines, and cancer metastasis (see the Figure).

Recent Publications

- Kikuta J, Kawamura S, Okiji F, Shirazaki M, Sakai S, Saito H, Ishii M. S1P-mediated osteoclast precursor monocyte migration is a critical point of control in antibone-resorptive action of active vitamin D. ***Proc. Natl. Acad. Sci. USA.*** 110:9009-131, 2013.

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- Kotani M, Kikuta J, Klauschen F, Chino T, Kobayashi Y, Yasuda H, Tamai K, Miyawaki A, Kanagawa O, Tomura M, Ishii M. Systemic circulation and bone recruitment of osteoclast precursors tracked by using fluorescent imaging techniques. ***J. Immunol.*** 190:605-12, 2013.

- Ishii M, Kikuta J, Shimazu Y, Meier-Schellersheim M, Germain RN. (2010) Chemorepulsion by blood S1P regulates osteoclast precursor mobilization and bone remodeling in vivo. ***J. Exp. Med.*** 207:2793-98, 2010.

- Ishii M, Egen JG, Klauschen F, Meier-Schellersheim M, Saeki Y, Vacher J, Proia RL, Germain RN. (2009) Sphingosine-1-phosphate mobilizes osteoclast precursors and regulates bone homeostasis. ***Nature*** 458:524-28, 2009.

Nuclear Medicine

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Research Assistant	1
Support Staff	3

Jun Hatazawa, MD/PhD

RESEARCH REPORT

Nuclear medicine is a field of great potential which can evaluate the in-vivo 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 new methodology of in-vivo PET imaging, evaluation of patients receiving therapy with new metabolic based criteria, and new imaging modalities. Followings are the activities in FY2012.

In-vivo imaging of activated microglia and macrophage with translocator protein radioligands.

^{11}C-DPA-713 is a translocator protein (TSPO), which is up-regulated in the activated microglia and macrophages. We evaluated relationship between the accumulation of ^{11}C-DPA-713 and severity of the cerebral infarction in the rat model of brain ischemia. The figure shows the amount of the activated microglia and macrophages by PET images and infarction area by MR images. Accumulation of ^{11}C-DPA-713 showed different uptake level depending on the severity of the infarction. The interesting point is that less uptake was observed in the most severe infarction group than in moderate infarction group at day4 after ischemia.

We have confirmed the safety of this TSPO tracer for the clinical use and preparing for the clinical PET research with patients in the next fiscal year.

Quantitative in-vivo evaluation of cerebral blood flow and oxygen metabolism by positron emission tomography.

Cerebral blood flow and metabolic rate of oxygen have been extensively studied in humans by positron emission tomography (PET) with ^{15}O labeled gases and $H_2{}^{15}O$, to elucidate the brain functions and hemodynamic and metabolic compromise in stroke patients. However, a number of difficulties restrict the application of this method to experimental small animals. We established a standard methodology to evaluate the cerebral circulation and oxygen metabolism in rats by the original steady-state inhalation method of ^{15}O-CO_2 and ^{15}O-O_2 gases (T.Watabe et al). This method enabled to allow the evaluation of hemodynamic state in a rat model of cerebral infarction, especially in the simultaneous evaluation of the activated microglia by TSPO tracer in the same individual.

Evaluation of response to neoadjuvant chemotherapy for esophageal cancer patients by new PET-based criteria.

Recently, PET response criteria in solid tumors (PERCIST) have been proposed as a new standardized method to assess chemotherapeutic response metabolically and quantitatively (Wahl et al., 2009). We evaluated therapeutic response to neoadjuvant chemotherapy for locally advanced esophageal cancer, comparing PERCIST with the currently widely used response evaluation criteria in solid tumors (RECIST). RECIST based on the anatomic size reduction rate did not demonstrate the correlation between therapeutic responses and prognosis in patients with esophageal cancer receiving neoadjuvant chemotherapy. PERCIST was found to be the strongest independent predictor of outcomes, which suggested metabolic-based criteria by PET is more suitable for evaluation of chemotherapeutic response than size-based criteria.

Development of high resolution integrated PET/MR system.

The simultaneous measurement of PET and magnetic resonance (MR) imaging is an emerging field for molecular imaging research. Although optical fiber based PET/MR systems (Yamamoto et al., 2010) have advantages on less interference between PET and MR, there is a drawback in reducing the scintillation light due to the fiber. To reduce the problem, we have newly developed flexible optical fiber bundle based block detectors and employed them for a high resolution integrated PET/MR system and successfully performed simultaneous PET/MR imaging of small animals. The developed high resolution PET/MR system is promising device for molecular imaging research.

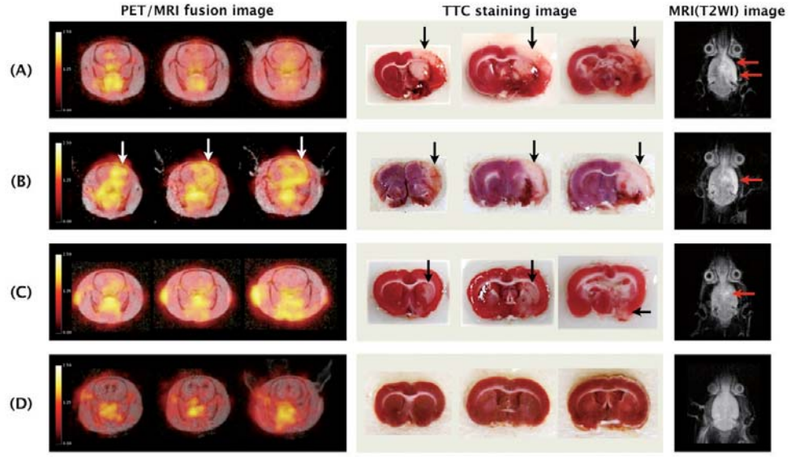

Figure. PET/MRI fusion images (transaxial), TTC staining slices, and T2 weighted MRI (coronal).

Recent Publications

- Watabe T, Shimosegawa E, Watabe H, Kanai Y, Hanaoka K, Ueguchi T, Isohashi K, Kato H, Tatsumi M, Hatazawa J. Quantitative Evaluation of Cerebral Blood Flow and Oxygen Metabolism in Normal Anesthetized Rats: 15O-Labeled Gas Inhalation PET with MRI Fusion. ***J. Nucl. Med.*** 54:283-90, 2013.
- Yamamoto S, Watabe H, Kato K, Hatazawa J. Performance comparison of high quantum efficiency and normal quantum efficiency photomultiplier tubes and position sensitive photomultiplier tubes for high resolution PET and SPECT detectors. ***Med. Phys.*** 39:6900-07, 2012.
- Yamamoto S, Watabe H, Kanai Y, Watabe T, Aoki M, Sugiyama E, Kato K, Hatazawa J. Development of a flexible optical fiber based high resolution integrated PET/MRI system. ***Med. Phys.*** 39:6660-71, 2012.
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Chemical Imaging Techniques

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RESEARCH REPORT

Protein fluorescence labeling is a powerful method for imaging the subcellular localization and dynamics of proteins. Currently, popular tools for live-cell imaging are fluorescent proteins (FPs) that are utilized by fusing with the proteins of interest (POIs). Although FPs are useful to many biological studies owing to its technical easiness, their application are sometimes not preferable as fusion tags because of their relatively large size. Moreover, FPs are not suitable to in-vivo imaging experiments, which require near-infrared fluorophore for visualizing the deep region of tissues, because no bright fluorescent proteins emitting fluorescence around 700-900 nm are not available.

Recently, protein labeling methods using synthetic fluorescent probes have been reported as a possible solution to these limitations. In these methods, a fluorescent probe is designed to specifically bind a protein tag, and the probe is used to image POIs fused with the protein tag. One of advantages in this approach is that any molecule, including NIR fluorescent dyes or other functional compounds, can be incorporated into a probe by replacement of the fluorophore moiety of the probe. Another advantage is that the pulse-chase analysis of protein movement or expression can be easily carried out by labeling the proteins with the probes at a specific time. Furthermore, it is possible to use a protein tag with smaller size than that of FPs. On the other hand, this labeling system has a potential problem that the fluorescence of free probes inside cells hinders the specific detection of labeled proteins. Therefore, a wash-out process is required to remove the free probes from cells. However, the problem arises when the probes are not completely washed out and the remaining probes cause the reduction of the signal-to-noise ratio. In our group, to solve this problem, a novel protein labeling system is developed utilizing photoactive yellow protein (PYP) tag and fluorogenic probes.

PYP-tag is a small protein composed of 125 amino acids and originally found in purple bacteria. This protein binds to a natural ligand, 4-hydroxycinnamic acid, which forms a thioester bond with Cys69. PYP-tag is also known to bind a 7-hydroxycoumarin derivative. This coumarin ligand was utilized as a ligand part and static quencher of a fluorogenic probe for labeling PYP-tag. FCTP was designed by connecting the coumairn ligand with fluorescein and in this probe, the fluorescein emitted only weak fluorescence due to the intramolecular interaction between the coumarin and the fluorescein, and enhanced fluorescence intensity upon the labeling reaction that disrupted the interaction. However, the labeling reaction was unexpectedly slow and needs more than 24 hours for the complete reaction. Hence, the improvement of the kinetic property was necessary for this protein labeling system.

The slow kinetics of FCTP was attributed to steric hindrance generated around the ligand as a result of the association with the fluorophore moiety. To promote the labeling rate, a quenching mechanism to avoid the steric hindrance was required. Considering this point, the second generation probe was developed using a new strategy that focused on the leaving group of the ligand. When PYP-tag is bound to a probe, thiophenol is released from the ligand moiety. By connecting a fluorescence quencher into the thiophenol, a probe, FCANB, containing fluorescein was designed (Figure 1). The key of this strategy is that nitrobenzene was selected as a quencher. This compound is known to interact with various fluorophores and quenches their fluorescence. Owing to this effect, the probe was assumed to reduce the fluorescence intensity, and become fluorescent by the labeling reaction as a result of the dissociation of the quencher. In fact, FCANB was weakly fluorescent in a free state and the labeling reaction triggered the fluorescence enhancement. The kinetic property of the probe was significantly improved by 110-fold in comparison with FCTP, as expected. Live-cell imaging experiments were also conducted without a washout step by utilizing the construct of PYP-tag fused to EGFR expressing on cell surface. The fluorescence was clearly detected along the plasma membrane of cells expressing PYP-tag by incubation of the probe with the cells for 30 min. This no-wash labeling system is unique in small protein tag and will be a valuable tool for the precise spatio-temporal analyses of proteins.

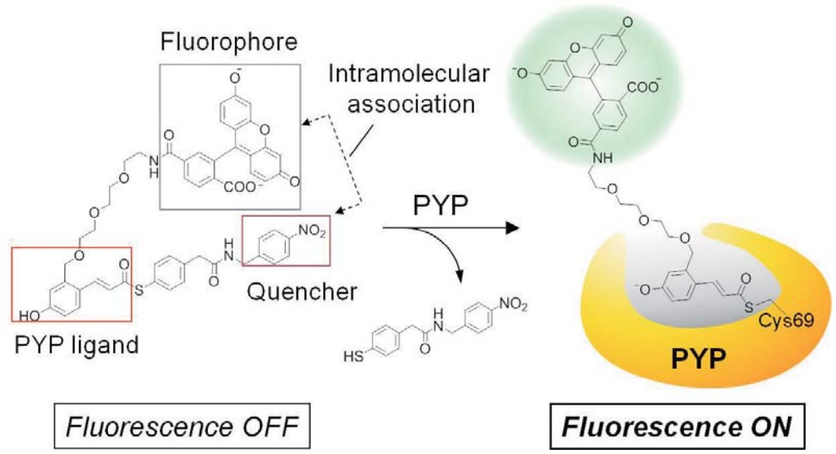

Figure. Principle of fluorogenic labeling with FCANB.

Recent Publications

- Hori Y, Nakaki K, Sato M, Mizukami S, Kikuchi K. Development of protein-labeling probes with a redesigned fluorogenic switch based on intramolecular association for no-wash live-cell imaging. ***Angew. Chem. Int. Ed.*** 51:5611–14, 2012.
- Baba R, Hori Y, Mizukami S, Kikuchi K. Development of Fluorogenic Probe with Transesterification switch for Detection of Histone Deacetylase Activity. ***J. Am. Chem. Soc.*** 134:14310-13, 2012.
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Biophotonics Laboratory

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RESEARCH REPORT

Raman spectroscopic imaging is a valuable label-free modality for measuring chemical component distributions in living cells, and allows a degree of quantitative measurement of the composition, distribution and changes in cell molecules, all without using labeling techniques. In the Biophotonics laboratory, we use recently developed highly sensitive detector technology and appropriate laser excitation sources to push Raman measurement and analysis towards label-free chemically specific imaging, allowing not only label-free imaging, but also the measurement of chemical changes which are difficult to observe by other means (Okada *et al* 2012).

Over the last year we made progress in three main areas: technical innovation, label-free measurement of disease progression and nanoparticle-assisted Raman analysis. Firstly, we created the first simultaneous Raman and quantitative phase imaging system. One challenge with Raman is that the imaging rate is limited by the scattering probability of the incident light. While some approaches to get around this are being developed, there is no "magic bullet" to solve the low signal emission. Instead we added an additional mode using coherent light to spectrally isolate the two imaging systems. This multimodal imaging system retains the label-free aspects of both approaches and gives us rapid and quantitative phase imaging so that we can see how cell is moving and determine how the light is retarded during transmission through the cell, while still collecting the cellular component information provided by the Raman mode. Finding that certain types of cellular components produce different sources of contrast (e.g. proteins appear stronger in the phase mode while lipids appear stronger in the Raman mode), we can use both these complementary label-free modalities together and benefit from the advantages of each.

We also applied nanoparticle-enhancement to amplify our measurement of cellular components. Nanoparticle antenna can boost the Raman signal by many orders of magnitude and do not have to be targeted to a particular molecule and can reveal detailed information about what molecules are in proximity to it during or after the particle entry to the cell (Ando *et al,* 2011). An additional finding was that surface treatments of the nanoparticle cell can be used to control the surface charge and this in turn affects the immunological reaction to the nanoparticles. We clarified an important point this year: the use of Raman and nanoparticle-enhanced Raman is not common in immunology and effects of the nanoparticle on the immune response hadn't been well studied. We found that at high enough concentrations the particles could indeed affect normal cellular reactions to pathogens or stimuli such as LPS, however at the concentrations relevant for our experimental approach, they do not interfere with the immune response (Pissuwan *et al* 2013). This paves the way for further uses in label-free single-molecule sensitivity detection in the cell.

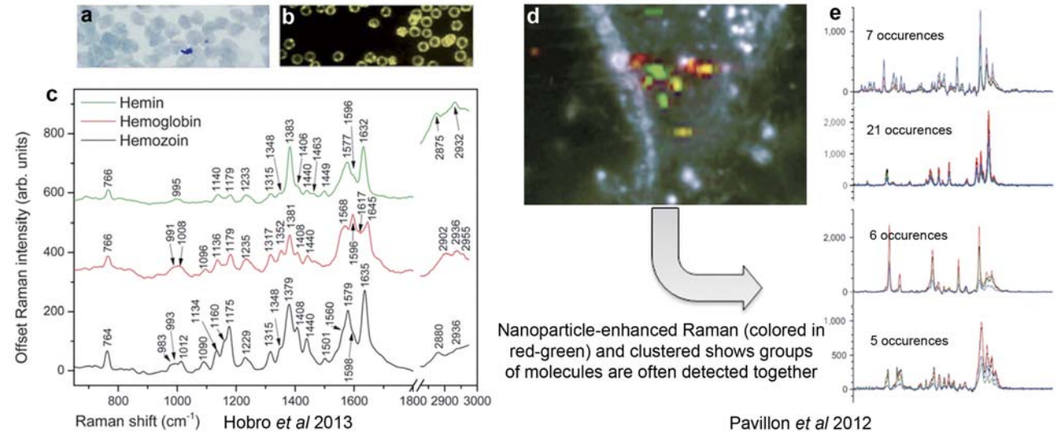


Figure. (a) Giemsa staining vs (b)Raman analysis of P. yoelii NL-infected erythrocytes. Free heme and aggregated heme are spectrally distinct (c) and can be used for disease diagnosis. (d) shows how nanoparticles detect groups of molecules in a cell. We use mathematical models to detect and group signals (e) from molecules in proximity to the nanoparticles.

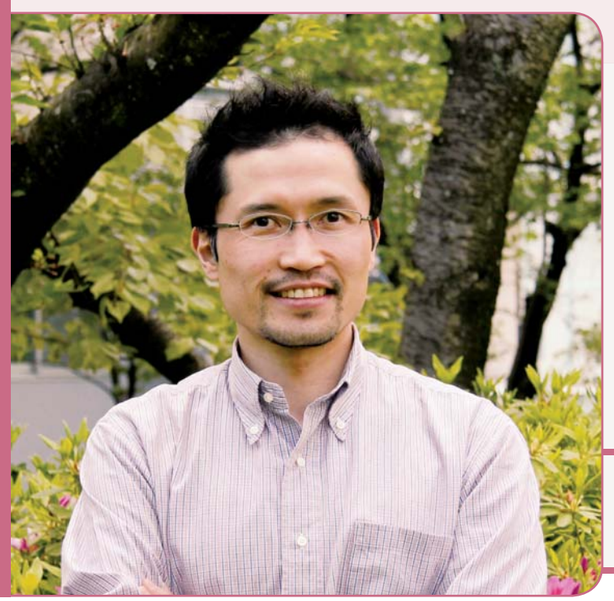
When we perform surface-enhanced Raman imaging, we typically end up with thousands or tens of thousands of spectra. We created a system (Pavillon *et al* 2012) for automatically identifying signals of interest from within our data and by further clustering the measured spectra we found that we could isolate a number of different molecules in the cell, measured with the aid of nanoparticles. This is an important step towards interpreting and identifying measured spectra, and together with our cell component spectral database is helping improve the specificity of Raman measurements.

We also saw the emergence of new projects using Raman not in an imaging mode but in a more analytic mode where we fully utilize spectral details by linking them robustly to molecular structure. Although protein and RNA structure can be quantitatively determined by a combination of existing experimental techniques and emerging computational predictive methods, Raman has the capability to significantly add to the available structural information for small molecules (Hobro *et al* 2013a). In particular molecules that are difficult to analyze by NMR or other methods can be well-suited to Raman analysis. This will be an on-going project over the next year. We also applied Raman analysis of blood and plasma samples for malaria detection in collaboration with colleagues in the Laboratory of Malaria Immunology. During the progression of the disease, malaria parasites form hemozoin which can be detected by Raman or other methods. However, at very early stages of the disease, the amount of hemozoin can be too low to be detectable by imaging since there may be no hemozoin particle in the field of view. We found that the sensitivity of Raman measurements were high enough to detect very small changes in heme content in plasma, showing the possibility of detection of disease at very early stages, which is advantageous for treatment (Hobro *et al* 2013b).

Recent Publications

- Hobro AJ, Standley DM, Ahmad S, and Smith NI. Deconstructing RNA: optical measurement of composition and structure. ***Phys. Chem. Chem. Phys.***, 2013. in press.
- Hobro AJ, Konishi A, Coban C and Smith NI. Raman spectroscopic analysis of malaria disease progression via blood and plasma samples. ***Analyst*** 138:3927-33, 2013.
- Pissuwan D, Kumagai Y and Smith NI. Effect of surface-modified gold nanorods on inflammatory cytokine response in macrophage cells. ***Part. Part. Syst. Charact.*** 30:427-33, 2013.
- Pavillon N, Bando K, Fujita K and Smith NI. Feature-based recognition of Surface-enhanced Raman spectra for biological targets. ***J. Biophotonics***, 2012. in press.
- Okada M, Smith NI, Palonpon AF, Endo H, Kawata S, Sodeoka M. Label-free Raman observation of cytochrome c dynamics during apoptosis. ***Proc. Nat. Acad. Sci. USA.*** 109:28-32, 2012.

Immune Response Dynamics



Associate Professor
Kazuhiro Suzuki
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Support Staff 2

Kazuhiro Suzuki, MD/PhD

RESEARCH REPORT

Looking deeper and deeper

The recent advances of two-photon microscopy allowed direct visualization of cell behaviors in deep tissues. Since the introduction of two-photon microscopy in the field of immunology, various aspects of immune responses have been visualized, which has strongly promoted our understanding of the immune system. Immune responses are mediated by multimodal cellular interactions in specific microenvironments within lymphoid tissues. Therefore, for comprehensive understanding of the immune responses, it is important to assess the localization of cells involved in the responses throughout the lymphoid tissues. However, current two-photon microscopy penetrates only to a depth of ~300 μm below the surface of lymphoid organs, which allows visualization of only limited regions of the organs. Thus, complementary optical techniques are required to image cell populations deeper in intact lymphoid tissues.

3D reconstruction of lymphoid organs

One of the major causes that limit depth penetration in optical imaging is light scattering in tissues. To overcome this limitation, Atsushi Miyawaki and colleagues recently developed a reagent called 'Sca*l*e' that renders biological samples optically transparent without losing fluorescent signals in the clarified tissues (Hama, H., *et al. Nat. Neurosci.* 14: 1481, 2011), and succeeded to visualize brain structures in three dimensions (3D). Thus, we introduced this technique to visualize deeper regions of lymphoid organs and reconstruct the whole organs in 3D. In initial experiments, we tried 3D reconstruction of lymph nodes, which had been my major imaging object in two-photon microscopy. Mice were transferred with B and T cells that were labeled with different fluorescent dyes, and their peripheral lymph nodes were subjected to 3D reconstruction. By the treatment with the Sca*l*e solution for 10 days, the lymph nodes became almost transparent. Two-photon microscopy penetrated the lymph nodes of ~1.5 mm thickness from top to bottom. The 3D reconstruction of the whole lymph nodes allowed us to visualize cell localization all over the lymph node from various angles (Figure). We are now applying this method to other lymphoid organs, including spleen and thymus.

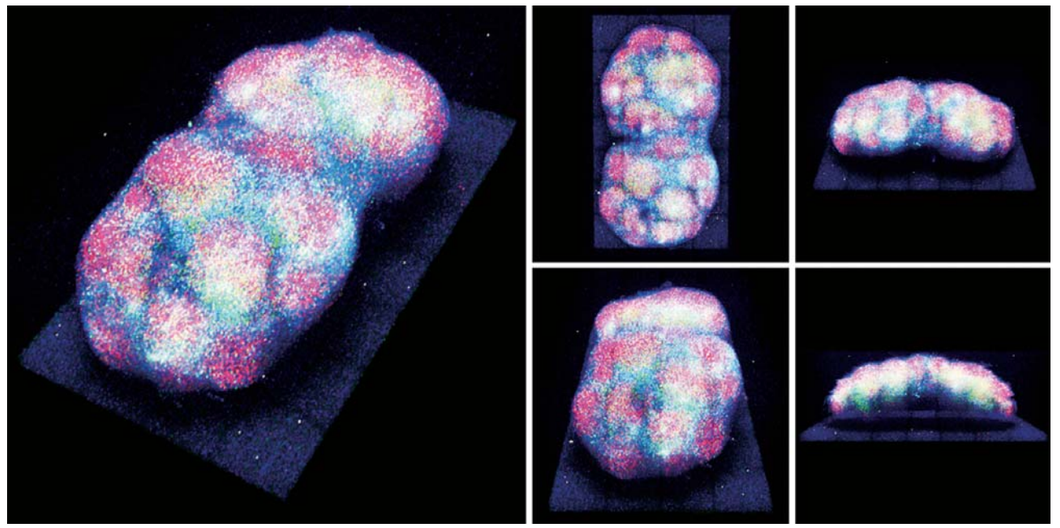

Figure. 3D reconstruction of a whole lymph node.
B (red) and T (green) lymphocytes were differentially labeled with fluorescent dyes and transferred to a mouse. Peripheral lymph nodes were collected from the mouse and subjected to optical clearing using the Sca*l*e solution. Multiple *z*-stacks were acquired by two-photon microscope and stitched together to generate a 3D image of a whole lymph node.

Perspectives

In combination with selective and efficient fluorescent reporters, the technique of 3D reconstruction of lymphoid organs would be useful to define the localization of rare cell populations that could be overlooked in sectioned organs. We are trying to visualize all of the memory lymphocytes, which usually account for less than 1% of total cells in lymphoid organs, in the whole spleen and lymph nodes. Revealing the positional relationships among memory lymphocytes in the whole lymphoid organs would provide a clue to solve a long-standing question why memory immune responses are induced much more efficiently than primary responses. In addition, we are investigating the relationships between lymphatic vessels and lymphoid stroma, including follicular dendritic cells. This would enable global analysis of antigen transport in lymphoid organs and help comprehensive understanding of how immune cells encounter antigens.

Recent Publications

- Gray EE, Friend S, Suzuki K, Phan TG and Cyster JG. Subcapsular sinus macrophage fragmentation and CD169^{+} bleb acquisition by closely associated IL-17-committed innate-like lymphocytes. ***PLoS One*** 7:e38258, 2012.
- Green JA, Suzuki K, Cho B, Willison D, Palmer D, Allen CD, Schmidt TH, Xu Y, Proia R, Coughlin SR and Cyster JG. The sphingosine 1-phosphate receptor $S1P_2$ maintains the homeostasis of germinal center B cells and promotes niche confinement. ***Nat. Immunol.*** 12:672-80, 2011.
- Gray EE, Suzuki K and Cyster JG. Identification of a motile IL-17-producing gamma delta T cell population in the dermis. ***J. Immunol.*** 186:6091-95, 2011.
- Suzuki K, Grigorova I, Phan TG, Kelly LM and Cyster JG. Visualizing B cell capture of cognate antigen from follicular dendritic cells. ***J. Exp. Med.*** 206:1485-93, 2009.
- Suzuki K, Kumanogoh A and Kikutani H. Semaphorins and their receptors in immune cell interactions. ***Nat. Immunol.*** 9:17-23, 2008.

Information Systems

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Assistant Professor
Syoji Kobashi
Kei Kuramoto
Assistant Professor
Manabu Nii
Support Staff 1

Yutaka Hata, PhD

RESEARCH REPORT

We did the following four studies in Information Systems.

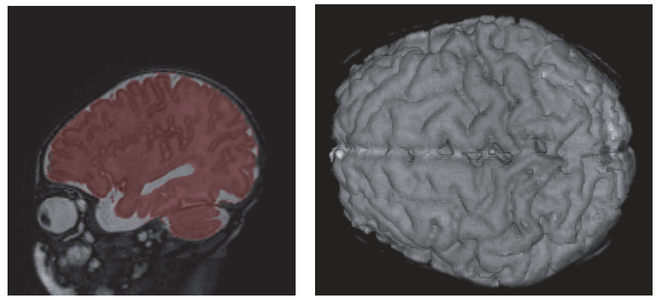
Ultrasonic Imaging

Azoospermia is one of the causes of male infertility. It is the symptom that there is complete absence of sperm in the ejaculate. Fifteen-20 % of infertile men turn out to be azoospermia. Sperm is made from seminiferous tubule in the testicular. To obtain sperm from Azoospermia patient, doctors perform Micro-TESE surgery. Azoospermia patient have two types of seminiferous tubule healthy and unhealthy. There are differences in diameter; healthy seminiferous tubule has diameter of 250 μm ~300 μm, and unhealthy seminiferous tubule has much smaller 150μm. In this surgery, doctors cut open the testicular and brings out the healthy seminiferous tubule. The rate of sperm extraction is 44.0 %. Failed patients did not have healthy tubule. However, today the physician confirms the tubule during the surgery. So no one could foretell the presence of the normal tubule before surgery. Therefore, the physician requires a measurement system that evaluates the presence of normal tubules before surgery.

We solved this problem by using ultrasonic device. Ultrasonic measurement of 150μm ideally requires wavelength with at least 150μm (=10MHz). However, we cannot observe 3cm testicular object because of the 10MHz wave penetrating power. To solve this problem, we found a relationship between diameter of line shape object and frequency of ultrasonic reflected wave. From this relationship, we proposed a method that identifies the diameter on frequency domain. The proposed method successfully evaluates the diameter of seminiferous tubules. This research could be applicable to the immune-related diseases on male infertility.

Magnetic Resonance (MR) image processing

Image understanding is a characteristic behavior of the human. In this FY, we studied a method to embed human knowledge to automated image understanding in computer. As a solution, we introduced fuzzy object model (FOM) into image segmentation. FOM is a set of fuzzy degrees stored in 3-D voxel space, and fuzzy degrees mean how belong to an object at the location. Also, we proposed a novel image segmentation method which is based on fuzzy connectedness image segmentation and FOM. The proposed method has been applied to brain extraction of newborn brain MR images. The method was validated by using leave-one-out-cross-validation (LOOCV) in 10 newborn subjects (revised age; 22.56±27.5 days). The quantitative evaluation using manual delineation results (grand truth)



Results (boy, -1month old)

Figure. Comparison with conventional method

shows a mean false-positive-volume-fraction (FPVF) of 1.33±0.97%, a mean false-negative-volume-fraction (FNVF) of 2.90±2.81%, and G-mean of 1.42±0.60%. The results concluded that use of human knowledge represented by FOM has improved image understanding. (see the attached figure)

Regional analysis and predictive modeling for asthmatic attacks

Asthma causes the bronchus inflammation, and makes breathing impossible. In this study, we perform the regional analysis and predictive modeling for asthmatic attacks in Himeji city using the data from 2001 to 2011. We divided Himeji city into inland and coastal areas, and predicted the number of asthmatic attacks in each area by Fuzzy-AR model considering the weather information. The coefficient of correlation was 0.786 in inland, 0.552 in coastal. In coastal, the prediction by AR model was more accurate than by Fuzzy-AR model. The results showed that Fuzzy-AR model considering the weather information is inappropriate in coastal. We also develop the cloud system and ICT system for the prediction and analysis using various statistical data of immune system disease.

Evaluation system for nursing-care text data

Nursing-care quality improvement is very important for our QOL. Instead of actual observation for evaluating nursing-care process, freestyle Japanese texts which contain real nursing-care were collected by using web-based application. In this study, we constructed a nursing-care text classification system to improve nursing-care quality. We proposed a novel feature definition by using the morphological and the dependency relation analysis. First, nursing-care texts are parsed into every word and extracted every relation between words. Then, feature vectors are constructed by using the above mentioned information. For one third of 12 nursing-care text sets collected in 2009, the proposed classification system classified the texts into four classes with 79.52-82.55% accuracy. From the results, we can conclude that the structure of data has importance for representing features.

Recent Publications

- Yagi N, Hata Y and Shibanuma N. Fuzzy support system for total hip arthroplasty stem by ultrasonic intraoperative measurement. ***J. Hindawi - Advances in Fuzzy Systems***, 2012. Online.
- Hashioka A, Kobashi S, Kuramoto K, Wakata Y, Ando K, Ishikura R, Ishikawa T and Hata Y. A neonatal brain MR image template of 1 week newborn. ***J. Computer Assisted Radiology and Surgery*** 7:2:273-280, 2011.
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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 and Kurosaka M. Nondestructive evaluation of cell numbers in bone marrow stromal cells/beta-tricalcium phospate composites using ultrasound. ***Tissue Engineering, Part C: Methods*** 16:3:347-353, 2010.
- Hata Y, Kobashi S and Nakajima H. Human health care system of systems. ***IEEE Systems Journal*** 3:2:231-238, 2009.

Systems Immunology

- **Associate Professor** Daron M Standley
- **Associate Professor** Kazutaka Katoh
- **Assistant Professor** Shide Liang, Alexis Vandenbon
- **Postdoctoral Fellow** 5
- **Research Assistant** 3
- **Visiting Scientist** 1
- **Support Staff** 1

Daron M Standley, PhD

RESEARCH REPORT

Our Laboratory uses computers to address biological questions that cannot easily be elucidated by experiment. We collaborate closely with a number of experimental groups at IFReC 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 cytokines, transcription factors, and other RBPs. While there is much interest in the mechanism by which RBPs recognize their RNA targets, the structures of protein-RNA complexes are notoriously difficult to solve because of the flexibility and reactivity of the RNA molecules. As an alterative approach, we are developing an *in silico* method for predicting the structures of protein-RNA complexes. The first step in the method is to predict the RNA binding sites and nucleotide specificity on the molecular surface of a protein structure of interest (Fernandez, 2011). The second step is to dock the RNA molecule of interest into the predicted binding sites. Protein-RNA complexes we are actively working on include Regnase-1 with IL6 (Matsushita, 2009) and other mRNA targets (Iwasaki, 2011; Uehata, 2013) (figure 1).

Gene expression and epigenetics analysis.

The importance of regulation of gene expression on the level of epigenetics and initiation of transcription is widely recognized. However, detailed regulatory mechanisms remain poorly understood. We investigated combinatorial regulation of the immune response by C/EBPα and NF-κB and confirmed our predictions by wet experiments (Vandenbon, 2012). Also, as part of our collaborations with the Host Defense laboratory, we clarified the functional roles of several important molecules. These include: Jmjd3 and IRF4 in macrophages (Satoh, 2010); IκBζ in NK cells (Miyake, 2010); JDP2 in neutrophils (Maruyama, 2012); Regnase-1 in macrophages and T-cells (Matsushita, 200; Iwasaki, 2011; Uehata, 2013). In addition, we are collaborating with the Experimental Imunology laboratory on Treg cell epigenetics. Finally, we are carrying out large-scale analysis of the regulation of immune responses by epigenetics and transcription factors (Vandenbon, 2012, Vandenbon 2013).

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, there has yet to be a validated model of the MyD88- or TRIF-dependent signaling complex that considers more than 2 proteins. As a first step, we have constructed a structure of the trimer complex formed by one TRIF and two TRAM molecules based on mutagenesis and yeast two-hybrid (Y2H) data. This model can be extended to a hexamer structure (figure 2) by including TLR4. We have since generalized our methodology so that it can be used for any protein complex, and now we're applying it to the MyD88 signaling complex composed of TLR4, Mal, and MyD88.

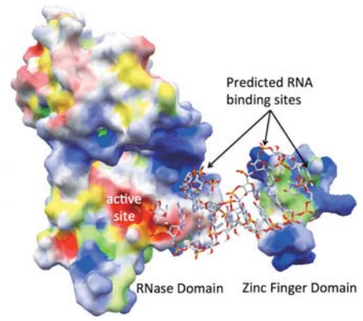

Figure 1. Model of Regnase-1 bound to IL6 3'UTR mRNA stem-loop.

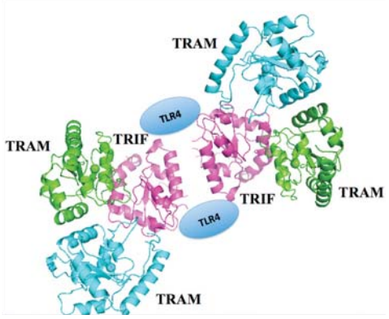

Figure 2. TRAM-TRIF Hexamer model.

Mechanism of STIM1 calcium sensing.

The protein STIM1 is a key regulator of Store-operated Ca^{2+} 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 (figure 3).

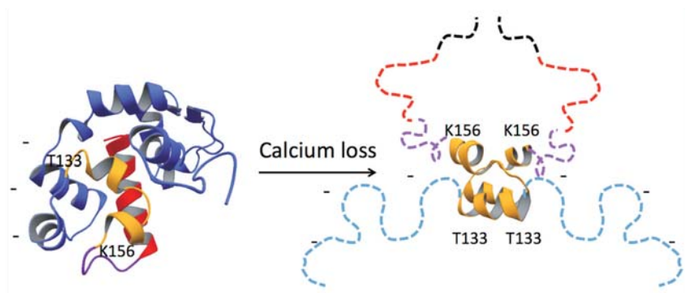

Figure 3. Mechanism of STIM1 signaling.

Recent Publications

- Katoh K & Standley DM. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. ***Mol. Biol. Evol.*** 30:772-80, 2013.
- Vandenbon A, Kumagai Y, Teraguchi S, Amada KM, Akira S & Standley DM. A Parzen window-based approach for the detection of locally enriched transcription factor binding sites. ***BMC Bioinformatics*** 1: 14-26, 2013.
- Liang S, Zhang C, Sarmiento J & Standley DM. Protein loop modeling with optimized backbone potential functions. ***J. Chem. Theory Comput.*** 8:1820–27, 2012.
- Teraguchi S, Kumagai Y, Vandenbon A, Akira S & Standley DM. Stochastic binary modeling of cells in continuous time as an alternative to biochemical reaction equations. ***Phys. Rev. E Stat. Nonlin. Soft Matter Phys.*** 8:062903, 2011.
- Matsushita K, Takeuchi O, Standley DM, Kumagai Y, Kawagoe T, Miyake T, Satoh T, Kato H, Tsujimura T, Nakamura H & Akira S. Zc3h12a is an RNase essential for controlling immune responses by regulating mRNA decay. ***Nature*** 458:1185-90, 2009.

Bioinformatics and Genomics

Associate Professor
Diego Miranda-Saavedra

Assistant Professor
Diego Diez

Postdoctoral Fellow 2

Diego Miranda-Saavedra, PhD

RESEARCH REPORT

In the Bioinformatics and Genomics laboratory we develop and combine bioinformatic methods with experimental research to address specific questions that could not be easily tackled otherwise. In line with IFReC's vision of fusing specific disciplines, we show that the synergies created between experimental science and bioinformatics are yielding new insights into important questions in immunology.

One research interest of the laboratory concerns the mechanistic dissection and manipulation of the interleukin 10-mediated anti-inflammatory response (AIR). The IL-10 response is essential in the control of inflammation and no other pathway can compensate for its loss. Still to this date, we do not comprehend the mechanisms that allow this JAK-STAT pathway (IL-10/JAK1/STAT3) to restrict inflammation. To this end, we previously used ChIP-seq in combination with RNA-seq to characterize the genome-wide targets of STAT3 in cytokine-stimulated macrophages (Hutchins *et al* (2012) Blood). Among the STAT3 target genes are found the AIR factors, defined as genes transcribed by STAT3 and which function to abrogate the transcriptional expression of pro-inflammatory cytokine genes. Through our efforts we expect to describe the entire STAT3-controlled network of AIR factors responsible for the AIR not only in macrophages, but also in other myeloid cell types. Although the LPS-mediated pro-inflammatory response is rather similar across a diversity of myeloid cells, the AIR is not, suggesting that therapeutic opportunities exist for the spatio-temporal control of inflammation.

Besides identifying the elusive AIR factors, we are also exploring other important control mechanisms of the AIR. These include (i) the selection of specific genomic binding sites by STAT3; and (ii) epigenetic control mechanisms. By exploiting published STAT3 ChIP-seq data in various cell types besides macrophages we have shown that most STAT3 binding sites are cell type-specific, thus endowing each type of cell with specific functions. Most interestingly, however, is the finding of an evolutionarily conserved core of 35 STAT3 binding sites in all cell types. This set of 35 sites encodes a mode of auto-regulation for STAT3 signaling, including a number of negative regulators of the JAK-STAT3 pathway that have clear therapeutic potential in the treatment of inflammatory conditions (Hutchins *et al* (2012) Nucleic Acids Research; Hutchins *et al* (2013) JAK-STAT, *in press*) (Figure 1). We also showed that an important mechanism whereby STAT3 selects its genomic binding sites during the AIR is by associating with specific co-factors into assemblies called 'transcriptional regulatory modules' (Figure 2). Indeed, some factors appear to mark specific STAT3 binding sites, such as E2F1. We are also exploring the epigenetic mechanisms that regulate the AIR, both by macrophage-specific developmental factors such as PU.1 (as happens during the pro-inflammatory response) and by the dynamic creation of active enhancers, which can be explored with our tool ChromaGenSVM (Fernandez and Miranda-Saavedra (2012) Nucleic Acids Research). ChromaGenSVM is capable of predicting enhancers very precisely from a small combination of histone epigenetic marks.

We have developed a number of computational tools that complement our experimental program in the laboratory. These include rTRM, a platform for the systematic reconstruction of transcriptional regulatory modules from experimental data. rTRM has been instrumental in predicting specific associations of STAT3 with certain co-factors that are important regulators of the AIR (Figure 2). The selective advantage of rTRM over other methods lies in the integration of protein-protein interaction data, as proteins need to engage in physical interactions with other proteins to perform their functions (Diez, Hutchins and Miranda-Saavedra, *in review*).

In summary, our detailed analyses using a combination of bioinformatics and experiments have identified some key nuclear and cytoplasmic regulatory mechanisms of the AIR. We continue to explore the complexity of the AIR and have started to explore specific avenues with a view to translating our discoveries into treatments for inflammatory diseases.

As a final note, our laboratory is also interested in the role that reversible protein ubiquitination plays in the regulation immune system. To this end, we have developed the foremost bioinformatic method for the genome-wide identification of ubiquitinating and deubiquitinating enzymes and report one-third more such genes in the human genome, which had not been annotated hitherto (Hutchins *et al* (2013) Mol Biol Evol). The systematic exploration of ubiquitination pathways in the immune system, both in physiological and disease states, should uncover novel regulatory mechanisms.

We have active collaborations with a number of laboratories, including those of Profs. Murakami (IFReC) and Michel Tremblay (McGill).

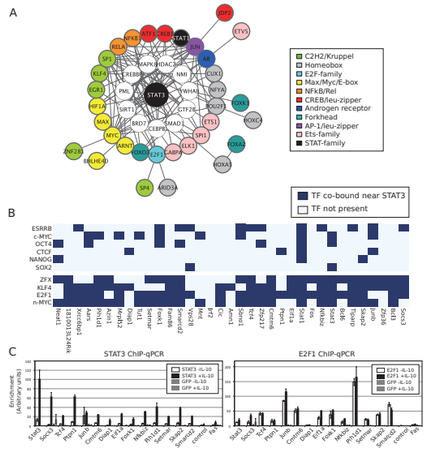

Figure 1. The shared overlap of STAT3 binding in all 4 cell-types forms a cell type-independent regulatory network with MYC and E2F1. (A) HOMER was used to generate *de novo* motifs from the list of 35 STAT3 binding sites common to all four cell-types. Motifs resembling STAT3 or a STAT3 half-site were removed and over-enriched motifs were collected and annotated to genes. Interaction networks were constructed by interrogating the PPI network for proteins interacting with those representing the enriched motifs. TFs were clustered together by motif similarity and coloured by the cluster they belong to: white-colored nodes do not have a representative motif in the databases or do not bind to DNA directly; proteins with a bold circle have a motif enriched in that cell type, whilst proteins with no bolded circle have no discovered motif but were linked to STAT3 through the PPI network. Proteins in the network were filtered by gene expression and here we present the union of the network in all four cell-types (the separate networks are presented in Supplementary Figure 6) (B) ChIP-seq data from ESCs (GSE11431) were re-analyzed and binding sites overlapping within 400 bp were collected. The heatmap shows the 35 STAT3 binding sites together with the other TFs bound in the vicinity of STAT3. (C) We designed primers for 14 STAT3 binding sites shared between all four cell types and performed ChIP-qPCR. Macrophages were treated for 4 h with IL-10 (white bars), or left untreated (black bars), and chromatin was harvested. ChIP was performed using antibodies against STAT3 (left panel), E2F1 (right panel) or GFP (as a control in both panels). Each group of bars represents a STAT3 binding site and is labeled with the name of the nearest gene. (Hutchins *et al* (2013) Nucleic Acids Research).

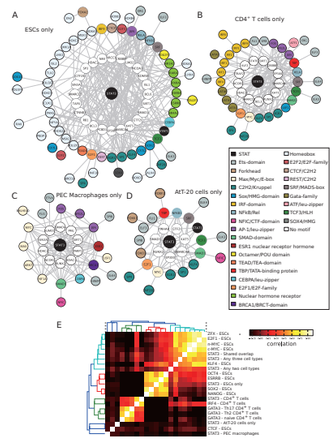

Figure 2. Discreet Transcriptional Regulatory Modules (TRM) determine STAT3 cell type-specific functions. The networks are divded into the cell type-specific lists for ESCs (A), CD4$^+$ T cells (B), macrophages (C) and AtT-20 cells (D). (E) Pairwise correlation heatmap of the ChIP-seq peaks from ESCs, CD4$^+$ T cells and the STAT3 binding sites described here. The lists of peaks were assessed for overlap in an all-against-all comparison, and Pearson correlation scores were used to measure the frequencies of overlapping peaks when compared to all other overlaps.

Recent Publications

- Hutchins AP, Liu S, Diez D, Miranda-Saavedra D. The repertoires of ubiquitinating and deubiquitinating enzymes in eukaryotic genomes. ***Mol. Biol. Evol.*** 30:1172-87, 2013.
- Hutchins AP, Diez D, Takahashi Y, Ahmad S, Jauch R, Tremblay ML, Miranda-Saavedra D. Distinct transcriptional regulatory modules underlie STAT3's cell type-independent and cell type-specific functions. ***Nucl. Acids Res.*** 41:2155-70, 2013.
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- Fernández M, Miranda-Saavedra D. Genome-wide enhancer prediction from epigenetic signatures using genetic algorithm-optimized support vector machines. ***Nucl. Acids Res.*** 40:e77, 2012.
- Hutchins AP, Poulain S, Miranda-Saavedra D. Genome-wide analysis of STAT3 binding in vivo predicts effectors of the anti-inflammatory response in macrophages. ***Blood*** 119: e110-19, 2012.

Symposia & Seminars

Immunology Frontier Research Center

International Symposium
“Dynamism of Immune Reactions & Regulation”

The “Dynamism of Immune Reactions & Regulation” Symposium was originally scheduled for May, 2011, as one of the events to celebrate the 80th anniversary of Osaka University in Japan, but it was cancelled due to the Great East Japan Earthquake.

This symposium was organized by IFReC with the support of the Kishimoto Foundation and Funding Program for World-Leading Innovative R&D on Science and Technology, the FIRST Program AKIRA Project.

In the symposium, world leading scientists discussed the current progress in elucidating immune reactions and mechanisms that mediate and regulate immune responses. In addition to this, on the second day of the symposium, a special session was scheduled in remembrance of Professor Yuichi Yamamura (1918–1990).

DATE May 22-23, 2012

VENUE Osaka International Convention Center
(OICC GRAND CUBE OSAKA)

■ May 22, 2012

Speaker	Title	Chair
Xuetao Cao (Chinese Academy of Medical Sciences)	MHC molecules in the regulation of innate inflammatory responses	Tomohiro Kurosaki
Shizuo Akira (Osaka University)	Regulation of the immune response by the zinc finger domain containing nuclease, Regnase-1/Zc3h12a	
Shigekazu Nagata (Kyoto University)	Exposure of phosphatidylserine to the cell surface	
Ronald Germain (National Institute of Allergy and Infectious Diseases)	Using advanced imaging and systems biology to understand immunity	Takashi Saito
Michael Dustin (New York University)	Signaling complexes controlling human regulatory T cell function	
Mark Davis (Stanford University)	T cell recognition and repertoire	
Scott Fraser (California Institute of Technology)	Imaging, gene traps and conditional mutagenesis for dynamic analyses of embryogenesis and organogenesis	Atsushi Kumanogoh
Alexander Rudensky (Memorial Sloan-Kettering Cancer Center)	Thymic and extrathymic differentiation of regulatory T cells	
Shimon Sakaguchi (Osaka University)	Roles of concurrent epigenetic conversion and Foxp3 expression for the development and function of regulatory T cells	

■ May 23, 2012

Speaker	Title	Chair
Diane Mathis (Harvard Medical School)	Tissular Tregs	Kiyoshi Takeda
Fiona Powrie (University of Oxford)	IL-23-driven cellular and molecular pathways that promote intestinal inflammation and colon cancer	
Josef Penninger (Institute of Molecular Biotechnology, Austria)	RANKL - from a bone loss and osteoimmunology to breast cancer	
Special session : In memory of Professor Yuichi Yamamura (1918-1990)		
Tadamitsu Kishimoto (Osaka University)	IL-6: All the way to therapy	Takehiko Sasazuki
Max Cooper (Emory University)	Evolution of adaptive immune responses	
Tadatsugu Taniguchi (The University of Tokyo)	Regulation of immune responses by old and new cytokines	
Fritz Melchers (Max Planck Institute for Infection Biology)	Microenvironments of B cell development	

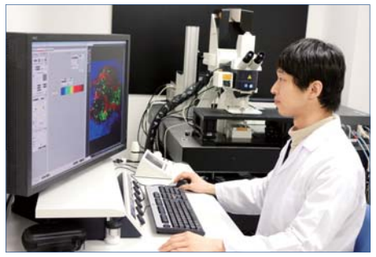
"LICHT Leica Center" Opening Seminar

Osaka University and Leica Microsystems have signed a joint research contract and established a joint research and development center, the "LICHT Leica Center," on the 7th floor of the IFReC Research Building.

To commemorate its opening, the "LICHT Leica Center" Opening Seminar was held. At the start of the seminar, IFReC Director Shizuo Akira, Leica Microsystems K. K. President Takashi Ueno, and Osaka University Trustee Akio Baba gave opening addresses. In the seminar, four researchers presented their current research progress related to bioimaging technology. Afterward, a tour to the "LICHT Leica Center" was arranged for participants of the seminar.

*LICHT : Leica-Osaka University Interdisciplinary Collaboration Hub for Techno-development on bioimaging

Speaker	Title
Andrea Pfeifer (Product Manager, Leica-Microsystems CMS GmbH)	Multiphonton Microscopy beyond 1000 nm
Junichi Kikuta (Cellular Dynamics, IFReC)	Dynamic in vivo imaging of osteoclast function and its regulation by immune system
Michio Tomura (Center for Innovation in Immunoregulative Technology and Therapeutics, Kyoto University)	Approaches to visualizing immunological events in vivo by novel fluorescent proteins
Kazuhiro Suzuki (Immune Response Dynamics, IFReC)	Looking deep into the lymph node



Kishimoto Foundation Lecture

The "Kishimoto lecture" is a seminar series supported by the Kishimoto Foundation. It started in 2010 as a part of the "Advanced Seminar Series on Microbiology and Immunology" organized by Research Institute for Microbial Diseases (RIMD), Osaka University. In the 2nd and 3rd lectures co-organized by RIMD and IFReC, the world's leading scientists gave talks.

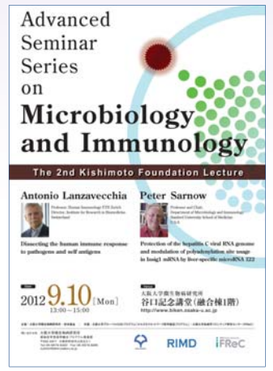
2nd

Antonio Lanzavecchia
(Professor, Human Immunology ETH Zurich
Director, Institute for Research in Biomedicine, Switzerland)

"Dissecting the human immune response to pathogens and self antigens"

Peter Sarnow
(Professor and Chair, Department of Microbiology and Immunology, Stanford University School of Medicine, USA)

"Protection of the hepatitis C viral RNA genome and modulation of polyadenylation site usage in Insig1 mRNA by liver-specific microRNA 122"



DATE September 10, 2012

VENUE Taniguchi Memorial Hall, Osaka University

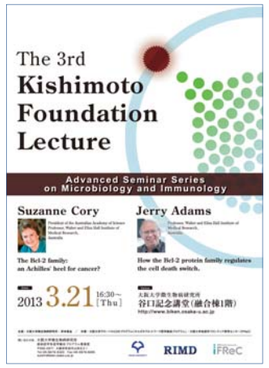
3rd

Suzanne Cory
(President of the Australian Academy of Science
Professor, Walter and Eliza Hall Institute of Medical Research, Australia)

"The Bcl-2 family : an Achilles' heel for cancer?"

Jerry Adams
(Professor, Walter and Eliza Hall Institute of Medical Research, Australia)

"How the Bcl-2 protein family regulates the cell death switch."



DATE March 21, 2013

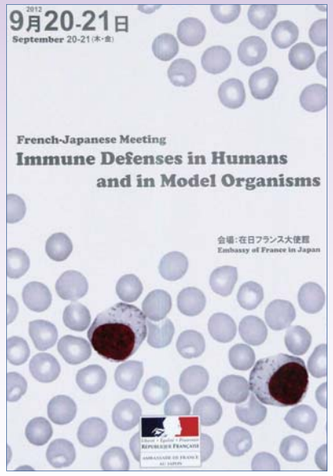
VENUE Taniguchi Memorial Hall, Osaka University

French-Japanese Immunology Meeting

In this French-Japanese meeting "Immune Defenses in Humans and in Model Organisms", various immunology fields such as innate immunity and immune therapy etc. were discussed. Japanese and French researchers have greatly contributed to the important progress made in these fields. The immunologists who represented both countries at this event presented their high-level research results in a closed meeting.

From IFReC, Shizuo Akira, Shimon Sakaguchi and Tadamitsu Kishimoto (keynote speaker) were invited. Dr. Jules A. Hoffman, who is a 2011 Nobel Prize laureate, was among the participants from France. Both Dr. Hoffman and Dr. Akira were jointly awarded Robert Koch Prize and Canada Gairdner Awards in the past.

In the evening of Sep. 20, Mr. Christian Masset, Ambassadeur de France au Japon hosted a reception to celebrate the symposium and Dr. Hoffman's visit to Japan. Using this unique opportunity, all the participants involved held active discussions on various topics.



DATE September 20-21, 2012

VENUE Embassy of France in Tokyo

Speaker	
Tadamitsu Kishimoto	IFReC
Tasuku Honjo	Kyoto University
Jean-Claude Weill	INSERM, Université Paris Descartes
Jules Hoffmann	CNRS
Jean-François Bach	Academie des Sciences, France
Tadatsugu Taniguchi	The University of Tokyo
Sebastian Amigorena	INSERM
Shimon Sakaguchi	IFReC
Bernard Malissen	Centre d'Immunologie de Marseille-Luminy
Alain Fischer	INSERM, Université Paris Descartes
Shigekazu Nagata	Kyoto University
Shizuo Akira	IFReC
Kensuke Miyake	The University of Tokyo

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 conducted, and to promote collaborations between all the immunology, imaging and informatics groups. About 100 people on average attend each time.

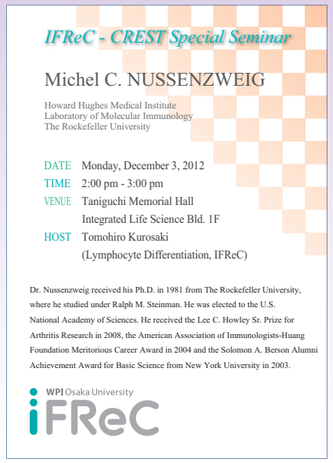
DATE The 4th Colloquium: June 20, 2012
The 5th Colloquium: August 29, 2012
The 6th Colloquium: October 17, 2012
The 7th Colloquium: December 19, 2012
The 8th Colloquium: February 20, 2013

VENUE Taniguchi Memorial Hall, Osaka University

Speaker	Title
Kazuya Masuda (Immune Regulation)	Protein X: A molecule which stabilizes IL-6 mRNA. Balance between Protein X and Regnase-I may be important in the pathogenesis of autoimmune diseases
Barry Ripley (Immune Regulation)	Negative feedback regulation of IRF-7-IFN-α/β pathway by PPAR-γ. PPAR-γ agonists may represent novel therapeutics for treating IFN dependent autoimmune diseases
Morihisa Fujita (Immunoglycobiology)	Shedding of GPI-anchored proteins by a novel GPI cleaving enzyme
Toshiyuki Kowada (Chemical Imaging Techniques)	Chemical probes for the imaging of bone-resorbing osteoclasts
Shuhei Sakakibara (Molecular Immunology)	Generation and selection of virus- and self-reactive B cells during herpesvirus infection
Toshiro Tanaka (Immunopathology)	IL-6 blockade strategy for the treatment of various immune-mediated diseases
Wataru Ise (Lymphocyte Differentiation)	Cell-intrinsic and extrinsic factors which support efficient activation of memory B cells during secondary antibody response
Andrew Hutchins (Bioinformatics and Genomics)	STAT3: mechanisms of transcription and the myeloid antiinflammatory response
Kazuo Yamashita (Systems Immunology)	In silico modeling of protein-protein docking
Alexis Vandenbon (Systems Immunology)	Identification of microRNA biomarkers for prediction of adverse reaction to immunization
Hideki Ogura (Developmental Immunology)	IL-6 amplifier, a chemokine inducer in non-immune cells, is associated with various human diseases
Hideaki Fujita (Single Molecule Imaging)	Using Imaging in immunology: some examples
Rikinari Hanayama (Immune Network)	Identification of a novel fusion mediator in macrophages
Naganari Ohkura (Experimental Immunology)	The development of regulatory T cells
Makoto Kinoshita (Mucosal Immunology)	Analysis for the pathogenesis of inflammatory bowel diseases
Yuki Mori (Biofunctional Imaging)	Monitoring of immune cell dynamics in mouse brain with MRI
Takuma Misawa (Host Defense)	Microtubule-driven spatial mitochondria arrangement promotes NLRP3-inflammasome activation
Nicolas Pavillon (Biophotonics)	Multimodal Label-free imaging of living cells
Kazuhiro Suzuki (Immune Response Dynamics)	Crosstalk between chemokine and adrenergic receptors: a mechanism for neural regulation of lymphocyte dynamics

IFReC Seminars

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



VENUE Meeting Room #1, IFReC Research Building
Taniguchi Memorial Hall, Osaka University

Speaker	Affiliation	Title	Date
Timothy J. Stasevich	Graduate School of Frontier Biosciences, Osaka University	Visualizing live-cell epigenetic modifications of endogenous rna polymerase Ⅱ and histones at an activated gene array	May.18
Takanari Inoue	Johns Hopkins University	Synthetic cell biology of primary cilia	Jun.11
Hideaki Fujita	Laboratory for Comprehensive Bioimaging, RIKEN Quantitative Biology Center	Optical methods for understanding biosystems	Jun.13
Florent Ginhoux	Singapore Immunology Network (SIgN)	Immuno-biology of dendritic cells	Jun.18
Michael L. Tremblay	Jeanne and J.-Louis Levesque Chair in Cancer Research Fellow of the Royal Society of Canada James McGill Professor Chercheur National du FRSQ	A review of Protein Tyrosine Phosphatases (PTP) gene family and their roles in diseases and immunity	Aug.30
Zoltan Fehervari	Senior Editor, Nature Immunology	How to get published	Nov.1
Jeroen Roose	Department of Anatomy, University of California, San Francisco	A gain-of-function mutation in Rasgrp1's calcium binding domain cause Lupus like autoimmunity	Nov.16
Adriana Montano	Department of Biochemistry and Molecular Biology, Edward A. Doisy Research Center, Saint Louis University School of Medicine	Induction of oral tolerance to N-acetylgalactosamine 6-sulfate sulfatase (GALNS) used for enzyme replacement therapy (ERT) in Morquio A syndrome	Nov.20

Speaker	Affiliation	Title	Date
Richard Culleton	Malaria unit, Institute of Tropical Medicine, Nagasaki University	Quantitative whole genome resequencing and genetic linkage analyses identify genes controlling medically important phenotypes of malaria parasites	Nov.26
Michel C. Nussenzweig	Howard Hughes Medical Institute Laboratory of Molecular Immunology, The Rockefeller University	Toward an HIV vaccine	Dec.3
Fritz Melchers	Max Planck Institute for Infection Biology, Germany, Lab. of Lymphocyte Development, IFReC	Microenvironments that regulate B cell development	Dec.12
Jingsong Xu	Dermatology and Pharmacology, University of Illinois College of Medicine at Chicago	Cell polarity and directed migration	Jan.16
Roman Jerala	National Institute of Chemistry, Ljubljana, Slovenia	Synthetic biology shaping the cellular response and molecules	Mar.28

Events

Immunology Frontier Research Center

IFReC Retreat 2012

The first IFReC Retreat was held with 239 IFReC members and collaborators. It was an event to deepen the mutual understanding of all members through friendly interactions as well as to confirm to the aims and obligations that IFReC is to fulfill.

DATE November 12-13, 2012

VENUE Royal Oak Hotel, Otsu, Shiga

Program [DAY 1]

Time	Program	
13:30 – 15:20	Plenary Session	Opening Remarks (Kazuhiro Suzuki)
		Talk (Shizuo Akira)
		Talk (Shimon Sakaguchi)
		One-Minute Presentations by Poster Presenters
15:20 – 17:20	Poster Session	
17:30 – 20:00	Get-Together (Dinner Party & Laboratory Introductions)	

Program [DAY 2]

Time	Program	
9:30 – 12:15	Group Sessions	Oral Presentations (in 10 parallel sessions)
12:30	Closing Remarks (Rikinari Hanayama)	

Message to IFReC members

As the director of the Immunology Frontier Research Center, I am very happy to announce that the first IFReC retreat will be held in Otsu by Lake Biwa... The retreat is an excellent occasion not only to confirm the main WPI program requirements (scientific achievement, globalization, interdisciplinary research activities, etc.), but also to deepen the mutual understanding of all members through friendly interactions for two days. I sincerely wish this is a great opportunity for IFReC members to meet each other and help further the collaborative nature of research at IFReC.

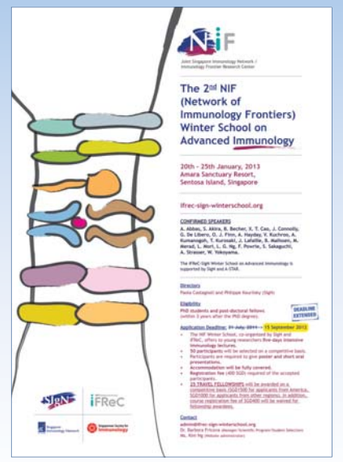
Shizuo Akira
Director of IFReC

NIF Winter School on Advanced Immunology 2013

The second Winter School on Advanced Immunology was jointly organized with Singapore Immunology Network (SIgN) and held in Singapore. Fifty four young researchers selected from 256 applicants discussed about progress and new ideas in immunology with 20 internationally renowned immunologists.

DATE January 20-25, 2013

VENUE Amara Sanctuary Resort, Sentosa Island, Singapore



Speaker	Title
Abul Abbas (The University of California)	Activation and Regulation of T Lymphocytes
Shizuo Akira (IFReC, Osaka University)	mRNA Stability in the Immune Response
Burkhard Becher (University of Zurich)	Cytokine Networks in Autoimmune Disease
Xuetao Cao (Chinese Academy of Medical Sciences)	Regulation of PRR-Triggered Innate Response and Inflammation
John Connolly (SIgN, A*STAR)	Pathways of Antigen Trafficking
Gennaro de Libero (SIgN, A*STAR)	Antigen Recognition by Immune Cells
Olivera J. Finn (University of Pittsburgh)	Tumor Immunosurveillance
Adrian Hayday (King's College London)	Layers of Protection: T Cells in the Skin and in the Gut
Vijay Kuchroo (Harvard Medical School)	T Cell Differentiation and Autoimmunity
Atsushi Kumanogoh (IFReC, Osaka University)	Immune Regulation by Semaphorins and their Receptors
Tomohiro Kurosaki (IFReC, Osaka University)	Humoral Memory Responses
Juan Lafaille (New York University School of Medicine)	Induced Tregs in Tolerance and Inflammation
Bernard Malissen (Centre d'Immunologie de Marseille-Luminy)	Genetic and Proteomic Dissection of T Cell Activation
Miriam Merad (Mount Sinai School of Medicine)	The Mononuclear Phagocyte Lineage
Lucia Mori (SIgN, A*STAR)	Lipid Antigen Presentation to T Cells
Lai Guan Ng (SIgN, A*STAR)	Neutrophil Trafficking
Fiona Powrie (University of Oxford)	Gut Reactions: Immune Pathways that Control Intestinal Homeostasis
Shimon Sakaguchi (IFReC, Osaka University)	The Molecular Basis of Treg-Mediated Suppression
Andreas Strasser (Walter and Eliza Hall Institute of Medical Research)	The Role of Programmed Cell Death in Positive and Negative Selection During T and B Lymphocyte Development
Wayne M. Yokoyama (Washington University in St. Louis)	Natural Killer Cell Biology

Explanatory Meetings for Members

In order to enhance IFReC members' awareness of regulatory compliance, explanatory seminars were held in both Japanese and English in collaboration with RIMD and other Osaka University departments.

DATE April 4, 2012: Orientation for New Comers (Japanese/English)

DATE September 26, 2012: Stop Misuse of Research Funds (Japanese/English)

DATE March 22, 2013: Fire Prevention Workshop (Japanese)
March 25, 2013: Fire Prevention Workshop (English)

VENUE Meeting Room #1, IFReC Research Building
Taniguchi Memorial Hall, Osaka University

Extra-Curricular Activities by Liaison Office

The IFReC Liaison Office (see pp.6) has been conducting the following activities, which have attracted favorable comments, in order to support the overseas staff.

Japanese Lessons

A) Class for absolute beginners

Ms. K. Tajima, a professional Japanese teacher teaches basic grammar, hiragana, katakana etc., aiming to provide basic knowledge necessary for conversations in daily life.

B) Japanese café for beginners & intermediate-level

This is an opportunity to speak Japanese in a casual manner aiming at learning practical Japanese to improve participants' daily life, and communication skills with Japanese staff at IFReC. Participants bring their own questions regarding Japanese language to discuss with a teacher or other participants.

Happy Hour

IFReC Happy Hour was started in April, 2012, so that researchers, especially young researchers, could have an opportunity to meet their fellow researchers in a casual atmosphere. The aim is to expand the range of extra-research activities to in an effort to promote collaboration between laboratories.

Outreach Activities

Immunology Frontier Research Center

WPI Joint Outreach Symposium

The WPI joint outreach symposium started in 2011 to encourage a close relationship between WPI scientists and high school students. The second symposium “Let’s enjoy the world’s advanced science!” was held in Tsukuba-city co-organized by the International Center for Materials Nanoarchitectonics (WPI-MANA) and other WPI institutes, an audience of 670 in total attended.

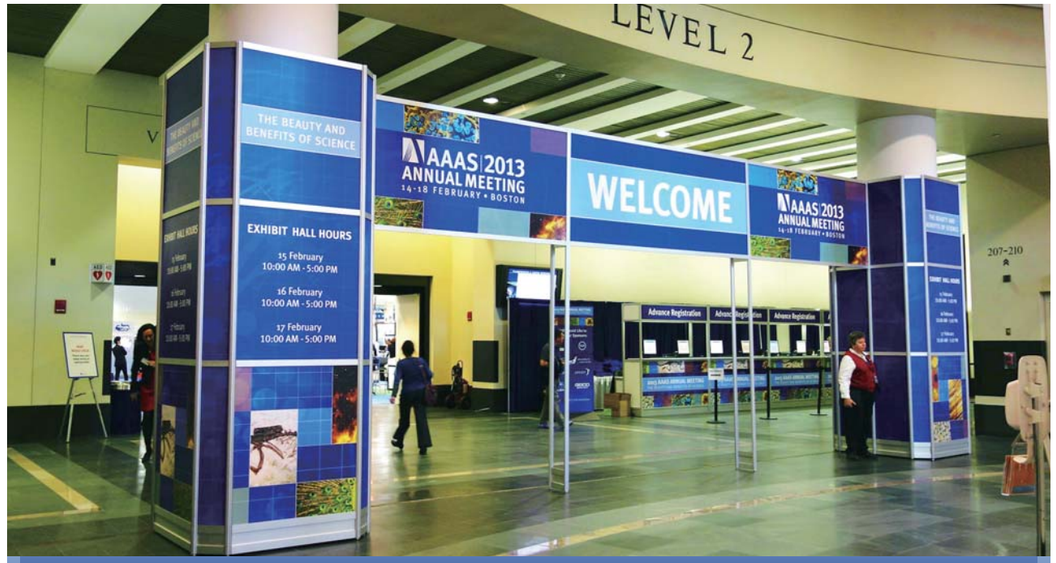
From IFReC, Prof. Tomohiro Kurosaki gave a talk titled “Answer to the mystery behind immune memory”. In the second half, the students participated in the quiz event about science topics. At the end of the event, students and scientists spoke freely with one another and enjoyed experiments at each booth.

DATE November 24, 2012

VENUE Tsukuba International Congress Center, Tsukuba-city

HOST International Center for Materials Nanoarchitectonics (WPI-MANA), WPI institutes

SUPPORT Ibaraki Prefecture



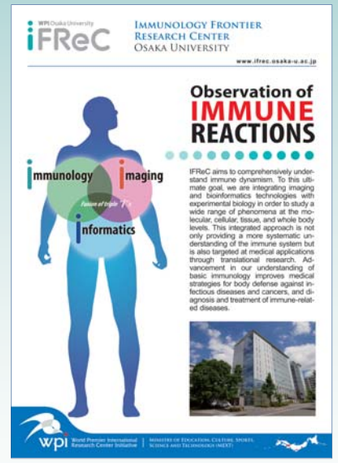
AAAS 2013 in Boston

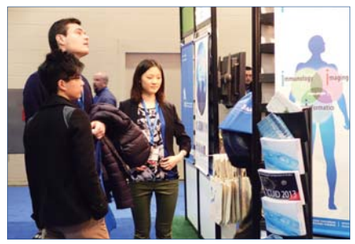
The Annual Meeting of the American Association for the Advancement of Science (AAAS) has been a widely recognized as the scientific event, with various networking opportunities and global media coverage. In the WPI exhibition as a part of “Japan pavilion at AAAS 2013”, the Press Information Officers from the WPI institutes introduced the collaborative researches bridging various research fields in our international environment of the institutes. The staff from Japan including WPI members held a press conference with the theme, “Japan: Your next career destination” on Feb. 15. In the conference, Mr. Ueda (MEXT WPI director) and other staff introduced “the internationally-opened institutes in Japan”, and the new job information by 3 WPI institutes starting from 2012 was shown to the press.

DATE February 13-18, 2013

VENUE Hynes Convention Center, Boston, MA, USA

HOST American Association for the Advancement of Science





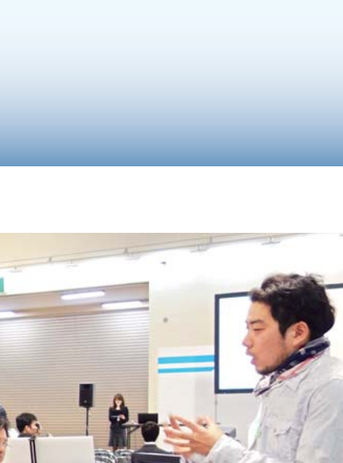
Science and Technology Festa in Kyoto

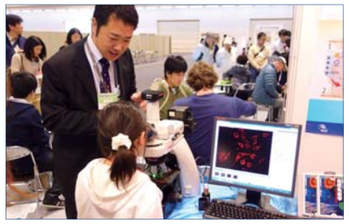
"Science & Technology Festa in Kyoto", a scientific event for high school students began in 2010 organized by the Cabinet Office and others. IFReC ran a booth at "Festa 2013" with other WPI institutes. In the IFReC booth, we introduced the researches of IFReC to students and various attendances. And, we demonstrated a biological microscope in cooperation with the Nikon Instech co. About 6100 participants visited "Festa" in two days, and Mr. Ichita Yamamoto, the Minister of State for science and technology Policy inspected WPI booths on March 16.

DATE March 16-17, 2013

VENUE Kyoto PULSE PLAZA

HOST Cabinet Office, WPI Institutes





Science Café Series "Café on the Edge"

"Café on the Edge" started in FY2010 and co-organized by IFReC and FIRST Program AKIRA Project. We held "Café on the Edge" four times in FY2012 with speakers chosen from the researchers of IFReC and other institutions. The number of participants totaled 350 over the four Cafés.

8th

DATE April 30, 2012

VENUE Techno Alliance Building, Osaka University

TITLE "Dendritic cell; A key player in the immune system"

GUEST Tsuneyasu Kaisho (Immune Regulation, IFReC)

9th

DATE July 28, 2012

VENUE Architect Café (Umeda, Osaka)

TITLE "Why vaccines are effective against pathogens?"

GUEST Wataru Ise (Lymphocyte Differentiation, IFReC)

10th

DATE September 25, 2012

VENUE Art Area B1 (Naniwabashi station, Osaka)

TITLE "The history of immunology traced via Nobel Prize"

GUEST Satoshi Uematsu
(The Institute of Medical Science, University of Tokyo)

11th

DATE November 10, 2012

VENUE Café Comment Allez Vous (Hakata, Fukuoka)

TITLE "The hot topics in immunology"

GUEST Tomohiro Kurosaki (Lymphocyte Differentiation, IFReC)
Sho Yamasaki (Kyushu University)

Lectures for Career Development

In Japan, education for young people, in particular girls who want to become scientists is required. IFReC designed “Lectures for Career Development for the younger generation” to let them know about “true scientists” and “scientists’ lives”.

1st
- DATE: November 10, 2012
- VENUE: Suma Gakuen High School, Kobe, Hyogo
- TITLE: “Biological science revealed by live-imaging”
- LECTURER: Fuminori Sugihara (Biofunctional Imaging, IFReC)

2nd
- DATE: November 10, 2012
- VENUE: Suma Gakuen Junior High School, Kobe, Hyogo
- TITLE: “Biological science revealed by live-imaging”
- LECTURER: Fuminori Sugihara (Biofunctional Imaging, IFReC)

3rd
- DATE: February 20, 2013
- VENUE: Mukogawa Women’s University, Nishinomiya, Hyogo
- TITLE: “The story of a woman researcher’s life”
- LECTURER: Masako Kohyama (Immunochemistry, IFReC)

Lectures at High Schools

Professor Shizuo Akira, Director of IFReC gave lectures at two high schools. Shuyukan Senior High School and Kozu High School are leading schools in Fukuoka and Osaka area, respectively, and they have given plenty of eminent persons. Prof. Akira lectured his audience “The mystery of immunity; a newly opened door to immunology”, which explains his brilliant achievements in an easy-to-understand manner. The audience more than 1500 in total included not only students, but also their parents and teachers. Even after the lecture, students gathered around Prof. Akira, and they were keenly asking questions for an hour.

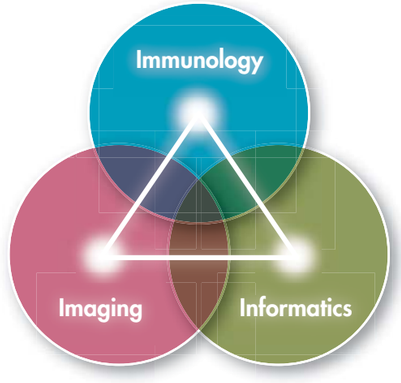
DATE: July 4, 2012 (Shuyukan), February 2, 2013 (Kozu)

Research Projects

Immunology Frontier Research Center

Research Support Program for Combined Research Fields

IFReC is focused on the advancement of the field of Immunology by combining three different "i"s: Immunology, Imaging and Informatics. To help facilitate this direction, a program called the "Research Support Program for Combined Research Fields" was launched in October 2009. This program provided financial support for the internationally recognized notion of the importance of bridging research proposals between different research areas. Project teams must have consisted of researchers from different IFReC groups/backgrounds. The annual budget for each project is ¥3 million/yr for 3 years, with an internal evaluation once a year. As of March 31, 2013, a total of 12 projects are currently underway in IFReC.

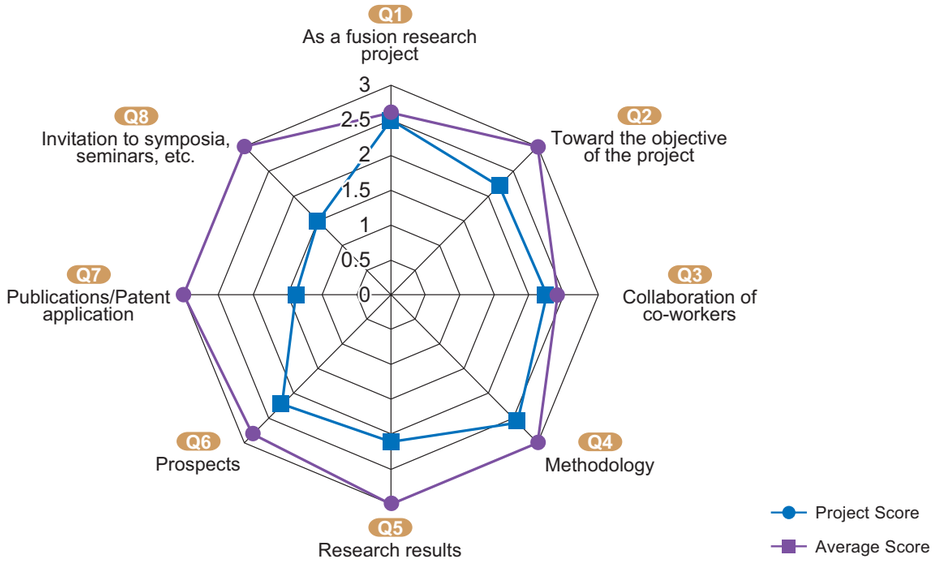


Ongoing Projects

Date Started	Principal Researcher	Collaborators	Titles of Proposed Project
Oct. 2010	●T Suenaga	●H Arase ●J Uemori ●D Standley ●Y Kawaguchi ●K Maenaka	Modulation of immune response by viral infection
	●S Simmons	●M Ishii ●F Melchers ●K Ohnishi	The hemotopoietic microenvironment in the bone: Bone marrow stromal cell niches of early hematopoiesis
	●M Jang	●M Ishii ●H Kang ●J Doh	Development of techniques for high-resolution imaging of intestinal F4/80(+)cells
	●C Coban	●Y Yoshioka ●K Ishii	Immune response to, and regulation by, novel magnetic nanoparticles
	●T Kowada	●K Kikuchi ●O Takeuchi	Visualization of osteoclast function using pH-activatable fluorescent probes
	●S Jin (-Mar. 2011) ●H Kang (Apr. 2011 - Oct. 2011)	●H Fujii	Elucidation of roles of GARP, an activated T-reg-specific cell surface protein, in the regulation of gastrointestinal immunity
	●D Miranda-Saavedra	●H Fujii ●A Hutchins	Transcriptional regulation of Fas : an integrated pipeline for the bottom-up reconstruction of gene regulatory networks
Oct. 2012	●M Yamamoto	●D Standley ●E-M 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 Immune activation and regulation

Researchers from the groups of <●Immunology>, <●Imaging>, <●Bioinformatics>, and <●Other institutions>

Evaluation for Each Project



Quality Index

Level of Achievement
- Q1 As a fusion research project
- Q2 Toward the objective of the project
- Q3 Collaboration of co-workers

Scientific/Technical Merits
- Q4 Methodology
- Q5 Research results
- Q6 Prospects

Outcomes
- Q7 Publications/Patent application
- Q8 Invitation to symposia, seminars, etc.

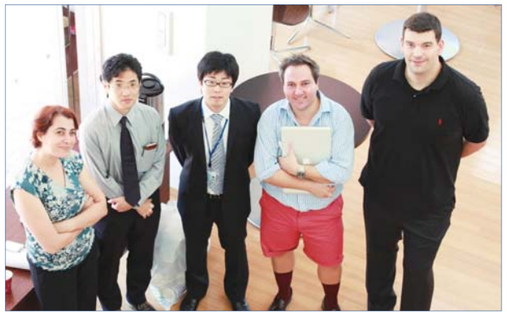
On a scale of : Good=3, Fair=1, Poor=0, Perfect Score 24Pts.

Workshop for the "Research Support Program for Combined Research Fields"

A workshop for the "Research Support Program for Combined Research Fields FY2012" was held. This internal event is regarded as an important meeting for the promotion of interdisciplinary research activities in IFReC. In the meeting, a total of 12 project leaders presented their research progress over two days.

DATE September 27-28, 2012

VENUE Taniguchi Memorial Hall, Osaka University



Funds for Young Researchers

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; the first project started in October, 2012.

IFReC Young Scientist Support Program for Research Abroad

To strengthen our international research network and our basis for international collaborative research, IFReC has provided financial support to young researchers who wish to participate in research activities at overseas institutions. The program aims to develop the practical skills and abilities of young researchers towards international collaborative research and to develop their networking skills with researchers overseas. In total, 16 researchers have used this support program; 6 in FY2011 and 10 in FY2012.

FIRST Program
AKIRA Project

FIRST Program: AKIRA Project

The Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST Program), AKIRA Project aims to comprehensively understand dynamic mechanisms of the immune system from innate to acquired immunity by using imaging techniques and systems biology methods. The ultimate goal of the project is, based on such understanding, to establish a method of manipulating immune responses by effectively regulating intracellular and intercellular systems.

Toward the achievement of the project goal, many important findings, including the discovery of the function of Regnase-1 in innate immunity, were obtained. In addition, the development of elements for biological imaging techniques at the molecular and cellular levels has been progressing well. In FY2012, the Council for Science and Technology Policy (CSTP), Cabinet Office, Government of Japan, evaluated the above results and decided to continue this research project.

In order to strengthen the support system for the acquisition of intellectual property rights, we created a new post of Intellectual Property Strategy Coordinator. In FY2012, the four invention reports were submitted, of which, three of them are currently being applied for.

Date	FY2012 Activities of the AKIRA Project (Except outreach activities)
Jun. 30	Publishing "Report of Research Results in the AKIRA Project from FY2009 to FY2011" (in Japanese)
Sep. 11	The Third Steering Committee Meeting
Sep. 18	The First Patent Committee Meeting
Sep. 25	Hearing for the Interim Evaluation by CSTP, Cabinet Office
Oct. 2	The Second Patent Committee Meeting
Oct. 29-31	International Symposium TCUID 2012
Oct. 31	The Fourth Steering Committee Meeting
Nov. 20	The Site Visit by CSTP, Cabinet Office
Jan. 31	Publishing the Leaflet "Immunology On the Edge" (in English)
Feb. 26	The AKIRA Project Research Meeting 2013
Feb. 26	The Fifth Steering Committee Meeting
Mar. 11	The Third Patent Committee Meeting
Mar. 12	Publishing the Leaflet "Immunology On the Edge" (in Japanese,revised edition)

Novel Findings

Shizuo AKIRA ▸ Osaka University

- Novel function of macrophage such as the controlling mechanism of the metabolic disorder.
- Mechanism of microtubule-dependent assembly of the NLRP3 inflammasome.

Masaru ISHII ▸ Osaka University

- Dynamic visualization of RANKL and Th17-mediated osteoclast function.
- Dynamic behavior of osteoclasts and their monocytoid precursors visualized by intravital microscopy.

Kazuya KIKUCHI ▸ Osaka University

- Development of protein labeling probes with tunable chemical switches and no-wash live-cell imaging.
- ^{19}F MRI monitoring of gene expression in living cells via cell surface ß-lactamase activity.

Nicholas I. SMITH ▸ Osaka University

- Label-free Raman imaging and analysis of T and B cells.
- Effect of surface-modified gold nanorods on inflammatory cytokine response in macrophage cells.

Fuyuhiko INAGAKI ▸ Hokkaido University

- Specific RNA recognition and degradation mechanism of Regnase-1.
- 3D reconstruction of protein-protein complexes from site-directed mutagenesis and yeast two-hybrid screening.

Kenta NAKAI ▸ The University of Tokyo

- Functional annotation of intrinsically disordered domains by their amino acid content using IDD Navigator.
- Time course analysis of transcription start site distribution changes in mice dendritic cells after LPS stimulation.

Activities

Outreach

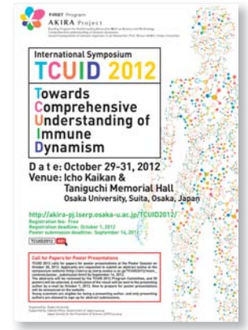
- In FY2012, the AKIRA Project has been carrying out several kinds of outreach activities to enhance communication with the public.

  Our project held a series of science classes by foreign researchers, titled "In Touch with Science" at Osaka International School of Kwansei Gakuin as a novel outreach activity. Dr. Smith (Oct. 9) and Dr. Standley (Jan. 16) gave exciting experiment-based lectures for students.
- Prof. Akira gave a short lecture, and talk with high school students at "FIRST Science Forum 3" in Kyoto. Our project also opened booths at events held at this forum in Tokyo and Kyoto.
- We held the science café series, "Science Café on the Edge" four times (See "Outreach Activities").

Intellectual property (IP)

To raise researchers' awareness about IP, our project held IP seminars (8 times) and IP search seminars (2 times) both in English and Japanese.

International Symposium TCUID 2012



The International Symposium "Towards Comprehensive Understanding of Immune Dynamism" (TCUID 2012) was held on the 29th-31st of October at the Icho Kaikan, Osaka University. This symposium was supported by the Cabinet Office and the Japan Society for the Promotion of Science with 162 people participating. Ten speakers from our project and 6 guest speakers from abroad presented their current research data. The aim of the symposium was to advance our understanding of the dynamic mechanisms regulating immune responses. Participants in various research fields such as immunology, imaging, structural and systems biology had active discussions through oral and poster presentations, and panel discussions. A Young Researcher's Workshop was also held on the first day to encourage interdisciplinary research among young researchers and students by having them introduce their research topics and exchange ideas.

Speaker	Title
Shizuo AKIRA (Osaka University)	The Zinc Finger Domain Containing Nuclease, Regnase-1/Zc3h12a, Controls T Cell Activation
Tatsuya SAITOH (Osaka University)	Microtubule-Driven Spatial Mitochondria Arrangement Promotes NLRP3-Inflammasome Activation
Dirk BUSCH (Technische Universität München)	Composing Robust $CD8^+$ T Cell Immunity from Individual Precursor Cells
Paul KUBES (University of Calgary)	Imaging Intravascular Immunity
Masaru ISHII (Osaka University)	Dynamic Behavior of Osteoclasts and Their Monocytoid Precursors Visualized by Intravital Microscopy
Marc BAJÉNOFF (Centre d'Immunologie de Marseille-Luminy)	Investigating Langerhans Cells Homeostasis
Nicholas I. SMITH (Osaka University)	Raman Imaging and Analysis of T and B Cells
Yoshichika YOSHIOKA (Osaka University)	*In vivo* Visualization of Immune Cells in the Central Nervous System
Matthew BOGYO (Stanford University School of Medicine)	Small Molecule Probes of Protease Function: Applications to Molecular Imaging and Drug Discovery
Kazuya KIKUCHI (Osaka University)	Design, Synthesis and Biological Application of *in Vivo* Imaging Probes with Tunable Chemical Switches
Jie-Oh LEE (Korea Advanced Institute of Science and Technology)	Structural Biology of the Toll-like Receptor Family
Fuyuhiko INAGAKI (Hokkaido University)	Structural Biology of Innate Immunity
Daron M. STANDLEY (Osaka University)	3D Reconstruction of Protein-Protein Complexes from Site-Directed Mutagenesis and Yeast Two-Hybrid Screening
Kenta NAKAI (The University of Tokyo)	Deducing the Nature of Immune Responses from Time-Course Changes of Transcriptional Start Sites
Yutaka SUZUKI (The University of Tokyo)	Towards System-Level Understandings of Transcriptional Regulations
Nicolas CHEVRIER (Harvard University)	System-Level Analysis of the Toll-Like Receptor Network

Data

Immunology Frontier Research Center

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. To allow researchers to focus more on their work, various kinds of equipment are run and maintained by skilled technicians providing a professional service. The instruments other than Core Facilities and Animal Resource Center are located in specific laboratories of IFReC or RIMD. Collaboration is key to the goals of IFReC; all facilities are accessible to any researchers, facilitated with help from the core facilities management group.

IFReC-RIMD Research Complex at Suita campus of Osaka University

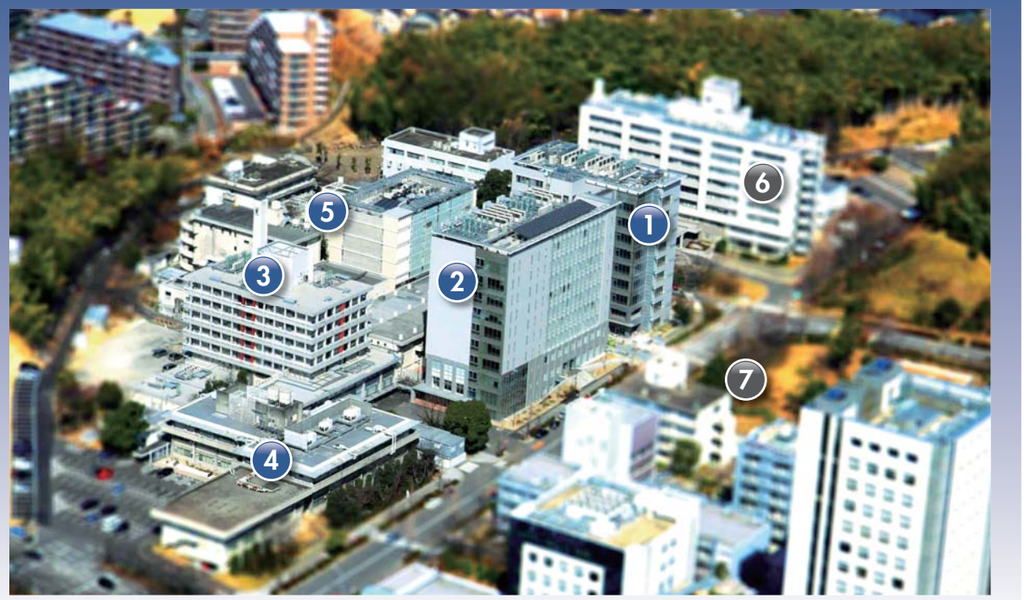


Photo: S. Higashiyama

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

Immunology Groups

- DNA sequencers
- Cell sorters and Flow cytometers
- Real-time PCRs
- Spectrometers
- Two-photon microscopes
- Super-high resolution microscope
- Basic facilities for biological experiments at each lab

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

Bioinformatics Groups

- Server for bioinformatics

Animal Resource Center (A, B, C)

- Facilities for breeding and maintenance of specific pathogen-free animals
- Facilities for embryo freezing and preservation
- Facilities for generating transgenic and knock-out animals
- Facilities for long-term and short-term *in vivo* experiments

Core Facilities with Skilled Technicians

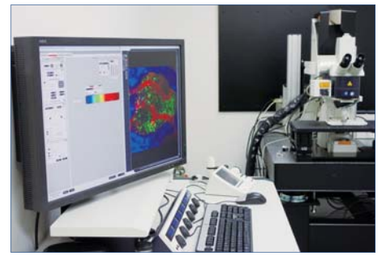
- Animal facility A (for infected animal experiments)
- Animal facility B&C (specific pathogen-free)
- Radio isotope experiment facilities
- DNA chip facilities
- Next generation DNA sequencer
- DNA sequencers
- Cell sorters and Flow cytometers
- TEM
- Mass spectrometers

Facilities are owned, operated and maintained by the management group for the core facilities.

LICHT* Leica Center

At this joint research center on the 7th floor of the IFReC building, Leica Microsystems introduces its latest optical microscope systems, and the research groups at IFReC employs them in their research, providing feedback for further developments for the system. The aim of this arrangement is to meet the needs of future research through the development of next generation microscopic systems.

*LICHT: Leica-Osaka University Interdisciplinary Collaboration Hub for Techno-development on bioimaging



Microscope systems

- Leica TCS MP5 multiphoton microscope
- Ti-sapphire laser (680nm-1080nm)
- Optical microscope
- Objective lens (HCX IRAPO L 25x/0.95W, PL FLUOTAR 5x/0.15)
- Tandem scanner

Kishimoto Foundation Fellowship

IFReC launched the Kishimoto Foundation Fellowship program for researchers in various fields of immunology in 2010. The program is supported by the Kishimoto Foundation and designed to support overseas researchers in order to promote and develop immunological research and international exchanges at IFReC. The fellowships are open to 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.

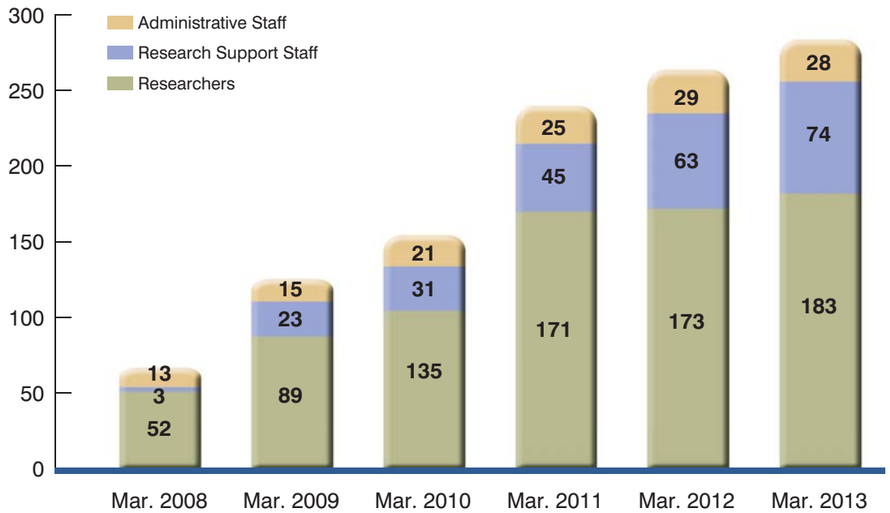
The Kishimoto Foundation was established in 2008 in honor of Tadamitsu Kishimoto, who, during the 1980s and 90s, elucidated the function of interleukin-6 (IL-6), a key molecule for stimulating immune responses. He later developed the anti-IL6 receptor-based therapy, tocilizumab, to treat immune disorders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

FY2012 Kishimoto Fellowship Recipients

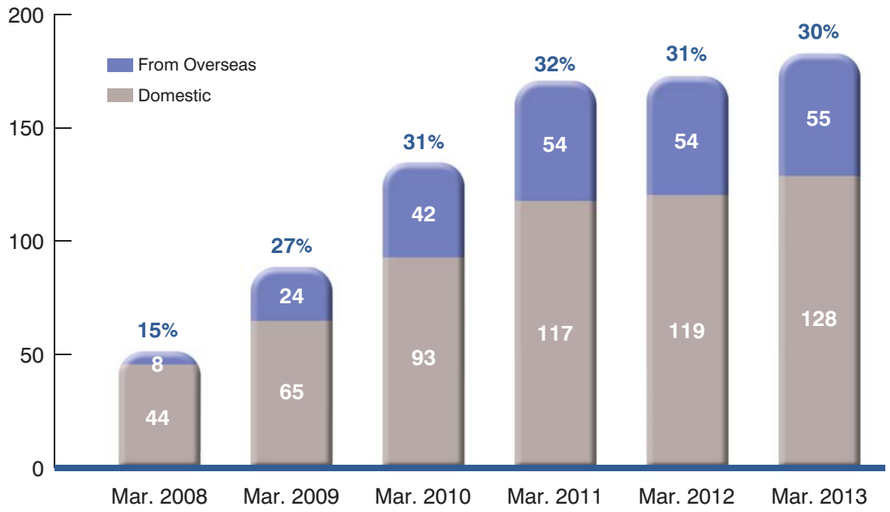
Position of Recipient	Nationality	Host researcher	Period
Specially Appointed Researcher	China	Coban	Jul. 1, 2010 - Jun. 30, 2013
	India	Murakami	Aug. 16, 2010 - Aug. 15, 2013
	Germany	M. Ishii	Sep. 1, 2010 - Mar. 31, 2013
	Taiwan	Kikutani	Apr. 1, 2011 - Mar. 31, 2014
	India	Akira	Apr. 1, 2011 - Mar. 31, 2014
	Korea	Kishimoto	Apr. 16, 2011 - Mar. 31, 2014
Research Fellow	Germany	Kishimoto	Aug. 29, 2011 - Sep. 14, 2012
Specially Appointed Researcher	China	Kishimoto	Sep. 1, 2011 - Jun. 30, 2013
	Nigeria	Sakaguchi	Sep. 16, 2011 - Sep. 15, 2012
	Tunisia	Hanayama	Jan. 16, 2012 - Jan. 15, 2015
	India	Kishimoto	Jun. 1, 2012 - Sep. 15, 2013
	India	Kishimoto	Nov. 1, 2012 - Oct. 31, 2013

Members

Number of IFReC Staff



Number of Researchers



The number of researchers in IFReC from overseas has been favorably increased, their percentage comfortably exceeding the 30% WPI-program target level.

Visitors

Below is the list of visitors to IFReC. Participants to scientific events are not included in this table (See Symposia and Seminars).

Name	Position	Institute	Country	Date
Jean Marchal	Vice-Director for International Relations	University de Liege	Belgium	Jun.14
Pierre Duysinx	Professor, Automotive Engineering	University de Liege	Belgium	Jun.14
Andreas Thele	Professor, Director of the Center for Japanese Studies	University de Liege	Belgium	Jun.14
Marie Clotuche	Project Manager at the International Office	University de Liege	Belgium	Jun.14
Didier Viviers	Director, President of FNRS	University Libre de Bruxelles	Belgium	Jun.14
Serge Jaumain	Vice-Director International Relations, President of CIRI	University Libre de Bruxelles	Belgium	Jun.14
Françis Reniers	Dean of the Faculty of Sciences	University Libre de Bruxelles	Belgium	Jun.14
Jean-Louis Moortgat	International Relations Officer for Asia	University Libre de Bruxelles	Belgium	Jun.14
Bruno Delvaux	Director, President of CReF	University Catholique de Louvain	Belgium	Jun.14
Genevieve Schamps	Professor, Faculty of Law	University Catholique de Louvain	Belgium	Jun.14
Jean-Luc Capron	Associate Professor, Architect	University Catholique de Louvain	Belgium	Jun.14
Samia Patsalides	International Relations Officer for Asia	University Catholique de Louvain	Belgium	Jun.14
Pierre Dehombreux	Vice-Director International Relations	University de Mons	Belgium	Jun.14
Barbara Marchi	Technology Transfer Officer	University de Mons	Belgium	Jun.14
Philippe Toint	Vice-Director for Research	Faculties Universitaires Notre-Dame de la Paix	France	Jun.14
Lise-Anne Hondekyn	International Relations Officer	Conseil Interuniversitaire de la Communaute Francaise	France	Jun.14
Jin-Kyung Kim	Professor	Catholic University of Daegu	Korea	Jul.11
Engel Vrieling	Lecturer	University of Groningen	Netherlands	Sep.11
Jodien Houwers	Staff	University of Groningen	Netherlands	Sep.11
Gilles Bloch	Directeur	Direction des Sciences du Vivant, CEA	France	Nov.15
Eric Quemeneur	Directeur de Recherche	Direction des Sciences du Vivant, CEA	France	Nov.15
Renaud Blaise	Relations Internationales	Direction des Sciences du Vivant, CEA	France	Nov.15
Denis Le Bihan	Directeur de Neuro Spin	Direction des Sciences du Vivant, CEA	France	Nov.15
Stefan Noreen	Senior Adviser, Office of the President, The University of Tokyo, Former Swedish Ambassador to Japan	The University of Tokyo	Japan	Nov.16
Gunnar Svedberg	Professor	KTH Royal Institute of Technology	Sweden	Nov.17
Eva Regårdh	Communications Manager	Swedish Foundation for Strategic Research	Sweden	Nov.17
Joakim Amorim	Research Programmes Manager	Swedish Foundation for Strategic Research	Sweden	Nov.17
Sonja Buchegger	Researcher	KTH Royal Institute of Technology	Sweden	Nov.17
Carlota Canalias	Researcher	KTH Royal Institute of Technology	Sweden	Nov.17
Marie Dacke	Researcher	Lund University	Sweden	Nov. 27
Alexander Dmitriev	Researcher	Chalmers University of Technology	Sweden	Nov. 27
Johan Elf	Researcher	Uppsala University	Sweden	Nov. 27
Daniel Fällman	Researcher	Interactive Institute, Umeå	Sweden	Nov. 27
Martin Högbom	Researcher	Stockholm University	Sweden	Nov. 27
Tobias Larsson	Researcher	Karolinska Institutet	Sweden	Nov. 27
Arne Lindqvist	Researcher	Karolinska Institutet	Sweden	Nov. 27
Richard Lundmark	Researcher	Umeå University	Sweden	Nov. 27
Johan Malmström	Researcher	Lund University	Sweden	Nov. 27
Johan Mauritsson	Researcher	Lund University	Sweden	Nov. 27
Thomas Nolte	Researcher	Mälardalen University	Sweden	Nov. 27
Anders Nordström	Researcher	Umeå University	Sweden	Nov. 27
Rickard Sandberg	Researcher	Karolinska Institutet	Sweden	Nov. 27
Camilla Svensson	Researcher	Karolinska Institutet	Sweden	Nov. 27
Sebastian Westenhoff	Researcher	Göteborg University	Sweden	Nov. 27
Anders Karlsson	Counsellor, Science and Innovation / Head of Office	Embassy of Sweden, Office of S&I	Sweden	Nov. 27
Shigeyuki Naito	Officer, Science and Innovation, ICT	Embassy of Sweden, Office of S&I	Sweden	Nov. 27
Setsuko Hashimoto	Program Manager, Life Sciences	Embassy of Sweden, Office of S&I	Sweden	Nov. 27

Awards

Foreign Associate of the National Academy of Sciences USA

Shimon Sakaguchi

National Academy of Sciences (NAS) is a private organization of scientists and engineers dedicated to the furtherance of science and its use for the general welfare. NAS consists of about 2,300 American members and 400 foreign members, and about 200 NAS members were awarded the Nobel Prize in the past. Election to the NAS means one is recognized as one of the best researchers among scientists.

On May 1, 2012, NAS elected Professor Shimon Sakaguchi of IFReC to the foreign associate of NAS. Prof. Sakaguchi has greatly contributed to the understanding of the molecular basis of the development and function of regulatory T cells (Treg) and their roles in controlling a variety of physiological and pathological immune responses, including autoimmune disease, transplantation tolerance, and tumor immunity.

At present, IFReC has the three NAS members, Professors Tadamitsu Kishimoto, Shizuo Akira and Shimon Sakaguchi.

International

The Royal Decoration from Thai Kingdom

Tadamitsu Kishimoto

Thinking Inside the Box Award

Ken Ishii

Mochida Memorial Science Prize

Atsushi Kumanogoh

SNM2012 (Society of Nuclear Medicine) Poster Award

Jun Hatazawa

Inoue Prize for Science

Kazuya Kikuchi

Astellas Award for the Best Biomedical Research

Rikinari Hanayama

Domestic

Young Investigator Award, 20th International Symposium on Molecular Cell Biology of Macrophage

Masanaka Sugiyama

Young Investigator Award, The Japanese Society of Carbohydrate Research

Morihisa Fujita

Young Investigator Award, Japanese Biochemical Society

Morihisa Fujita

Prizes for Science and Technology, Minister of Education, Culture, Sports, Science and Technology

Tomohiro Kurosaki

The Young Scientists' Prize, Minister of Education, Culture, Sports, Science and Technology

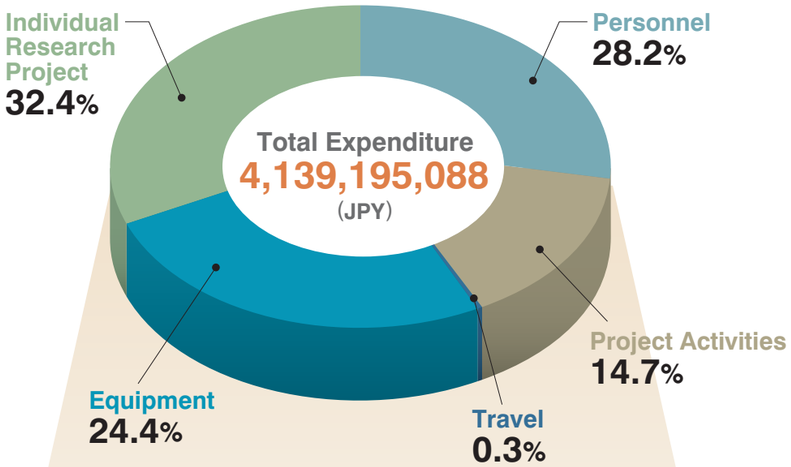
Shin Mizukami

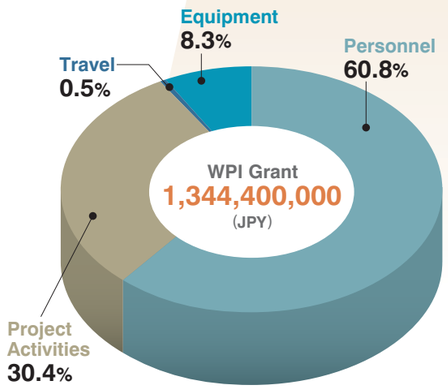
Young Investigator Award, Japanese Society for Immunology

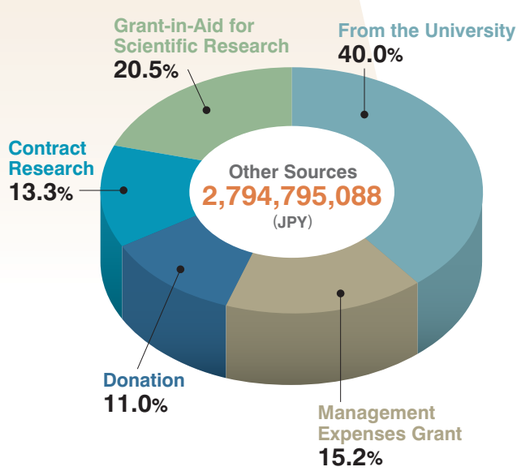
Wataru Ise
Tatsuya Saitoh
Kazuhiro Suzuki

Finance

Break Down of Total Expenditure at IFReC







Research Outputs

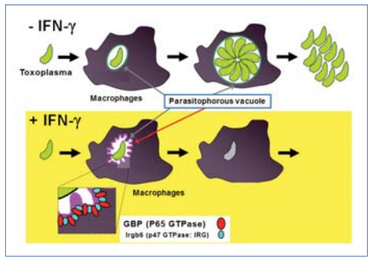
Immunology Frontier Research Center

Selected Articles

A cluster of interferon-γ-Inducible p65 GTPases plays a critical role in host defense against *Toxoplasma gondii*.

Immunity 37:302-313, 2012

Masahiro Yamamoto, Megumi Okuyama, Taishi Kimura, Naganori Kamiyama, Hiroyuki Saiga, Jun Ohshima, Miwa Sasai, Hisako Kayama, Toru Okamoto, David C.S. Huang, Dominique Soldati-Favre, Kyoji Horie, Junji Takeda, Kiyoshi Takeda



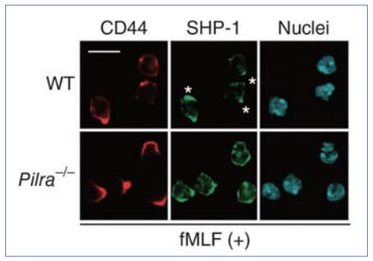
Gbpchr3-deleted cells are defective in IFN-γ-mediated killing of *T. gondii*.

Interferon-γ (IFN-γ) is essential for host defense against intracellular pathogens. Stimulation of innate immune cells by IFN-γ upregulates ~2,000 effector genes such as immunity-related GTPases including p65 guanylate-binding protein (Gbp) family genes. The authors show that a cluster of *Gbp* genes was required for host cellular immunity against the intracellular parasite *Toxoplasma gondii*. They generated mice deficient for all six *Gbp* genes located on chromosome 3 (Gbp^{chr3}) by targeted chromosome engineering. Mice lacking Gbp^{chr3} were highly susceptible to *T. gondii* infection, resulting in increased parasite burden in immune organs. Furthermore, Gbp^{chr3}-deleted macrophages were defective in IFN-γ-mediated suppression of *T. gondii* intracellular growth and recruitment of IFN-γ-inducible p47 GTPase Irgb6 to the parasitophorous vacuole. In addition, some members of Gbp^{chr3} restored the protective response against *T. gondii* in Gbp^{chr3}-deleted cells. These results suggest that Gbp^{chr3} play a pivotal role in anti-*T. gondii* host defense by controlling IFN-γ-mediated Irgb6-dependent cellular innate immunity.

Neutrophil infiltration during inflammation is regulated by PILRα via modulation of integrin activation.

Nature Immunology 14:34-40, 2013

Jing Wang, Ikuo Shiratori, Junji Uehori, Masahito Ikawa, Hisashi Arase



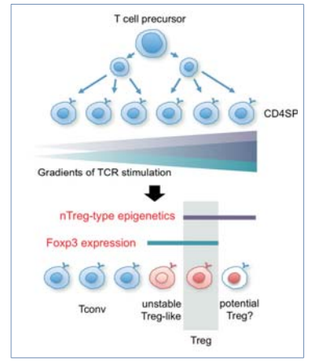
PILRα aggregates at the leading edge of polarized neutrophils.

Acute inflammatory responses are important in host defense, whereas dysregulated inflammation results in life-threatening complications. The authors found that paired immunoglobulin-like type 2 receptor alpha (PILRα), an inhibitory receptor containing immunoreceptor tyrosine-based inhibitory motifs (ITIMs), negatively regulated neutrophil infiltration during inflammation. PILRα$^{-/-}$ mice had increased neutrophil recruitment to inflammatory sites and were highly susceptible to endotoxin shock. PILRα$^{-/-}$ neutrophils showed enhanced transmigration ability and increased adhesion to the β_2 integrin ligand ICAM-1. 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 β_2 integrin inside-out activation. These data demonstrate that neutrophil recruitment in inflammatory responses is regulated by PILRα via modulation of integrin activation.

T cell receptor stimulation-induced epigenetic changes and Foxp3 expression are independent and complementary events required for Treg cell development.

Immunity 37:785-799, 2013

Naganari Ohkura, Masahide Hamaguchi, Hiromasa Morikawa, Kyoko Sugimura, Atsushi Tanaka, Yoshinaga Ito, Motonao Osaki, Yoshiaki Tanaka, Riu Yamashita, Naoko Nakano, Jochen Huehn, Hans Joerg Fehling, Tim Sparwasser, Kenta Nakai, Shimon Sakaguchi



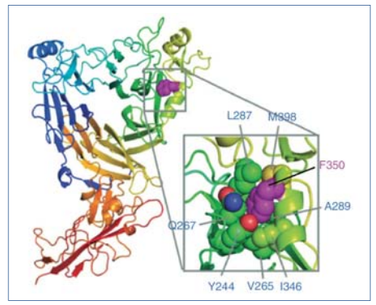
Treg cell development requires both Foxp3 induction and CpG hypomethylation.

The transcription factor Foxp3 is essential for the development of regulatory T (Treg) cells, yet its expression is insufficient for establishing the Treg cell lineage. The authors showed that Treg cell development was achieved by the combination of two independent processes, i.e., the expression of Foxp3 and the establishment of Treg cell-specific CpG hypomethylation pattern. Both events were induced by T cell receptor stimulation. The Treg cell-type CpG hypomethylation began in the thymus and continued to proceed in the periphery and could be fully established without Foxp3. The hypomethylation was required for Foxp3+ T cells to acquire Treg cell-type gene expression, lineage stability, and full suppressive activity. Thus, those T cells in which the two events have concurrently occurred are developmentally set into the Treg cell lineage. This model explains how Treg cell fate and plasticity is controlled and can be exploited to generate functionally stable Treg cells.

A point mutation in Semaphorin 4A associates with defective endosomal sorting and causes retinal degeneration.

Nature Communications 4:1406-1415, 2013

Satoshi Nojima, Toshihiko Toyofuku, Hiroyuki Kamao, Chie Ishigami, Jun Kaneko, Tatsusada Okuno, Hyota Takamatsu, Daisuke Ito, Sujin Kang, Tetsuya Kimura, Yuji Yoshida, Keiko Morimoto, Yohei Maeda, Atsushi Ogata, Masahito Ikawa, Eiichi Morii, Katsuyuki Aozasa, Junichi Takagi, Masayo Takahashi, Atsushi Kumanogoh

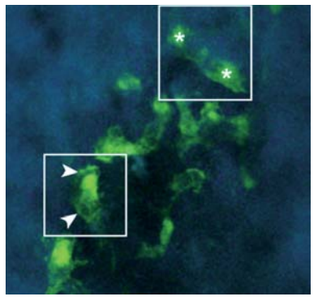


Structural modeling of mouse Sema4A ectodomain.

Semaphorin 4A (Sema4A) has an essential role in photoreceptor survival. In humans, mutations in Sema4A are thought to contribute to retinal degenerative diseases. The authors generate a series of knock-in mouse lines with corresponding mutations (D345H, F350C or R713Q) in the Sema4A gene and find that Sema4AF350C causes retinal degeneration phenotypes. The F350C mutation results in abnormal localization of the Sema4A protein, leading to impaired endosomal sorting of molecules indispensable for photoreceptor survival. Additionally, protein structural modeling reveals that the side chain of the 350th amino acid is critical to retain the proper protein conformation. Furthermore, Sema4A gene transfer successfully prevents photoreceptor degeneration in Sema4AF350C/F350C and Sema4A$^{-/-}$ mice. Their findings not only indicate the importance of the Sema4A protein conformation in human and mouse retina homeostasis but also identify a novel therapeutic target for retinal degenerative diseases.

Selected Articles

Dynamic visualization of RANKL and Th17-mediated osteoclast function.



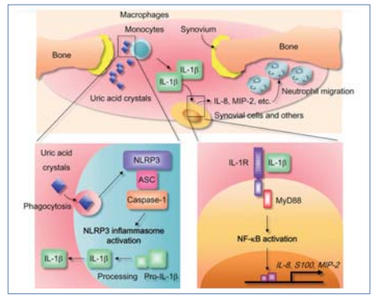
Visualization of living mature osteoclasts on the endosteum by using intravital multiphoton microscopy.

■ *J Clin Invest.* 123:866-873, 2013

Junichi Kikuta, Yoh Wada, Toshiyuki Kowada, Ze Wang, Ge-Hong Sun-Wada, Issei Nishiyama, Shin Mizukami, Nobuhiko Maiya, Hisataka Yasuda, Atsushi Kumanogoh, Kazuya Kikuchi, Ronald N. Germain, Masaru Ishii

Osteoclasts are bone resorbing, multinucleate cells that differentiate from mononuclear macrophage/monocyte-lineage hematopoietic precursor cells. Although previous studies have revealed important molecular signals, how the bone resorptive functions of such cells are controlled in vivo remains less well characterized. The authors visualized fluorescently labeled mature osteoclasts in intact mouse bone tissues using intravital multiphoton microscopy. Within this mature population, they observed cells with distinct motility behaviors and function, with the relative proportion of static – bone resorptive (R) to moving – nonresorptive (N) varying in accordance with the pathophysiological conditions of the bone. They also found that rapid application of the osteoclast-activation factor RANKL converted many N osteoclasts to R, suggesting a novel point of action in RANKL-mediated control of mature osteoclast function. Furthermore, we showed that Th17 cells, a subset of RANKL-expressing CD4+ T cells, could induce rapid N-to-R conversion of mature osteoclasts via cell-cell contact. These findings provide new insights into the activities of mature osteoclasts in situ and identify actions of RANKL-expressing Th17 cells in inflammatory bone destruction.

Microtubule-driven spatial arrangement of mitochondria promotes activation of the NLRP3 inflammasome.



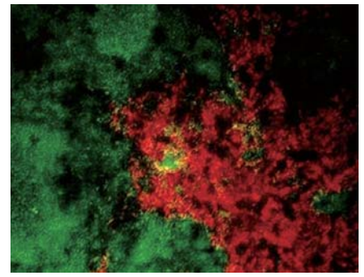
Gouty inflammation caused by the activation of NLRP3 inflammasome.

■ *Nature Immunology* 14:454-460, 2013

Takuma Misawa, Michihiro Takahama, Tatsuya Kozaki, Hanna Lee, Jian Zou, Tatsuya Saitoh, Shizuo Akira

NLRP3 forms an inflammasome with its adaptor ASC, and its excessive activation can cause inflammatory diseases. However, little is known about the mechanisms that control assembly of the inflammasome complex. Here we show that microtubules mediated assembly of the NLRP3 inflammasome. Inducers of the NLRP3 inflammasome caused aberrant mitochondrial homeostasis to diminish the concentration of the coenzyme NAD+, which in turn inactivated the NAD+-dependent a-tubulin deacetylase sirtuin 2; this resulted in the accumulation of acetylated a-tubulin. Acetylated a-tubulin mediated the dyneindependent transport of mitochondria and subsequent apposition of ASC on mitochondria to NLRP3 on the endoplasmic reticulum. Therefore, in addition to direct activation of NLRP3, the creation of optimal sites for signal transduction by microtubules is required for activation of the entire NLRP3 inflammasome.

Critical role of Trib1 in differentiation of tissue-resident M2-like macrophages.



B-cells (g) & tissue-resident M2-like macrophages (r) in the spleen.

■ *Nature* 495:524-528, 2013

Takashi Satoh, Hiroyasu Kidoya, Hisamichi Naito, Masahiro Yamamoto, Naoki Takemura, Katsuhiro Nakagawa, Yoshichika Yoshioka, Eiichi Morii, Nobuyuki Takakura, Osamu Takeuchi, Shizuo Akira

Macrophages consist of at least two subgroups, M1 and M2. Whereas M1 macrophages are proinflammatory and have a central role in host defense against bacterial and viral infections, M2 macrophages are associated with responses to anti-inflammatory reactions, helminth infection, tissue remodeling, fibrosis and tumor progression. Trib1 is an adaptor protein involved in protein degradation by interacting with COP1 ubiquitin ligase. Genome-wide association studies in humans have implicated TRIB1 in lipid metabolism. The authors show that Trib1 is critical for the differentiation of F4/80^{+}MR^{+} tissue-resident macrophages-that share characteristics with M2 macrophages (which they term M2-like macrophages)-and eosinophils but not for the differentiation of M1 myeloid cells. Trib1 deficiency results in a severe reduction of M2-like macrophages in various organs, including bone marrow, spleen, lung and adipose tissues. Aberrant expression of C/EBPα in Trib1-deficient bone marrow cells is responsible for the defects in macrophage differentiation. Unexpectedly, mice lacking Trib1 in haematopoietic cells show diminished adipose tissue mass accompanied by evidence of increased lipolysis, even when fed a normal diet. Supplementation of M2-like macrophages rescues the pathophysiology, indicating that a lack of these macrophages is the cause of lipolysis. In response to a high-fat diet, mice lacking Trib1 in haematopoietic cells develop hypertriglyceridaemia and insulin resistance, together with increased proinflammatory cytokine gene induction. Collectively, these results demonstrate that Trib1 is critical for adipose tissue maintenance and suppression of metabolic disorders by controlling the differentiation of tissue-resident M2-like macrophages.

Data of Research Activities

■ Articles, Lectures, and Awardees

FY	Article	Lecture at International Meeting	Awardee (International & Domestic)
2010	223	76	20
2011	214	96	13
2012	238	99	15

■ Publications on High-Impact Journals

Journal	Nature	Nat. Immunol.	Nat. Cell Biol.	Nat. Genet.	Nat. Med.	Nat. Neurosci.	Science	Cell	Immunity	JEM	High Impact Total
IF*	36.0	25.7	19.4	36.4	25.4	14.2	31.4	32.4	24.2	14.8	-
2010	4	4	1	1	Unissued		0	2	5	2	19/223
2011	0	4	0	0	3	1	1	1	6	5	21/214
2012	3	4	0	0	2	1	1	0	8	3	22/238

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

Research Outputs

Publications

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Lectures by PIs

Lecturer	Meeting	Country	Date
Shimon Sakaguchi	1st Cancer and Immunology Seminar	Japan	Apr. 12
Shizuo Akira	The 2012 Spring Conference of the Korean Association of Immunologists	Korea	Apr. 12
Shizuo Akira	Mexican Congress of Immunology	Mexico	Apr. 17
Shimon Sakaguchi	Tokyo 2012 Chronic Inflammation and Autoimmune Diseases	Japan	Apr. 18
Jun Hatazawa	The Celebration Meeting for the 50th Anniversary of Chonnam National University Nuclear Medicine	Korea	Apr. 20
Masaru Ishii	Gordon Research Conferences	Italy	Apr. 24
Cevayir Coban	Molecular Immunology & Immunogenetics Congress 2012	Turkey	Apr. 27
Ken Ishii	Immunology & Immunogenetics Congress 2012	Turkey	Apr. 27
Ken Ishii	GIGA DAY, The GIGA-Research Center	Belgium	May 4
Shimon Sakaguchi	10th Tohoku Blood Study Group Research Meeting	Japan	May 12
Shimon Sakaguchi	66th Japan Society of Nutrition and Food Science Meeting	Japan	May 19
Shizuo Akira	10th CIMT Annual Meeting	Germany	May 25
Shimon Sakaguchi	4th Brainstorming Medical Conference	Japan	May 27
Shimon Sakaguchi	14th Hakuba Symposium in Kyoto	Japan	Jun. 7
Shizuo Akira	5th International Singapore Symposium of Immunology	Singapore	Jun. 7
Ken Ishii	Asia-Pacific Congress of Medical Virology	Australia	Jun. 7
Taroh Kinoshita	8th International Symposium on Glycosy ltransferases	Germany	Jun. 7
Jun Hatazawa	52nd Society of Nuclear Medicine Annual Meeting	USA	Jun. 10
Ken Ishii	6th Annual World Vaccine Congress Asia 2012	Singapore	Jun. 12
Masaru Ishii	Cold Spring Harbor Asia	China	Jun. 12
Kiyoshi Takeda	Macrophage Molecular and Cellular Biology 2012	Japan	Jun. 15
Taroh Kinoshita	Tohoku PNH Conference	Japan	Jun. 17
Hisashi Arase	12th Annual Meeting of the Protein Science of Japan	Japan	Jun. 20
Atsushi Kumanogoh	Federation of Clinical Immunology Societies Meeting 2012	Canada	Jun. 21
Shimon Sakaguchi	Federation of Clinical Immunology Societies Meeting 2012	Canada	Jun. 22
Kiyoshi Takeda	7th RCAI International Summer Program	Japan	Jun. 22
Ken Ishii	7th RCAI-JSI International Symposium on Immunology	Japan	Jun. 28
Shimon Sakaguchi	12th Foundation for Biomedical Research and Innovation Monthly Lecture	Japan	Jul. 5
Ken Ishii	GTC Meeting for Influenza R & D	USA	Jul. 7
Kazuya Kikuchi	3rd International Conference on Molecular Sensors & Logic Gates (MSMLG)	Korea	Jul. 9
Toshio Yanagida	Gordon Research Conferences -Single Molecule Approaches to Biology	USA	Jul. 18
Tomohiro Kurosaki	JSI Immunology Summer School	Japan	Jul. 23
Shimon Sakaguchi	14th JSI Immunology Summer School	Japan	Jul. 23
Shimon Sakaguchi	2nd T-Cell Camp	Japan	Aug. 4
Toshio Yanagida	Vallee Foundation Symposium 2012	Iceland	Aug. 6
Toshio Yanagida	UK-Japan Symposium for Mechanochemical Cell Biology	UK	Aug. 23
Shizuo Akira	Les Treilles Meeting 2012	France	Aug. 31
Diego Miranda-Saavedra	University of Dundee College of Life Sciences Seminar	UK	Sep. 4
Shizuo Akira	European Congress of Immunology 2012	Scotland	Sep. 6
Diego Miranda-Saavedra	European Congress of Immunology 2012	UK	Sep. 6
Shimon Sakaguchi	3rd European Congress of Immunology	UK	Sep. 7
Diego Miranda-Saavedra	Glasgow University, Department of Chemistry and Polyomics Facility Seminar	UK	Sep. 10
Shimon Sakaguchi	National Institute for Medical Research Seminar	UK	Sep. 10
Shimon Sakaguchi	13th Japanese Society for Musculoskeletal Medicine	Japan	Sep. 14
Tadamitsu Kishimoto	French-Japanese Immunology Meeting	Japan	Sep. 20
Shizuo Akira	French-Japanese Immunology Meeting	Japan	Sep. 20
Shimon Sakaguchi	French-Japanese Immunology Meeting	Japan	Sep. 21
Jun Hatazawa	International Symposium on New Therapeutic Target and Molecular Mechanism of Metabolic Syndrome	Korea	Oct. 3
Shimon Sakaguchi	12th International Symposium on Dendritic Cells	Korea	Oct. 9
Shizuo Akira	12th International Symposium on Dendritic Cells	Korea	Oct. 9
Shimon Sakaguchi	3rd International Conference on Regulatory T Cells and Helper T Cell Subsets and Clinical Application in Human Diseases	China	Oct. 12
Ken Ishii	Vaccine & ISV Annual Global Congress	China	Oct. 16
Kiyoshi Takeda	34th Naito Conference on Infection, Immunity and their Control for Health	Japan	Oct. 16
Shimon Sakaguchi	34th Naito Conference on Infection, Immunity and their Control for Health	Japan	Oct. 16
Ken Ishii	34th Naito Conference on Infection, Immunity and their Control for Health	Japan	Oct. 19
Tsuneyasu Kaisho	12th Biennial International Endotoxin & Innate Immunity Society Meeting 2012	Japan	Oct. 23
Ken Ishii	12th Biennial International Endotoxin & Innate Immunity Society Meeting 2012	Japan	Oct. 23
Kiyoshi Takeda	12th Biennial International Endotoxin & Innate Immunity Society Meeting 2012	Japan	Oct. 23
Shimon Sakaguchi	Young Physician Workshop, 2012	Japan	Oct. 24
Kazuya Kikuchi	Germany-Japan Bilateral Meeting on Coordination Programming	Germany	Oct. 25
Toshio Yanagida	Workshop Molecular Functional Dynamics: Fundamental to Life Activity	Japan	Oct. 26
Toshio Yanagida	INCF Japan Node International Symposium Advances in Neuroinformatics 2012	Japan	Oct. 30
Shimon Sakaguchi	Graduate School of Osaka Medical College Special Lecture	Japan	Nov. 8
Kiyoshi Takeda	4th ASIAHORCs Joint Symposium	Korea	Nov. 11
Tomohiro Kurosaki	Graduate School Seminar at the University of Occupational and Environmental Health	Japan	Nov. 30
Jun Hatazawa	4th Trilateral Conference on Boron Neutron Capture Therapy	Taiwan	Nov. 30
Shimon Sakaguchi	Centennial of Hashimoto Disease International Symposium	Japan	Dec. 1
Tadamitsu Kishimoto	Centennial of Hashimoto Disease International Symposium	Japan	Dec. 1
Ken Ishii	DNA Vaccine 2012	USA	Dec. 5
Masahiro Yamamoto	41st Annual Meeting of Japanese Society of Immunology	Japan	Dec. 5
Shimon Sakaguchi	41st Annual Meeting of Japanese Society of Immunology	Japan	Dec. 7
Kiyoshi Takeda	6th Nagasaki Symposium on Tropical and Emerging Infectious Diseases, 11th Nagasaki-Singapore Medical Symposium	Japan	Dec. 10
Diego Miranda-Saavedra	McGill University, Immunology Department Seminar Programme	Canada	Dec. 13
Kiyoshi Takeda	5th India Probiotics Symposium	India	Dec. 15
Daron M Standley	RIKEN RCAI Seminar Series 2012: Toward Integrative Medical Biology	Japan	Dec. 18
Diego Miranda-Saavedra	CNIC (National Centre for Cardiovascular Research) Seminar	Spain	Dec. 20
Tomohiro Kurosaki	RCAI Michigan Joint Workshop	USA	Jan. 16
Cevayir Coban	Tulane University School of Public Health and Tropical Medicine	USA	Jan. 22
Tomohiro Kurosaki	2nd NIF Winter School on Advanced Immunology	Singapore	Jan. 24
Kazuya Kikuchi	Asian Chemical Biology Initiative, 2013 Bangkok Meeting	Thailand	Jan. 25
Ken Ishii	1st Symposium of International Immunological Memory and Vaccine Forum	Japan	Jan. 29
Kiyoshi Takeda	1st Symposium of International Immunological Memory and Vaccine Forum	Japan	Jan. 29
Tomohiro Kurosaki	1st Symposium of International Immunological Memory and Vaccine Forum	Japan	Jan. 29
Toshio Yanagida	57th Biophysical Society Annual Meeting	USA	Feb. 2
Shimon Sakaguchi	JST-CREST International Symposium Frontiers in Immunology and Inflammation From Molecules to Disease	Japan	Feb. 12
Tomohiro Kurosaki	Keystone Symposia: B Cell Development and Function	USA	Feb. 13
Shimon Sakaguchi	Tri-Institutional Immunology and Microbial Pathogenesis Program Research Seminar Series	USA	Mar. 4
Shimon Sakaguchi	Immunology Seminar Series Harvard Medical School	USA	Mar. 6
Shimon Sakaguchi	Immunology Seminar Series Massachusetts General Hospital	USA	Mar. 7
Tomohiro Kurosaki	1st Japan Society for Transplantation Young Researchers Training Seminar	Japan	Mar. 9
Taroh Kinoshita	14th International Membrane Research Forum	Japan	Mar. 17
Tomohiro Kurosaki	Mitsubishi Tanabe Pharma Special Lecture	Japan	Mar. 21
Shizuo Akira	14th Servier-IGIS Symposium	France	Mar. 21
Diego Miranda-Saavedra	Newcastle University, Medical Faculty Seminar Programme	UK	Mar. 27
Ken Ishii	Foundation Mérieux Conference 'Therapeutic Vaccines: Reprogramming Immunity in Infectious Diseases, Allergy and Cancer'	France	Mar. 27

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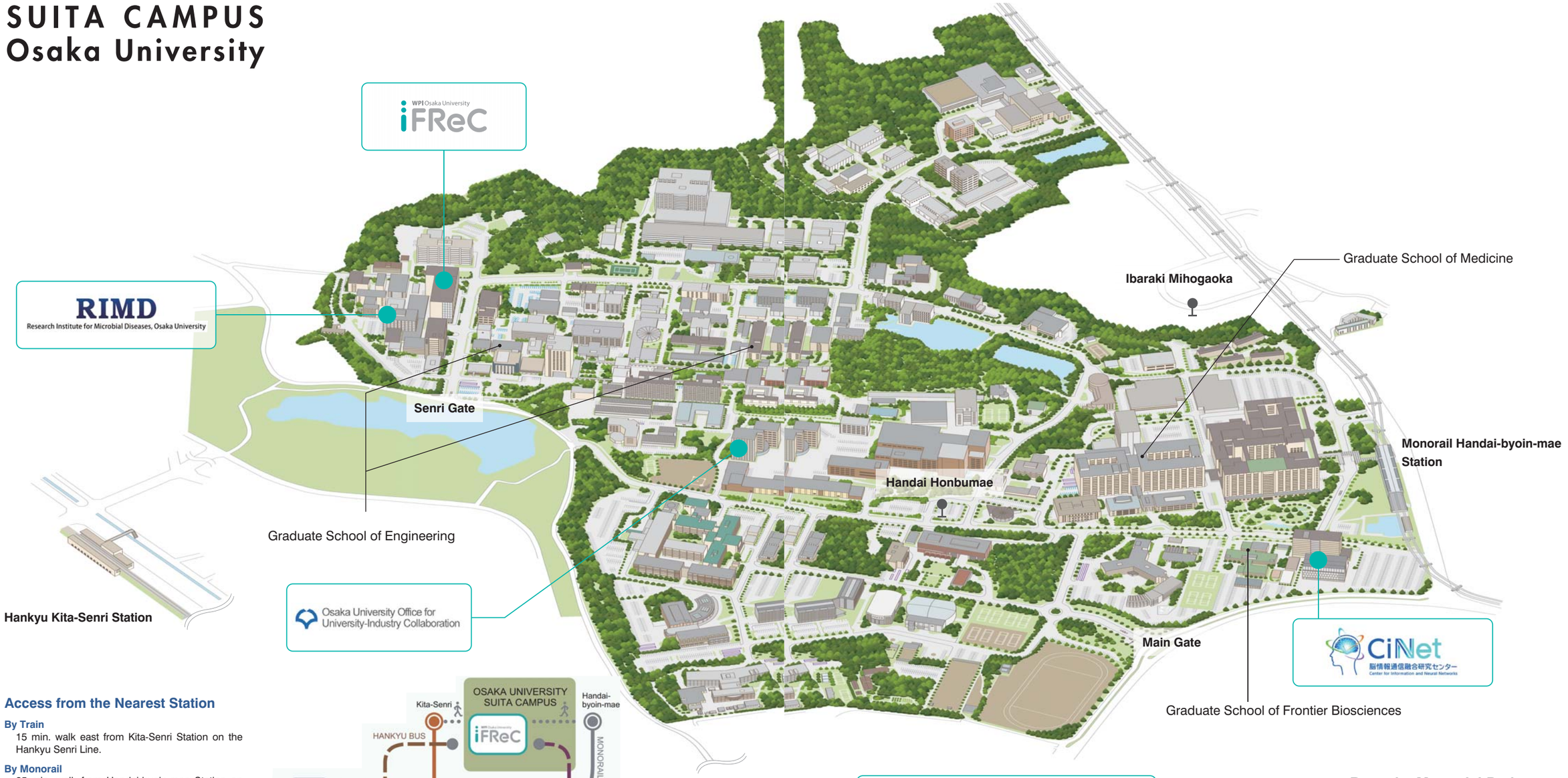

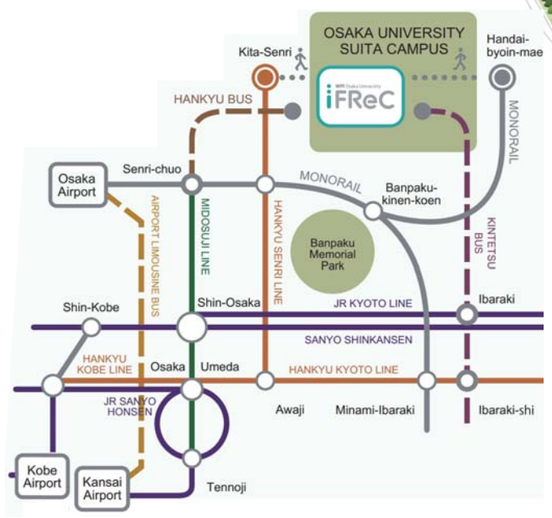

Access from the Nearest Station

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 Station. 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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