World Premier International
Research Center

Osaka University

# Immunology Frontier Research Center

WPI Immunology Frontier Research Center FY2014

Annual Report
of IFReC
FY 2014

Osaka University



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# Message from the Director

As the Director of the Immunology Frontier Research Center (WPI-IFReC) at Osaka University, I am very pleased to present the IFReC annual report for fiscal 2014.

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

Although IFReC was created as a part of the WPI program, a national project led by the Japanese Ministry of Education, Culture, Sports, Science and Technology, IFReC's management will be completely moved to Osaka University from FY2017 as a result of the decision in the WPI program committee meeting held in fall 2014. Even though the governing structure will change, our most important mission "Constructing a world-top immunology research center" remains the same. We will make unceasing efforts to develop immunology research to ensure translation to medical science.

IFReC has been running an original research grant Research Support Program for Combined Research Fields in order to promote collaborative studies between different fields. In 2014, the Immuno-Genomics Unit was newly established to advance this program. The unit comprises a group of young, talented researchers from different fields and we expect that lively debate will pave the way to new areas of research.

As an approach to nurturing young researchers, the fourth NIF Winter School co-organized by IFReC and Singapore Immunology Network was held in January, 2015. The school prides itself on offering not only productive educational content but also an opportunity for the young researchers to form a global network.

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

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

# Looking back on IFReC's activities over the years

Jun Sakanoue (Research Planning and Management Office, IFReC)

After the WPI follow up meeting held in November 2014, the WPI program committee decided that only Kavli IPMU would be nominated for a five-year extension of the WPI grant among the five research institutes launched in 2007[1]. This decision represents the termination of WPI funding for the other four institutes after 2017.

This was a great disappointment for researchers and office staff at IFReC after the intensive effort made to secure an extension for another five years.

The WPI committee did comment that all the five centers had fully met the goal of the WPI program and achieved a World Premier Status.

This World Premier Status should be used in the sense that IFReC has produced world-class achievements in science. Here, I would like to assess IFReC's research performance over the past period subject to evaluation by the WPI committee (2007-2013). The following data such as citation ranking are based on objective analysis[2].

The number of science papers in high-impact journals is used as a measure for high level science research. Since the establishment of IFReC, around 10% of the total papers have been published in high-impact journals such as Science, Nature, Cell and their affiliated journals (Table 1).

**Table 1 | Number of papers in High-Impact Journals in each Fiscal Year**

| Journals | Cell | Immunity | JEM | Science | New Eng J Med | Nature | Nature | | | | | High-Impact Journals |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Immunol | Cell Biol | Med | Neurosci | Rev Immunol | Total |
| IF | 33.1 | 19.7 | 13.9 | 31.5 | 54.4 | 42.4 | 25.0 | 20.1 | 28.1 | 15.0 | 33.8 | - |
| 2008 | 1 | 4 | 0 | 1 | 0 | 4 | 5 | 2 | 0 | 0 | 0 | 17/84 |
| 2009 | 3 | 3 | 7 | 0 | 0 | 2 | 4 | 1 | 0 | 0 | 0 | 20/99 |
| 2010 | 1 | 5 | 3 | 0 | 0 | 5 | 3 | 0 | 0 | 0 | 1 | 18/155 |
| 2011 | 1 | 9 | 3 | 1 | 0 | 0 | 4 | 0 | 2 | 0 | 0 | 20/153 |
| 2012 | 1 | 9 | 3 | 1 | 0 | 3 | 2 | 0 | 1 | 0 | 1 | 21/179 |
| 2013 | 2 | 3 | 1 | 1 | 1 | 2 | 2 | 0 | 0 | 1 | 3 | 16/181 |
| 2014 | 1 | 5 | 3 | 4 | 0 | 1 | 2 | 0 | 1 | 0 | 0 | 17/195 |

Data from WEB OF SCIENCE™ by Thomson Reuters
Each IF (Impact Factor) was calculated in 2013.

The research at IFReC has been maintained at a very high level in both quantitative and qualitative aspects ever since its establishment (Figure 1). The number of papers in each fiscal year by authors affiliated with IFReC has steadily increased from 2008 onward. As the number of papers increased, citation numbers have also increased. More than 800 papers have been published so far, and the average number of citations of these papers was 30.45 and the h-index[3] of IFReC as a whole was 66.

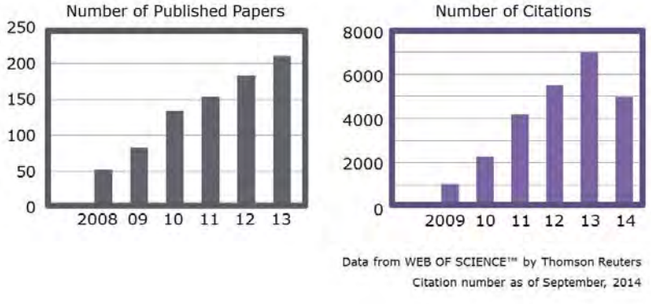



**Figure 1 | Research Outputs of IFReC in 2008-2013.** The number of papers by authors affiliated with IFReC has steadily increased from 2008 to 2013. More than 800 papers have been published over this time period. There were fewer papers published in 2007, since IFReC was established at the end of year 2007.

IFReC's paper productivity was compared with that of the La Jolla Institute for Allergy & Immunology (LIAI) in the USA (Table 2). The LIAI is one of only a few non-profit biomedical research institutes in the world focused on understanding the immune response to infectious agents and cancers and on advancing progress toward the prevention, treatment and cure of immune system diseases[4]. The scale of LIAI and IFReC in terms of researcher number is nearly identical. The achievements of IFReC in 2008-2013 compare favorably with those of LIAI, the research institution representing USA.

**Table 2 | Performance Comparison between the Two Institutes**

| | WPI Osaka University iFReC | La Jolla Institute FOR ALLERGY AND IMMUNOLOGY |
|---|---|---|
| Papers | 818 | 809 |
| Citation Number | 24,911 | 19,578 |
| Citation Impact | 30.45 | 24.20 |
| h-index | 66 | 65 |
| Number of papers cited > 50 | 91 | 85 |

Essential Science Indicators℠ for 2008-2013 by ©THOMSON REUTERS
Published in 2008 to 2013, Citation number as of September 4, 2014

The scientific achievements of IFReC have contributed to Osaka University's reputation as a research university. We can separate the papers from Osaka University into two types. One is those authored by researchers affiliated with IFReC, and the other by those outside of IFReC. In the field of Immunology, the number of papers, citation impact, and h-index by IFReC's researchers are all significantly higher than those by other researchers in Osaka University (Table 3). This high evaluation of IFReC leads to recognition of Immunology at Osaka University. In other research fields such as Molecular Biology, Virology, and Parasitology, papers from IFReC have also made remarkable contributions to the status of Osaka University.

**Table 3 | Contribution of IFReC to Osaka University**

| | Whole Osaka University | **IFReC** | Outside of IFReC |
|---|---|---|---|
| Paper Numbers in Immunology Field | 751 | **234** | 527 |
| Citation Impact | 29.7 | **57.3** | 17.0 |
| h-index | 64 | **53** | 43 |

Essential Science Indicators℠ for 2008-2013 by ©THOMSON REUTERS
Published in 2008-2013, Citation as of March 5, 2015

Osaka University was ranked first in citation impact among the top institutions in immunology all over the world (Table 4). Of course, the contribution of IFReC to Osaka University was restricted after 2007. However, IFReC undoubtedly contributes to the rise in the stature of Osaka University as a research university, and to Japan's international status in biosciences.

**Table 4 | TOP Institutions in Immunology**

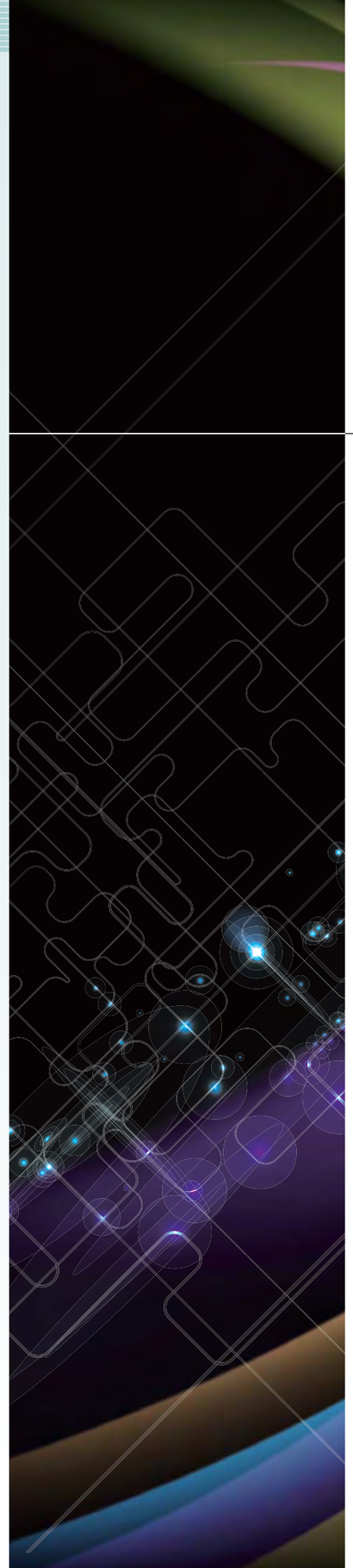
| RANK | INSTITUTION | PAPERS | CITATIONS | CITATION IMPACT |
|---|---|---|---|---|
| **1** | **Osaka Univ, Japan** | **1,005** | **56,048** | **55.77** |
| 2 | Yale Univ, USA | 1,352 | 57,783 | 42.74 |
| 3 | Brigham & Women Hospital, USA | 874 | 35,303 | 40.39 |
| 4 | Washington Univ in St. Louis, USA | 1,101 | 41,609 | 37.79 |
| 5 | Univ Washington, USA | 1,726 | 65,067 | 37.70 |
| 6 | NIAID, USA | 2,280 | 80,335 | 35.23 |
| 7 | Stanford Univ, USA | 1,013 | 34,988 | 34.54 |
| 7 | Univ Oxford, UK | 1,465 | 50,605 | 34.54 |

Essential Science Indicators℠ for 2003-2013 by ©THOMSON REUTERS
The data are sorted by Citation Impact in 2003-2013

## References

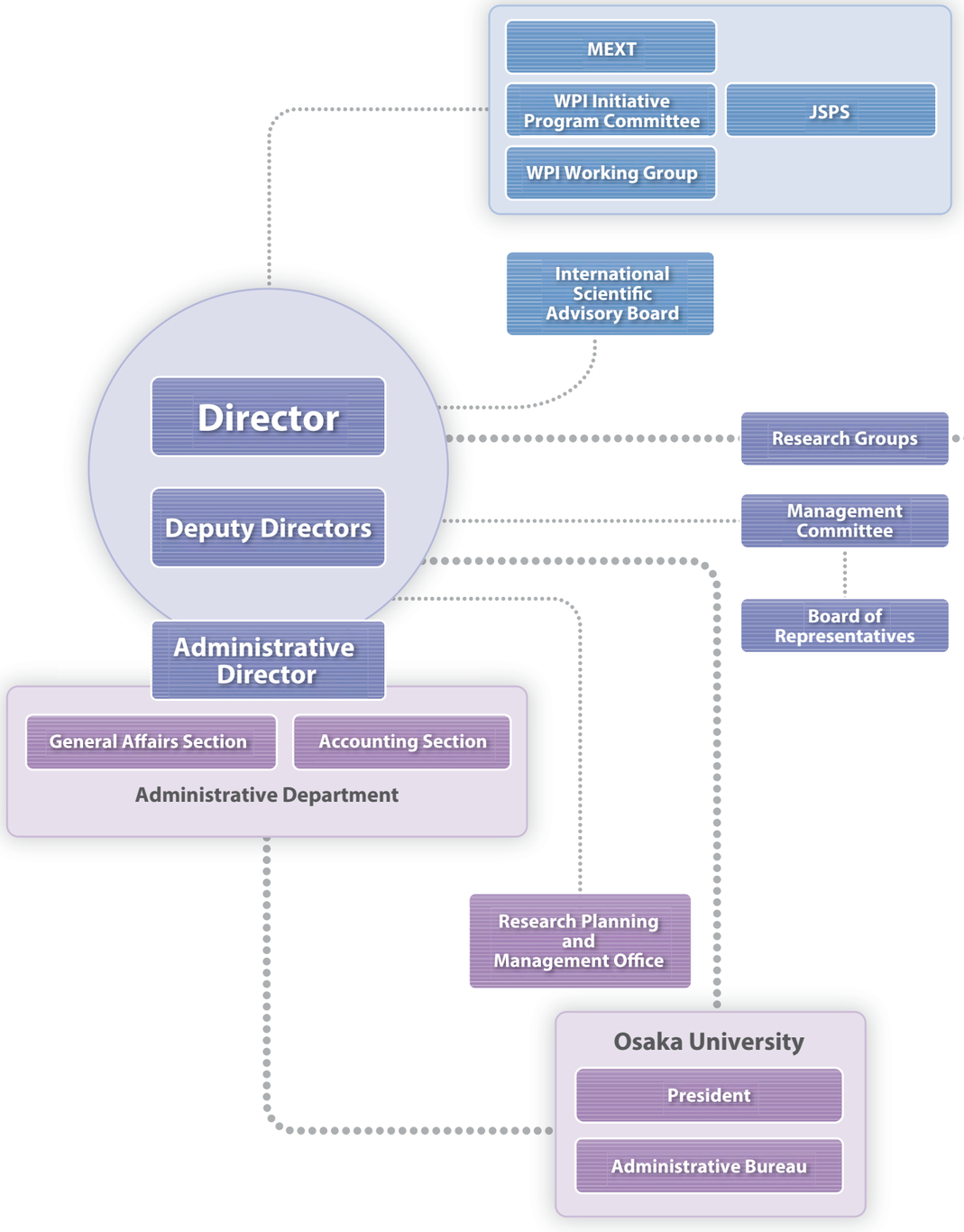

1) FY 2014 Follow-up of WPI Program
http://www.jsps.go.jp/english/e-toplevel/data/08_followup/FY2014result_e.pdf
http://www.mext.go.jp/a_menu/kagaku/toplevel/__icsFiles/afieldfile/2015/02/13/1355021_01.pdf
2) USING BIBLIOMETRICS in Research Evaluation © 2015 THOMSON REUTERS
http://researchanalytics.thomsonreuters.com/cu/inc-support/using-biblio/
3) An index to quantify an individual's scientific research output. Hirsch, JE, PNAS 102:16569–72, 2005.
4) Website of La Jolla Institute for Allergy & Immunology
http://www.liai.org/



# Organization

# Organization Chart




## Immunology Group

| | |
|---|---|
| Host Defense | Shizuo Akira |
| Immunoglycobiology | Taroh Kinoshita |
| Immunopathology | Atsushi Kumanogoh |
| Immunochemistry | Hisashi Arase |
| Immune Regulation | Tadamitsu Kishimoto |
| Mucosal Immunology | Kiyoshi Takeda |
| Molecular Immunology | Hitoshi Kikutani |
| Experimental Immunology | Shimon Sakaguchi |
| Cell Signaling | Takashi Saito |
| Lymphocyte Differentiation | Tomohiro Kurosaki |
| Lymphocyte Development | Fritz Melchers |
| Malaria Immunology | Cevayir Coban |
| Vaccine Science | Ken J. Ishii |
| Immune Regulation | Tsuneyasu Kaisho |
| Immune Network | Rikinari Hanayama |
| Immunoparasitology | Masahiro Yamamoto |
| Biochemistry and Immunology | Shigekazu Nagata |

## Imaging Group

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

## Informatics Group

| | |
|---|---|
| Information Systems | Yutaka Hata |
| Systems Immunology | Daron M. Standley |

## Units for Combined Research Fields

| | |
|---|---|
| Quantitative Immunology | Yutaro Kumagai |
| | Shunsuke Teraguchi |
| | Diego Diez |
| Next Generation Optical Immune-imaging | Noriko Takegahara |
| | Kazuaki Tokunaga |
| Immuno-Genomics | Alexis Vandenbon |
| | Hiromasa Morikawa |

## Common Facilities

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

## Cooperative Institutions

**Domestic**

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

**Overseas**

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

# Committee and Advisory Board for IFReC

## World Premier International Research Center Initiative (WPI)

### Program Committee Members

As of FY2014

| | |
|---|---|
| [Chair] Hiroo Imura | President, Foundation for Biomedical Research and Innovation |
| Toshiaki Ikoma | Representative Director, Executive Vice President & CTO, Canon Inc |
| Hiroto Ishida | President Emeritus, Kanazawa Gakuin University |
| Shinichiro Ohgaki | President, Japan Water Research Center (JWRC) |
| Tsutomu Kimura | Chairman, Tokyo Metropolitan Government Board of Education |
| Kiyoshi Kurokawa | Academic Fellow, National Graduate Institute for Policy Studies |
| Makoto Kobayashi | Director, Research Center for Science Systems, Japan Society for the Promotion of Science (JSPS)<br>Nobel Laureate in Physics (2008) |
| Ryozo Nagai | President, Jichi Medical University |
| Michiharu Nakamura | President, Japan Science and Technology Agency (JST) |
| Ryoji Noyori | President, RIKEN, Nobel Laureate in Chemistry (2001) |
| Robert Aymar | Senior Counsellor to the Administrateur General (CEO), French Atomic Energy Authority (CEA) |
| Rita Cowell | Professor, University of Maryland |
| Richard Dasher | Professor, Stanford University |
| Ian Halliday | Professor Emeritus, University of Edinburgh |
| Chuan Poh Lim | Chairman, Agency for Science, Technology and Research |
| Matthew Mason | Director, Robotics Institute, Carnegie Mellon University |

### Program Director

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

### Deputy Program Director

| | |
|---|---|
| Akira Ukawa | RIKEN Advanced Institute for Computational Science |

### Working Group Leader and Assigned Members

As of FY2014

| | |
|---|---|
| [Chair, Program Officer] Takehiko Sasazuki | 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 | Executive Vice-President for Research, Planning, and Hospital Administration, Kyoto University |
| Kazuhiko Yamamoto | Professor and Chairman, Department of Allergy and Rheumatology, Graduate school of Medicine, The University of Tokyo |
| Günter J. Hämmerling | Professor and Chairman, Division of the Molecular Immunology, German Cancer Research Center Heidelberg DKFZ, DEU |
| Hisataka Kobayashi | Associate (chief) scientist, Molecular Imaging Program, National Cancer Institute, National Institutes of Health, USA |
| Philippe Kourilsky | Professor, Collège de France / Honorary Director-General, The Institute of Pasteur Chairman, The Singapore Immunology Network, FRA |
| Diane Mathis | Morton Grove-Rasmussen Professor of Immunohematology, Division of Immunology, Department of Microbiology and Immunobiology, Harvard Medical School, Boston, USA |

### International Scientific Advisory Board

As of FY2014

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

IFReC has been the subject of evaluations including site visits and follow-ups by the WPI Program Committee and scientific evaluations on each PI by the International Scientific Advisory Board.

In close cooperation with the Program Director, the Program Officer and working group members, the WPI Program Committee conducts follow-up activities on progress being made by the WPI institutes including IFReC, with an eye to developing them into highly visible research centers. The working group, set up exclusively for IFReC, conducts an annual site visit and compiles evaluation results in the form of the Site Visit Report. Following the feedback received, IFReC has continued to make various efforts to develop and improve to meet the requirements of a WPI center.

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

# Administrative Office of IFReC

## General Affairs Section

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

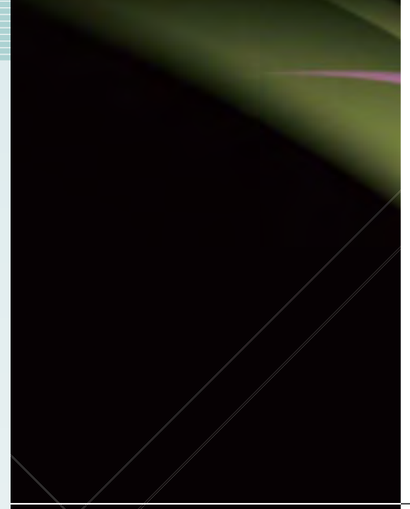
## Accounting Section

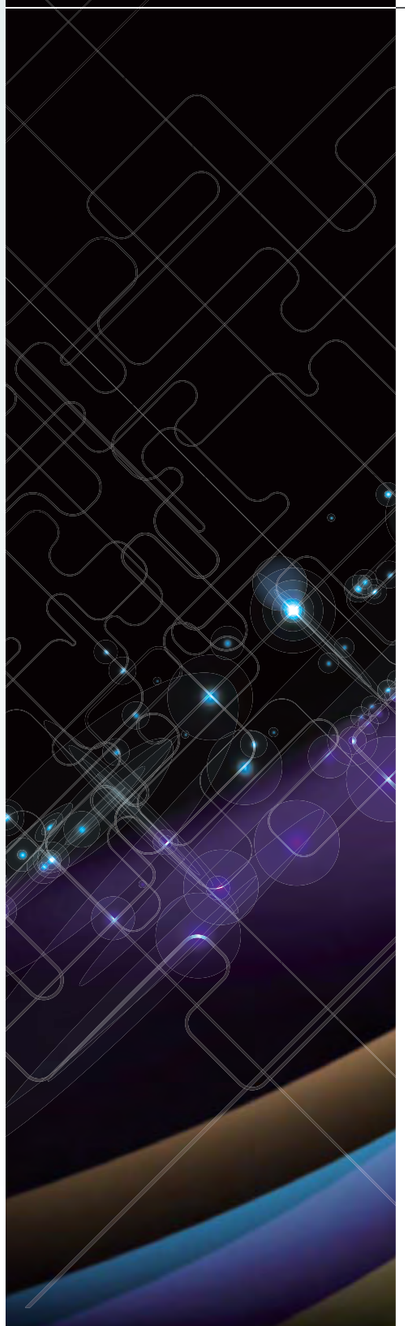
- Budget drafting / implementation / management
- Purchasing procedures
- Acceptance and implementation of third-party funding
- Payment of payroll, travel expense and honorarium
- Health insurance procedures
- Management of buildings and assets
- RI (Radio Isotope) procedures

## Research Planning & Management Office

- Research promotion & support
  (Consultation for grants & patents, Fusion research program, etc.)
- Establishing research environments
  (Facility & safety management, research agreement, etc.)
- Fostering young scientists
  (Winter School, Dual Mentor Program, etc.)
- Organizing scientific events
  (Symposia, colloquia, seminars, etc.)
- Public relations
  (Publishing, website, outreach to citizens, etc.)
- WPI evaluation issues
  (Progress report, advisory board meeting, etc.)

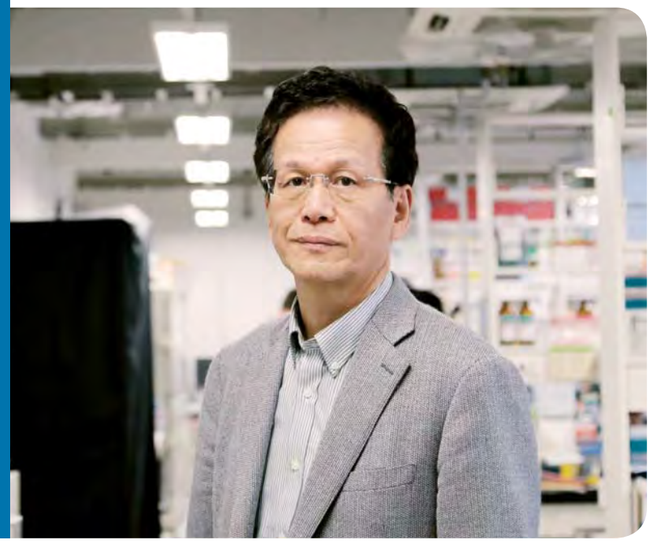






# Laboratories

# Host Defense



## Shizuo Akira, MD / PhD

| | |
|---|---|
| Professor | **Shizuo Akira** |
| Associate Professor | **Tatsuya Saitoh** |
| Assistant Professor | **Takashi Satoh** |
| | **Kenta Maruyama** |
| | **Mikaël Martino** |
| Postdoctoral Fellow | **4** |
| Research Assistant | **8** |
| Visiting Scientist | **2** |
| Support Staff | **7** |

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

Recently we focused on the role of zinc-finger antiviral protein (ZAP) against viral infection. We also showed that resveratrol suppresses NLRP3-inflammasome formation.

### 1. Zinc-finger antiviral protein mediates retinoic acid inducible gene I-like receptor- independent antiviral response to murine leukemia virus

Previously we found that zinc-finger antiviral protein (ZAP) acts as an RNA sensor and induces the degradation of the MLV transcripts by the exosome, an RNA degradation system, on RNA granules (Lee et al, PNAS, 2013). The loss of ZAP greatly enhances the replication efficiency of MLV. ZAP localizes to RNA granules, where the processing-body proteins assemble. ZAP induces the recruitment of the MLV transcripts and exosome components to the RNA granules. The CCCH-type zinc-finger domains of ZAP, which are RNA-binding motifs, mediate its localization to RNA granules and MLV transcripts degradation by the exosome. Thus, ZAP is the cytosolic RNA-sensing PRR that induces elimination of intracellular RNA viruses including MLV.

Next, we will investigate the role of ZAP against Sindbis virus.

### 2. Role of zinc-finger anti-viral protein in host defense against Sindbis virus

Accumulating evidence indicates that type I interferon (IFN) mediates the host protective response to RNA viruses. However, the anti-viral effector molecules involved in this response have not been fully identified. Here, we show that zinc-finger anti-viral protein (ZAP), an IFN-inducible gene, plays a critical role in the elimination of Sindbis virus (SINV) in vitro and in vivo. The loss of ZAP greatly enhances the replication of SINV but does not inhibit type I IFN production in primary mouse embryonic fibroblasts (MEFs). ZAP binds and destabilizes SINV RNA, thereby suppressing the replication of SINV. Type I IFN fails to suppress SINV replication in ZAP-deficient MEFs, whereas the ectopic expression of ZAP is sufficient to suppress the replication of SINV in MEFs lacking the expression of type I IFN and the IFN-inducible genes. ZAP-deficient mice are highly susceptible to SINV infection, although they produce sufficient amounts of type I IFN. Therefore, ZAP is an RNA-sensing anti-viral effector molecule that mediates the type-I-IFN-dependent host defense against SINV (Kozaki et al, Int Immunol 2015).

Although we have demonstrated the importance of ZAP in anti-viral responses, the molecular mechanisms underlying the elimination of viruses by ZAP are still unclear. ZAP suppresses the replication of SINV, Ebola virus, Marburg virus, MLV, HIV-1 and HBV, indicating that ZAP targets positive-sense RNA viruses, negative-sense RNA viruses, retroviruses and DNA viruses. However, ZAP does not suppress the replication of VSV, a negative-sense RNA virus. ZAP inhibits viral protein translation and viral RNA splicing and destabilizes viral RNA. These findings raise important questions. How does ZAP sense a wide variety of viruses? How does ZAP specifically identify its target viruses? How does ZAP distinguish the different modes of anti-viral actions? In future studies, we will address these questions to better understand the anti-viral innate immune response.

### 3. Suppression of NLRP3-inflammasome formation by resveratrol (Figure 1)

Previously we have clarified the molecular mechanism of colchicine, a drug for gout attack. In response to Nigericin, monosodium urate, or silica particles, NLRP3 forms an inflammasome with its adaptor protein ASC and mediates innate immune responses. NLRP3-inflammasome inducers cause aberrant mitochondrial homeostasis to reduce the NAD+ level, which in turn inactivates the NAD+-dependent α-tubulin deacetylase Sirtuin 2 (SIRT2), resulting in accumulation of acetylated α-tubulin. Accumulated acetylated α-tubulin mediates ASC-NLRP3 contact to promote NLRP3-inflammasome activation. Colchicine blocks the proximity of ASC and NLRP3 by disrupting tubulin structure. We recently showed that resveratrol also suppresses NLRP3-inflammasome formation by inhibiting acetylation of α-tubulin, and may be therapeutically useful for treatment of gout (Misawa et al. Int Immunol 2015 in press).

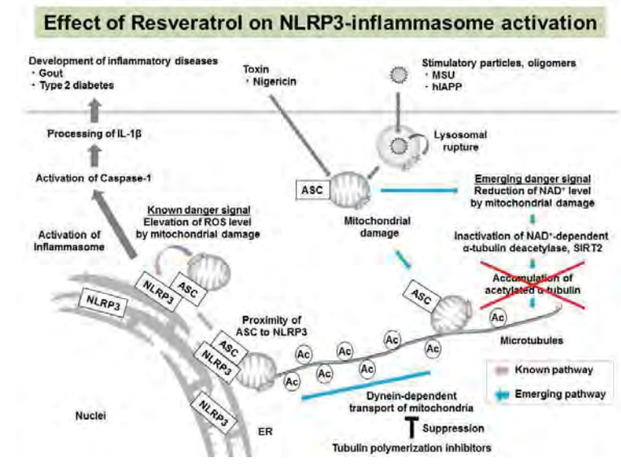



Figure 1. Effect of Resveratrol on NLRP3-inflammasome activation

## Recent Publications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# Immunoglycobiology

## Taroh Kinoshita, PhD

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

As many as 150 different proteins with various functions, such as receptors, adhesion molecules, enzymes, enzyme inhibitors and complement regulatory proteins, are anchored to the cell surface via glycosylphosphatidylinositol (GPI) that is covalently linked to the carboxyl-terminus. Our laboratory has been working to clarify the mechanism of biogenesis, biological functions and medical significance of GPI anchors and GPI-anchored proteins (GPI-APs). In 2014 we made a number of discoveries in studies of inherited GPI deficiency and regulation of GPI-AP functions.

We reported the first cases of inherited GPI-deficiency caused by mutations in *PGAP1* (*P*ost *G*PI *A*ttachment to *P*roteins *1*) gene (Murakami Y et al., *PLoS Genet*, 2014; Bosch DG et al., *Eur J Hum Genet*, 2015) in collaboration with research groups in Germany and Netherlands. We previously showed that *PGAP1* encodes a deacylase that removes a fatty acid from inositol moiety in nascent GPI-APs in the endoplasmic reticulum (ER) (Figure 1). The removal of the inositol-linked fatty acid by PGAP1 is critical for association of GPI-APs with transport cargo receptors and efficient exit from the ER. A lack of PGAP1 causes delayed transport of GPI-APs from the ER although the cell surface levels of GPI-APs are in general not affected. It is also known that the inositol-deacylation is prerequisite for GPI fatty acid remodeling that occurs in the Golgi. Therefore, structure of lipid moiety of GPI-APs on PGAP1-defective cells is abnormal (Figure 1). We have now found that two siblings with homozygous null mutation in *PGAP1* had intellectual disability and seizures, and an individual with compound heterozygous loss-of-function mutations in *PGAP1* had intellectual disability and cerebral visual impairment. It is suggested that structure of lipid moiety in GPI-APs is critical for normal function of neuronal cells. GPI-deficiency caused by mutations in 12 genes involved in GPI biosynthesis, remodeling and protein attachment have been found (Figure 2). GPI-deficiency caused by *PGAP1* mutations is unique among them in that expression of GPI-APs with abnormal GPI structure rather than reduction of cell-surface levels of GPI-APs is the major abnormality (Figure 1). Individuals with GPI-deficiency caused by mutations in genes involved in GPI biosynthesis have been found among children with early-onset epileptic encephalopathy. Five individuals with GPI-deficiency caused by PIGA mutations have been found in a cohort of 172 Japanese patients with early-onset epileptic encephalopathy (Kato M et al., *Neurology*, 2014).

A unique characteristic of GPI-APs is that intact proteins can be shed from the cell surface by cleavage of GPI-anchor by specific GPIases. We identified a novel GPI cleaving enzyme, termed PGAP6. PGAP6 is a GPI-specific phospholipase A2 mainly localized at the cell surface. PGAP6 sheds some but not all GPI-APs. CRIPTO, a GPI-AP, has a critical role in early embryonic development by acting as a co-receptor of Nodal, a TGF-β family protein. We found that CRIPTO is a sensitive substrate of PGAP6 and that PGAP6 initiates non-cell-autonomous action of CRIPTO via shedding while reducing cell-autonomous CRIPTO actions. In mice, Pgap6 and Cripto are co-expressed in day 6 embryonic epiblasts and *Pgap6*-knockout mice were embryonic lethal. These results suggest that PGAP6 plays an important role in Nodal signaling regulation through CRIPTO shedding.

Our current studies focus on mechanistic bases of intracellular trafficking of GPI-APs and of structural heterogeneity of GPI-anchors to better understand regulation of GPI-AP functions and bases of various phenotypes seen in individuals with GPI-deficiency.

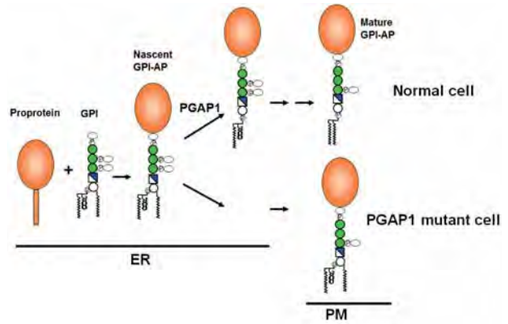


**Figure 1.**
**Abnormal lipid structure of GPI-APs expressed on PGAP1 mutant cells. PGAP1 removes palmitic acid from inositol of GPI in the endoplasmic reticulum (ER). GPI-APs expressed on the plasma membrane (PM) of PGAP1 mutant cells have palmitic acid linked to inositol and unsaturated fatty acid at sn2-position of glycerol due to a lack of fatty acid remodeling.**

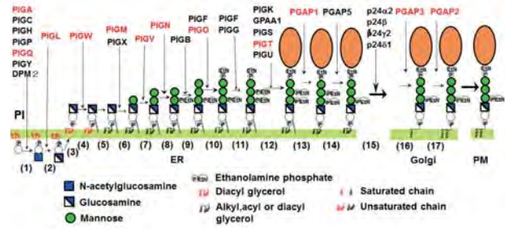


**Figure 2.**
**Genes involved in GPI biosynthesis, remodeling and protein attachment. Genes, for which mutations causing GPI-deficiency were identified, are in red.**

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- Theiler R, Fujita M, Nagae M, Yamaguchi Y, Maeda Y, Kinoshita T. The alpha helical region in p24γ2 subunit of p24 cargo receptor is pivotal for the recognition and transport of glycosylphosphatidylinositol-anchored proteins. J. Biol. Chem. 289:16835-43, 2014.
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# Immunopathology

## Atsushi Kumanogoh, MD/PhD

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|---|---|
| Professor | **Atsushi Kumanogoh** |
| Assistant Professor | **Hyota Takamatsu** |
| Research Assistant | **6** |

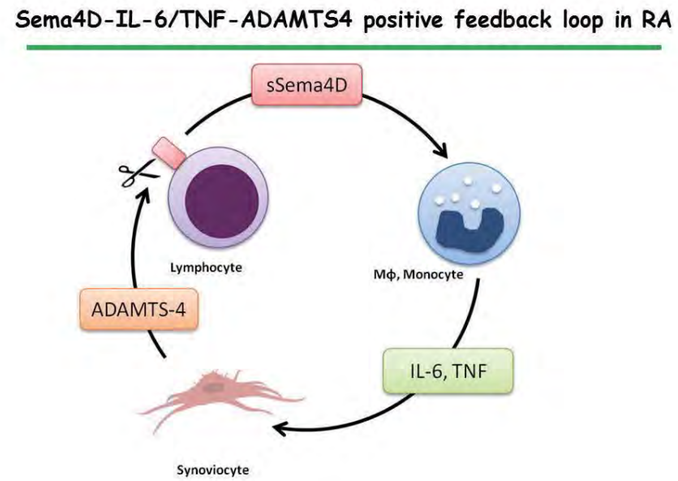
Our research team is involved in two approaches, that is, basic and clinical immunology. As basic aspects of our projects, our proposed study is the regulation of immune cell motility and migratory behavior in vivo by soluble and membrane-bound immune guidance molecules' such as semaphorins and their receptors. Semaphorins were originally identified as axon-guidance molecules that function during neuronal development. However, cumulative evidence indicates that semaphorins also participate in immune responses, both physiological and pathological, and they are now considered to be potential diagnostic and/or therapeutic targets for a range of diseases. Beyond such basic implications, we are trying to apply the findings from this proposed study into the diagnosis/therapy for human immunological disorders, such as autoimmunity, allergy, immune deficiency, cancer/metastasis, and neurodegenerative diseases. We here focus on the pathological implications of Sema4D in rheumatoid arthritis (RA).

*Sema4D and RA

RA is a common autoimmune disease that causes chronic inflammation of the synovium. RA synovitis evokes arthritic symptoms and leads to destruction of cartilage and bone in joints. Recent advances in pathogenesis of RA have revealed that complex interplays among genetic and environmental factors evoke autoimmunity, accompanied by the production of critical autoantigens such as citrullinated proteins. Once RA is developed, autoimmunity is sustained and leads to persistent synovitis, which in turn causes destruction of bone and cartilage. The mechanisms of sustained synovitis remain unclear. Recently, pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6) were shown to play key roles in RA. Biological disease-modifying anti-inflammatory drugs (bDMARDs), which can block these cytokines, constitute the current standard of care. However, a substantial proportion of RA patients are still unable to achieve drug-free remission of bDMARDs. In order to achieve true remission, it will be necessary to identify another key molecular player that identifies another key molecular player that contributes to autoimmunity, immune activation, and bone destruction in RA.

Semaphorin 4D/CD100 was the first semaphorin shown to play a role in the immune system, and was identified originally as a T-cell activation marker. Indeed, Sema4D is abundantly expressed on the cell surface of T cells; however, it is also expressed in a broad range of hematopoietic cells. Although Sema4D is a membrane-bound protein, it also exists as a functional soluble form (sSema4D) following proteolytic cleavage upon cellular activation. Cumulative studies have demonstrated that Sema4D plays crucial roles in the immune system. In addition, several studies have shown that Sema4D is relevant to the pathogenesis of autoimmunity. For instance, Sema4D-deficient mice are resistant to the development of experimental autoimmune encephalomyelitis, a murine model of MS. Sema4D is expressed on tumor-associated macrophages (TAMs), and Sema4D produced by TAMs is involved in tumor angiogenesis and vessel maturation. Notably, Sema4D derived from osteoclasts suppresses bone formation by osteoblasts, and blocking of Sema4D results in increased bone mass. Immune abnormality, angiogenesis, and bone destruction all play critical roles in the progression of RA, suggesting that Sema4D might exacerbate RA. However, the involvement of Sema4D in the pathogenesis of RA has not yet been determined.

In this study, we found that sSema4D levels were elevated in sera and synovial fluids from RA patients. The increased levels of sSema4D were produced by an inflammation-related proteolytic mechanism, and the resultant sSema4D in turn induced inflammation, suggesting the existence of an inflammatory activation loop in RA synovium. Inhibition of Sema4D ameliorated the symptoms of collagen induced arthritis. These results suggest that Sema4D represents a potential target for treatment of RA.

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# Immunochemistry

## Hisashi Arase, MD/PhD

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

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

### A) Interaction between PILRα 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). We found that PILRα associates with glycoprotein B (gB), an envelope protein of herpes simplex virus-1 (HSV-1), and the interaction between PILRα and gB is involved in membrane fusion during HSV-1 infection. This suggested that immune inhibitory receptors can be exploited by viruses to invade host cells for the first time (Satoh et al. *Cell* 2008; Wang et al. *J. Virol.* 2009). Furthermore, we found that PILRα is a unique receptor that has binding sites for both sugar chain and protein structures (Kuroki et al. *PNAS* 2014). We further analyzed host cell molecules that associate with HSV-1 gB and found that non-muscle myosin heavy chain (NMHC-IIA) associates with gB and is involved in HSV-1 infection (Arii et al. *Nature* 2010).

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

VZV belongs to α-herpesvirus similar to HSV, although the 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 with 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. *PNAS* 2010).

### C) PILRα plays an important role in neutrophil infiltration

In order to elucidate the function of PILRα in immune response, we generated inhibitory PILRα-knockout mice and analyzed the function of PILRα. PILRα-deficient mice were susceptible to LPS-induced endotoxin shock. Further analyses revealed that infiltration of neutrophils in liver and lung was significantly increased in PILRα-deficient mice. When we analyzed neutrophils from PILRα-deficient mice, we found that activation of integrin by chemokine stimulation is augmented in PILRα-deficient neutrophils (Wang et al. *Nat. Immunol.* 2012). Furthermore, PILRα-deficient mice showed severe DSS-induced colitis (Kishida et al. *Int. Immunol.* 2015). These findings indicated that PILRα plays an important role in the regulation of inflammation by regulating integrin function.

### D) Misfolded proteins complexed with MHC class II molecules are targets for autoimmune diseases

MHC class II allelic polymorphisms are associated with susceptibility to many autoimmune diseases. However, it has remained unclear how MHC class II molecules are involved in autoimmune disease susceptibility. We found that cellular misfolded autoantigens are rescued from protein degradation by MHC class II molecules (Jiang et al. *Int. Immunol.* 2013). Furthermore, we found that misfolded proteins complexed with MHC class II molecules can become targets for autoantibodies in autoimmune disease patients (Jin et al. *Proc. Natl. Acad. Sci. USA.* 2014). Autoantibody binding to misfolded proteins transported to the cell surface by MHC class II molecules was strongly correlated with susceptibility to autoimmune disease. Furthermore, we found that β2-glycoprotein I presented on MHC class II molecules are a major autoantibody target for antiphospholipid syndrome (Tanimura et al. *Blood.* 2015) (Figure 1). This suggested that misfolded proteins complexed with MHC class II molecules are natural autoantigens for autoantibodies. Indeed, most autoimmune-diseased tissues aberrantly express MHC class II molecules. Therefore, misfolded proteins, which normally would not be exposed to the immune system, can be targets for autoantibodies when they avoid protein degradation (Figure 2).

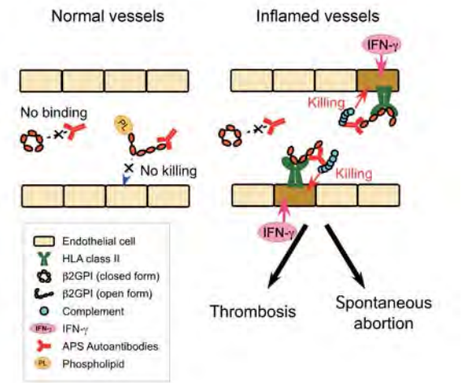


**Figure 1. β2GPI complexed with HLA class II molecules might play an important role in the pathogenesis of APS**
**A concept suggested by our findings. IFN-γ produced in inflamed tissues induces expression of HLA class II molecules on endothelial cells. When HLA class II molecules of the alleles with high affinity for linear or misfolded β2GPI are expressed on endothelial cells, the β2GPI is transported to the cell surface. Autoantibodies in APS patients bound to β2GPI complexed with HLA-DR damage endothelial cells in a complement-dependent manner (Tanimura et al. *Blood* 2015).**

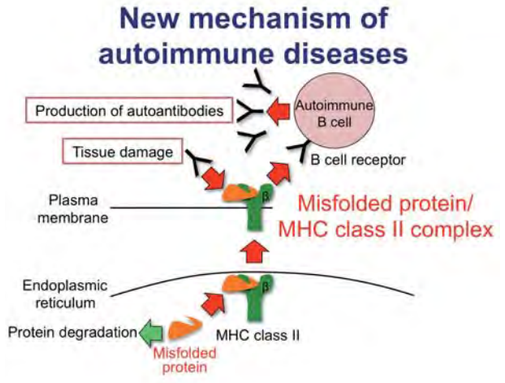


**Figure 2. Misfolded proteins transported to the cell surface by MHC class II molecules are targets for autoantibodies**
**Cellular misfolded proteins are transported to the cell surface by MHC class II molecules without processing to peptides when misfolded proteins are associated with MHC class II molecules at ER (Jiang et al. *Int. Immunol.* 2013). Furthermore, misfolded proteins complexed with MHC class II molecules of disease-susceptible alleles are specifically recognized by autoantibodies. Of note, autoantibody binding to misfolded proteins transported to the cell surface by MHC class II molecules was strongly correlated with susceptibility to autoimmune disease. This suggested that misfolded proteins complexed with MHC class II molecules are natural autoantigens for autoantibodies, which affects autoimmune disease susceptibility. (Jin et al. *Proc. Natl. Acad. Sci. USA.* 2014; Tanimura et al. *Blood* 2015).**

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- Tanimura K, Jin H, Suenaga T, Morikami S, Arase N, Kishida K, Hirayasu K, Kohyama M, Ebina Y, Yasuda S, Horita T, Takasugi K, Ohmura K, Yamamoto K, Katayama I, Sasazuki T, Lanier LL, Atsumi T, Yamada H, Arase H. β2-glycoprotein I / HLA class II complexes are novel autoantigens in antiphospholipid syndrome. Blood 125:2835-44, 2015.
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# Immune Regulation


## Tadamitsu Kishimoto, MD/PhD

| | |
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| Professor | Tadamitsu Kishimoto |
| Associate Professor | Barry Ripley |
| Assistant Professor | Kazuya Masuda |
| Postdoctoral Fellow | 5 |
| Research Assistant | 1 |
| Support Staff | 2 |

**1. Arid5a controls IL-6 mRNA stability, which contributes to elevation of IL-6 level in vivo (Kazuya Masuda)**

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 Th17 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. Regulation of expression levels of Arid5a in macrophages through TLR4 signaling (Kishan Nyati)**

Having established a critical role for Arid5a in macrophages in control of levels of IL-6 in vivo, we wished to explore how TLR4 signaling in macrophages regulates expression levels of Arid5a. We found that Arid5a protein is rapidly increased and then quickly degraded in LPS-treated macrophages. Interestingly, Arid5a protein is highly ubiquitinated when its protein levels reach peak maximal levels in macrophages following LPS stimulation. Our goal is to define precisely the regulatory pathways and the key proteins that control expression levels of Arid5a (and with respect to Regnase-1 expression levels) in macrophages.

**3. Arid5a directs the development of inflammatory CD4⁺ T cells through selective stabilization of STAT3 mRNA (Kazuya Masuda)**

Activation of STAT3 in T cells plays an important role in commitment of the differentiation of inflammatory CD4⁺ T cells. Here we demonstrate that Arid5a in T cells selectively stabilizes Stat3 (but not Stat1 and Stat5) mRNA in an IL-6-dependent manner, which drives naïve T cells to differentiate into inflammatory CD4⁺ T cells. Arid5a mRNA is specifically enhanced only under Th17-polarizing conditions but not in other distinct helper T (Th) cell subsets including Th1, Th2 and regulatory T cells. Loss of Arid5a in T cells led to diminished STAT3 activity in an IL-6-dependent manner, which results in the impairment of Th17 cell development, and also exhibits reduced pathogenicity for adoptive transfer EAE induction.

**4. Pathogenic role of Arid5a in endotoxin shock (Mahabub Zaman)**

Endotoxin is recognized as the most potent microbial mediator implicated in the pathogenesis of sepsis and septic shock. Our group has reported that Arid5a is expressed highly in macrophage cells in response to LPS. Therefore we used the endotoxin-induced shock murine model to assess involvement of Arid5a. Interestingly, Arid5a KO mice are found to be completely resistant to LPS-mediated shock. H&E staining of lung, liver and spleen tissue suggested that Arid5a KO mice are protected from tissue injury after LPS induced shock. We are currently studying the mechanistic role of Arid5a in LPS shock and generating cell-type specific Arid5a KO mice to identify which immune cells are important for Arid5a to augment endotoxin shock.

**5. Arid5a deficiency protects against the development of bleomycin-induced pulmonary fibrosis (Praveen Dubey)**

More than five million people are afflicted with idiopathic pulmonary fibrosis (IPF), a progressive and highly devastating interstitial lung disease. During bleomycin-induced lung injury several immunological cells including alveolar macrophages, neutrophils, Th cells are activated which induce lung tissue damage and fibrosis through the production of several cytokines. We have observed that Arid5a KO mice are highly resistant to bleomycin-induced lung injury-mediated mortality. Immunohistological data of lung tissue suggests that Arid5a deficiency could protect mice from bleomycin-induced lung fibrosis, which indicates an important role for Arid5a in lung tissue fibrosis.

**6. Type-I interferon controls its own production in immune homeostasis by inducing PPAR-γ expression and an inhibitory PPAR-γ/IRF7 complex (Barry Ripley)**

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. We also observed inhibition of type-I IFN-dependent responses in autoimmunity.

**7. Aryl Hydrocarbon Receptor (AHR) negatively regulates type 1 interferon production and the development of murine lupus (Soyoung Lee)**

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

**8. Molecular mechanisms of Thalidomide anti-Inflammatory effects (David Millrine)**

We investigated the mechanism underlying the anti-inflammatory properties of Thalidomide by studying the recently identified Thalidomide-binding protein, Cereblon. Thalidomide potently inhibited the TLR4-TRIF pathway (which acts in synergy with the MyD88-dependent pathway for cytokine production), involving inhibition of IRF3 transcriptional activity. We found that under homeostatic conditions cereblon exists in complex with Rabex-5, an established regulator of endosomal signaling. Intriguingly, Treatment with Thalidomide derivatives was found to disrupt this complex. Rabex-5 was also found to be critical for TLR4-induced signal transduction. Thus, we reason that disruption of the Cereblon-Rabex-5 complex underlies Thalidomide's anti-inflammatory properties.

**9. Therapeutic targeting of the interleukin-6 receptor**

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

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

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

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

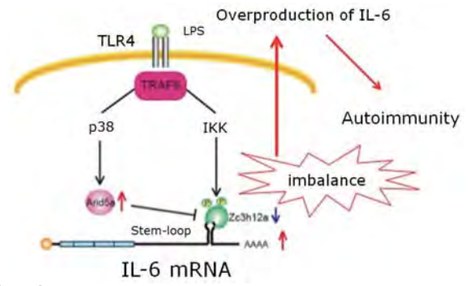


**Figure 1.**
**A balance between Arid5a and Regnase-1 tightly regulates production of IL-6 level**

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- Nakahama T, Kimura A, Nguyen NT, Chinen I, Hanieh H, Nohara K, Fujii-Kuriyama Y, Kishimoto T. Aryl hydrocarbon receptor deficiency in T cells suppresses the development of collagen-induced arthritis. Proc. Natl. Acad. Sci. USA. 108:14222-7, 2011.
- Masuda K. et al. Arid5a controls IL-6 mRNA stability, which contributes to elevation of IL-6 level in vivo. Proc. Natl. Acad. Sci. USA. 110:9409-14, 2013.
- Nakahama T. et al. Aryl hydrocarbon receptor-mediated induction of the microRNA-132/212 cluster promotes interleukin-17-producing T-helper cell differentiation. Proc. Natl. Acad. Sci. USA. 110:11964-9, 2013.
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# Mucosal Immunology

## Kiyoshi Takeda, MD/PhD

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|---|---|
| Professor | **Kiyoshi Takeda** |
| Associate Professor | **Eiji Umemoto** |
| Assistant Professor | **Hisako Kayama** |
| Postdoctoral Fellow | **3** |
| Research Assistant | **8** |
| Visiting Scientist | **1** |
| Support Staff | **3** |

We previously demonstrated that extracellular adenosine 5′-triphosphate (ATP) in the intestinal lumen contributes to development of intestinal Th17 cells via activation of a unique subset of intestinal dendritic cells. We have also found that the luminal ATP level is controlled by a member of ATP-hydrolyzing ecto-enzyme families, ecto-nucleoside triphosphate diphosphohydrolase 7 (E-NTPD7). E-NTPD7, which is highly expressed in the epithelial cells of the small intestine, regulates the luminal ATP level by hydrolysis, and thereby controls Th17 cell responses in the small intestine. In addition to E-NTPD7, E-NTPD1, also called CD39, was shown to regulate immune responses. ATP-hydrolyzing ecto-enzymes include a family of E-NTPDs, which convert ATP to ADP as well as ADP to AMP, and a family of ecto-nucleotide pyrophosphatase/phosphodiestrases (E-NPPs), which hydrolyze ATP to AMP.

### E-NPP3 negatively regulates ATP-dependent allergic inflammation

Among E-NPP family members, E-NPP3, which is also called CD203c, is well known as an activation marker of human basophils, and thus used as a diagnosis marker for allergic diseases. FcεRI crosslinking by antigen-bound IgE activates basophils and mast cells to induce immediate and late phases of allergic inflammation. In this context, E-NPP3 is rapidly induced by FcεRI crosslinking in mouse basophils and mast cells. However, its function has remained unknown. Therefore, we analyzed the physiological function of E-NPP3 by generating E-NPP3 knockout mice. Basophil and mast cell numbers increased in *Enpp3*$^{-/-}$ mice with elevated serum ATP levels. *Enpp3*$^{-/-}$ mice were highly sensitive to basophil- and mast cell-dependent chronic allergic pathologies, such as chronic allergic skin inflammation, experimental oral allergen-induced diarrhea, and experimental chronic asthma. ATP blockade reduced allergic responses in *Enpp3*$^{-/-}$ mice. FcεRI crosslinking induced ATP secretion from basophils and mast cells, and ATP clearance was impaired by the *Enpp3* deficiency. Extracellular ATP potently activated *Enpp3*$^{-/-}$ basophils and mast cells, which were blocked by P2X7 antagonists. Non-hydrolyzable ATP activated basophils and mast cells from wild-type mice to levels similar to those observed in *Enpp3*$^{-/-}$ cells. Introduction of the *P2rx7* deficiency into *Enpp3*$^{-/-}$ mice caused decreased responses of basophils and mast cells to FcεRI crosslinking. Thus, extracellular ATP released by FcεRI crosslinking stimulates basophils and mast cells via P2X7 in an autocrine manner for further activation causing allergic inflammation. E-NPP3 induced by FcεRI crosslinking decreases ATP levels and thereby negatively regulates basophil and mast cell activity (Figure 1).

### Regulation of immune responses by ATP-hydrolyzing ecto-enzymes

A series of studies on ATP-hydrolyzing ecto-enzymes have revealed that E-NTPD1, E-NTPD7, and E-NPP3 play mandatory roles in the regulation of immune responses. In addition to E-NTPDs and E-NPPs, ecto-5′-nucleotidases such as CD73, which produce adenosine from AMP, have also been shown to regulate immune responses. Thus, nucleotide-converting ecto-enzymes regulate a variety of aspects of immune responses through regulation of ATP hydrolysis (Figure 2).

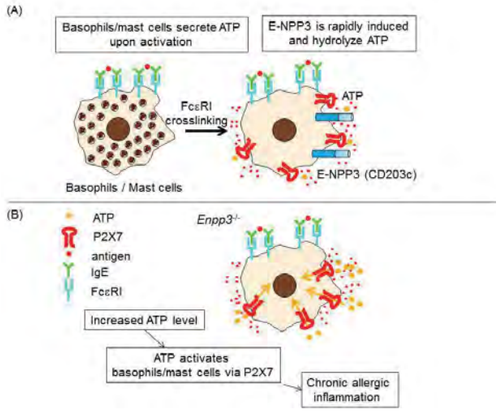


**Figure 1. E-NPP3 regulates chronic allergic responses by basophils and mast cells**
**(A)Basophils and mast cells secrete ATP upon FcεRI crosslinking. E-NPP3 is simultaneously expressed on basophils and mast cells and hydrolyzes extracellular ATP.**
**(B)In the absence of E-NPP3, ATP clearance activity is impaired in basophils and mast cells. Extracellular ATP, which level is increased, activates the cells in an autocrine manner via P2X7. This leads to development of enhanced chronic allergic inflammation.**

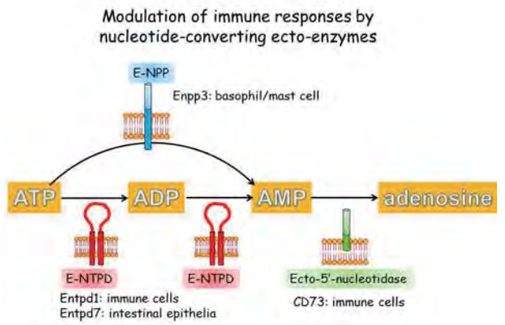


**Figure 2. Regulation of immune responses by nucleotide-converting ecto-enzymes**
**Members of the E-NTP family (E-NTPD1, E-NTPD7), the E-NPP family (E-NPP3), and ecto-5′ nucleotidase (CD73) have been shown to modulate the immune responses through hydrolysis of nucleotides such as ATP.**

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# Molecular Immunology

## Hitoshi Kikutani, MD/PhD

| | |
|---|---|
| Professor | Hitoshi Kikutani |
| Associate Professor | Teruhito Yasui |
| Assistant Professor | Shuhei Sakakibara |
| Postdoctoral Fellow | 1 |
| Support Staff | 2 |

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

EBV infects memory B cells for persistent infection. Although it has been shown that EBV-encoded LMP1 and 2a constitutively activate the CD40 and BCR signals respectively, effects of these viral proteins on the humoral immune responses remains largely unclear. In our laboratory, we generated conditional transgenic mice for EBV LMP1 or 2a to evaluate their function in vivo.

Our conditional LMP2a Tg mice exhibited impaired antigen-specific antibody production after immunization. In the spleen of LMP2a Tg mice, normal germinal center (GC) formation was observed whereas antigen-specific GC B cells were fewer at two weeks after immunization (Figure 1). In addition, plasma cell differentiation was significantly accelerated in LMP2a Tg mice. These results indicate that EBV LMP2a reduced the threshold for selection of high affinity B cells, which may contribute to the latent infection of EBV in memory B cells.

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

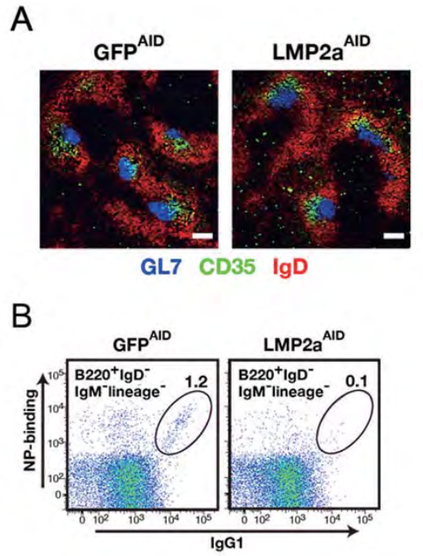


**Figure 1. EBV LMP2A reduces the threshold for selection of high-affinity B cells**
(A) The conditional expression of LMP2a by the activation-induced cytidine deaminase (AID) promoter-driven Cre did not alter GC size and structure in the spleen upon immunization. (B) Generation of antigen-binding B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

### 2) Generation and selection of autoreactive B cells in systemic lupus erythematosus (SLE)

SLE is a refractory disease characterized by a high-titer of serum IgG autoantibodies that are reactive to nuclear antigens such as DNA, histone, RNP and others. Despite extensive studies, the mechanisms in development of autoreactive B cells remain to be elucidated.

Anti-nuclear antibodies (ANAs) are one of the diagnostic markers for SLE and also considered as a pathogenic factor for this disease. We isolated several ANA monoclonal clones from acute SLE patients and characterized their properties. We found that most ANAs were antigen-specific and their specific reactivities to each antigen were highly dependent on somatic hypermutation (SHM). Furthermore, performing deep sequencing for the immunoglobulin variable region of representative clones, we found that there were many ANA sub-clones which share several mutated nucleotides in blood from acute patients. Phylogenetic analysis indicated that autoreactive B cells underwent the GC reaction for stepwise affinity maturation to self-antigens.

## Recent Publications


- Tada S, Okuno T, Hitoshi Y, Yasui T, Honorat JA, Takata K, Koda T, Shimagami H, Chi-Jing C, Namba A, Sugimoto T, Sakoda S, Mochizuki H, Kikutani H, Nakatsuji Y. Partial suppression of M1 microglia by Janus kinase 2 inhibitor does not protect against neurodegeneration in animal models of amyotrophic lateral sclerosis. JNI. 11:179, 2014.
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# Experimental Immunology

## Shimon Sakaguchi, MD/PhD

| | |
|---|---|
| Professor | **Shimon Sakaguchi** |
| Associate Professor | **Hiroyoshi Nishikawa**<br>**Keiji Hirota** |
| Assistant Professor | **James Badger Wing**<br>**Norihisa Mikami**<br>**Noriko Sakaguchi** |
| Postdoctoral Fellow | **1** |
| Research Assistant | **4** |
| Support Staff | **8** |

This laboratory studies: (i) The cellular and molecular basis of immunologic self-tolerance, in particular the roles of regulatory T cells; (ii) the strategy for eliciting effective immune responses to autologous tumor cells, or inducing immunologic tolerance to organ transplants, by manipulating the mechanism of immunologic self-tolerance; and (iii) the cause and pathogenetic mechanism of systemic autoimmune diseases, such as rheumatoid arthritis, by utilizing an animal model established in our laboratory.

One aspect of immunologic self-tolerance (i.e., immunological unresponsiveness of the normal immune system to normal self-constituents) is actively maintained through a T-cell-mediated dominant control of self-reactive T cells by naturally occurring regulatory $CD4^+$ T cells (Treg cells). Yet how Treg cells effectively control potentially hazardous self-reactive T cells in humans remains an open question. In particular, it is unknown whether Treg-cell-mediated suppression for a limited period has a critical long-lasting effect on cell fate and antigen reactivity of autoimmune T cells. This year, by addressing this issue, we have shown that Treg cells can render self-reactive human $CD8^+$ T cells anergic (i.e., hypo-proliferative and cytokine hypo-producing upon antigen re-stimulation) in vitro, likely by controlling the co-stimulatory function of antigen-presenting cells. Anergic T cells were naïve in phenotype, lower than activated T cells in T-cell receptor affinity for cognate antigen, and expressed several co-inhibitory molecules, including cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4). Using these criteria, we detected in healthy individuals, anergic T cells reactive with Melan A, a skin antigen targeted in the autoimmune disease vitiligo. Collectively, our results suggest that Treg-cell-mediated induction of anergy in autoimmune T cells is important for maintaining self-tolerance, and can be a key target in controlling autoimmunity and tumor immunity (Maeda et al., *Science*, 2014).

We have also attempted this year to determine the roles of Treg cells and BCL6-expressing T follicular regulatory (Tfr) cells in the control of humoral immune responses. We found that depletion of Treg cells, blocking of CTLA-4 or a Treg cell specific reduction in CTLA-4 expression, resulted in an increase in the formation of antigen-specific Tfh cells, germinal center (GC), plasma and memory B-cells following vaccination. In the absence of Treg-expressed CTLA-4, large proportions of Tfr cells are present but are unable to restrain Tfh and GC formation. Temporary Treg cell depletion during primary immunization was sufficient to enhance secondary immune responses. In addition, Tregs directly inhibited, via CTLA-4, B cell expression of CD80 and CD86, which was essential for Tfh formation. Taken together, Tregs and Tfr play a key role in the control of Tfh and germinal center development via CTLA-4-dependent control of CD80/CD86 expression (Wing et al., *Immunity*, 2014).

We have previously established an animal model of autoimmune arthritis, called SKG mice, which possess a ZAP-70 gene mutation and spontaneously develop T cell-mediated autoimmune arthritis immunopathologically similar to rheumatoid arthritis (RA) in humans. T cells mediating autoimmune diseases, such as RA, are technically difficult to characterize in healthy individuals because they are likely to be deleted or inactivated in the thymus if the self-antigens they recognize are ubiquitously expressed. This can be circumvented by altering TCR signaling intensity (for example, via mutated ZAP-70), which changes the sensitivity of developing T cells to thymic selection and results in the generation of new dominant self-reactive TCR specificities that are causative of systemic autoimmune diseases such as RA. With this strategy, we have isolated arthritogenic TCRs from SKG mice and characterized the self-antigens they recognized. One of them was the ubiquitously expressed 60S ribosomal protein L23a (RPL23A), with which T cells and autoantibodies from RA patients reacted. Our approach is instrumental in deciphering how T-cell autoimmunity to a ubiquitous self-antigen should trigger localized tissue damage in RA and other human autoimmune diseases, and devising effective ways of their systemic or local intervention (Ito et al., *Science*, 2014).

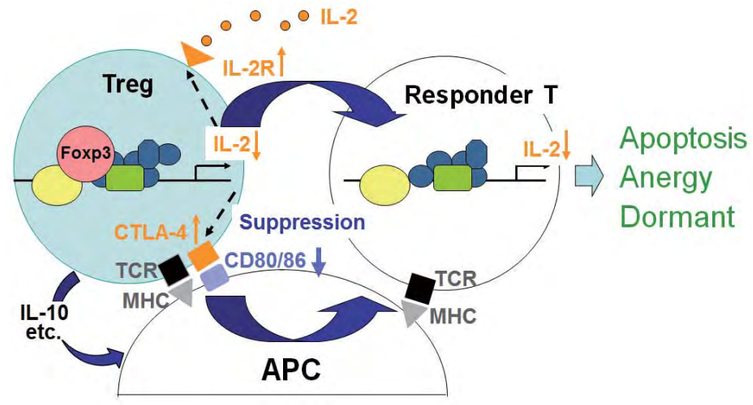


**Figure 1.**
**Treg cells, which express the transcription factor FoxP3, down-modulate the expression of CD80/CD86 by antigen-presenting cells (APCs). Responder T cells that recognize an antigen presented by such an APC are rendered anergic or apoptotic, or stay un-responsive (dormant or ignorant) depending of their TCR affinity for the antigen (Maeda et al., Science, 2014).**

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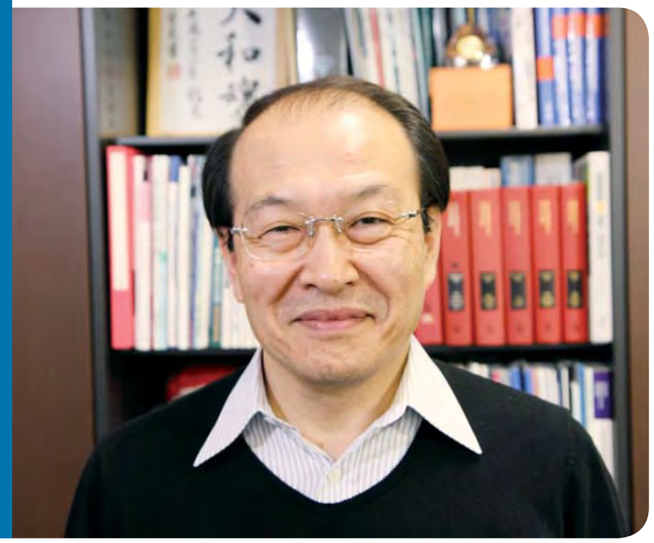
# Cell Signaling



## Takashi Saito, PhD

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| Professor | Takashi Saito |
| Postdoctoral Fellow | 1 |

T cells play critical roles in initiating and regulating immune responses. Deregulation of T cell activation and function leads to various immune diseases. We have analyzed the molecular mechanism of T cell activation upon antigen recognition and the subsequent homeostasis and differentiation of effector T cells. After we found that initial T cell activation is induced in TCR microclusters, which are generated by accumulation of signaling molecules critical for activation, we analyzed the regulation of and the relationship with other activation pathways to TCR microclusters, particularly the relationship with co-stimulation signals and cytoskeletal regulation.

### Dynamic regulation of NF-kB activation

TCR activation-induced NF-κB activation is mediated through the activation of the CARMA1-Bcl10-Malt1 (CBM) complex to activate IκB kinase. NF-κB activation is strongly enhanced by CD28-mediated co-stimulation signals. We showed that CD28 engagement induces costimulation signals by recruiting CD28-PKCΘ -CARMA1 to signaling competent region of cSMAC of immunological synapse (Fig.1 top). The accumulation of PKCΘ and CARMA1 to this region was inhibited when the CD28-CD80 interaction was blocked by the addition of CTLA4-Ig. It has been shown that many B cell lymphomas were induced by the mutations of CARMA1. When we introduced such mutant CARMA1, CARMA1 made spontaneous aggregation regardless of activation and induced NF-κB activation.

By searching binding molecules to GUK domain of CARMA1 by 2-hybrid method, we found that GUK domain directly binds to SH3 region of CARMA1 itself. Eventually we found that CARMA1 has a kind of closed structure with intra-molecular interaction in resting status while it has open structure inducing inter-molecular interaction upon activation, which induces cluster of CARMA1 (Fig.1 bottom). The SH3 mutant of CARMA1 that cannot bind to GUK failed to form CARMA1 cluster or NF-κB activation. T cells from the knock-in mice bearing the SH3 mutation did not respond to antigen in vivo or in vitro. The introduction of the SH3 mutation into the lymphoma-inducing CARMA1 mutant inhibits spontaneous formation of CARMA1 aggregates and NF-κB activation. Thus, appropriate cluster formation upon antigen stimulation is critical for correct activation of NF-κB and prevention of tumorigenesis through excess NF-κB activation.

### Cytoskeletal regulation of T cell activation

Spatial regulation of T cell activation is often regulated by cytoskeletal signaling. Our previous finding that TCR microclusters moved from the periphery to the center to form cSMAC through dynein-mediated translocation along microtubules and the TCR complex is associated with dynein complex suggested that not only the movement of TCR-microclusters but also its signaling is regulated by cytoskeletal dynamics. Accordingly, immunoprecipitation and mass-spectrometric analysis revealed that the TCR complex is associated with F-actin and actin-related molecules such as Arp2/3. Indeed, imaging analysis showed that F-actin and Arp2/3 are associated with TCR microclusters. Furthermore, TCR-microcluster/F-actin-Arp2/3 is surrounded by the ring structure composed of integrin and focal adhesion molecules. The adhesion ring structure is transiently formed and its formation is totally dependent on integrin and F-actin rearrangement. Since the central TCR and peripheral integrin is the principal of mature Immunological synapse, this structure is similar in micro-scale; therefore, we started to call it “microsynapse”. Microsynapse plays critical roles in promoting adhesion and activation particularly upon weak stimulation.

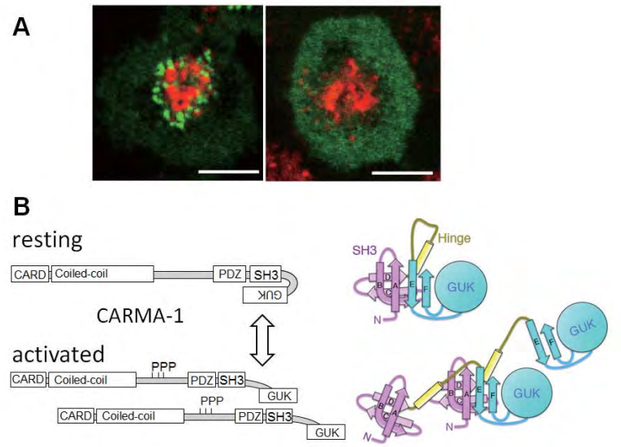


**Figure 1. Intra- and inter-molecular regulation of CARMA1 clustering for NF-kB activation upon T cell activation**
**A. CARMA1 was accumulated into cSMAC upon stimulation (left), while the mutant CARMA1 without the GUK-SH3 assembly failed to be accumulated (right). B. CARMA1 exhibits intra- and inter-assembly in resting and activated status, respectively. The inter-assembly induces CARMA1 aggregates.**

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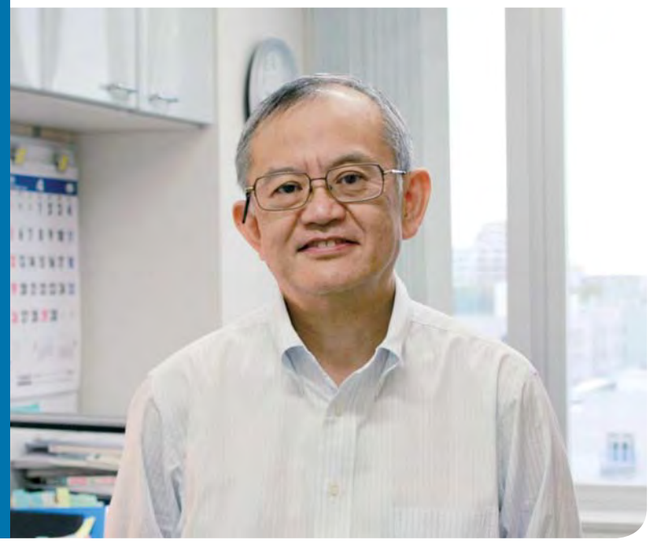
# Lymphocyte Differentiation



## Tomohiro Kurosaki, MD/PhD

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| ■ Professor | **Tomohiro Kurosaki** |
| ■ Associate Professor | **Yoshihiro Baba**<br>**Wataru Ise** |
| ■ Assistant Professor | **Takeshi Inoue**<br>**Masanori Matsumoto**<br>**Shinya Tanaka** |
| ■ Visiting Scientist | **1** |
| ■ Support Staff | **4** |

### Introduction

Signals propagated through the pre-B cell receptors (pre-BCR) and B-cell receptors (BCR) are crucial for the development of B lymphocytes and their antigen-triggered differentiation into memory B cells and antibody secreting plasma cells. The outcomes of the signaling events, for example, proliferation, apoptosis, or differentiation, are dependent on the developmental stage of the cell and quality of the signaling. When B cells recognize the same antigen for a second time, memory B cells get activated and subsequent memory antibody responses are 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. Our laboratory has focused on understanding the molecular mechanisms underlying crucial cell fate decisions during early B cell development as well as peripheral memory B cell development.

### Post-transcriptional regulation on early B cell development

The early B cell development steps are harmoniously regulated by transcriptional networks that integrate environmental cues to evoke gene expression programs appropriate to a particular developmental stage. Emerging evidence has demonstrated that these transcriptional regulatory mechanisms on their own are not sufficient for proper B cell development and that post-transcriptional mechanisms are also required. In regard to general post-transcriptional regulators, attention has been recently paid to the CCR4-NOT multi-protein complex, which serves as one of the major deadenylases on poly(A) in eukaryotes. Deadenylation is the initial and often a rate-limiting step in mRNA decay, resulting in repression of translation. Thus, we explored the role of one component of the CCR4-NOT complex, CNOT3, in B cell development and activation, and how, if at all, it participated in these processes. B-lineage specific CNOT3 deficiency results in a developmental block at the pro- to pre-B cell transition. This developmental defect was due primarily to impaired immunoglobulin heavy chain (Igh) gene rearrangement in pro-B cells and increased apoptosis in pro-/pre-B cells. Molecularly, CNOT3 turned out to regulate initiation of germline transcription of the Igh locus and to deadenylate p53 mRNA (Fig).

### Selection mechanisms of germinal center (GC) B cells into memory compartment

Antibody-mediated immunological memory relies on the development of memory B cells. Memory B cells can be generated through initial T-B interaction in a pre-GC period, at least to some extent. Nevertheless, the majority of memory B cells in wildtype setting responding to T-cell-dependent antigens are likely to arise from GC reaction. With successful selection inside GC, the GC B cells differentiate into two terminal fates, memory B cells and plasma cells. But, selection mechanisms into memory compartment have been obscure. We show that GC B cells with lower affinity BCRs express relatively higher Bach2 (a transcription factor) expression, controlled by strength of T cell help, being prone to enter memory pool from GC. Conversely, inhibition of Bach2 expression led to block of memory B cell generation. Hence, Bach2 functions as a rheostat to make a terminal cell fate decisions during GC reaction.

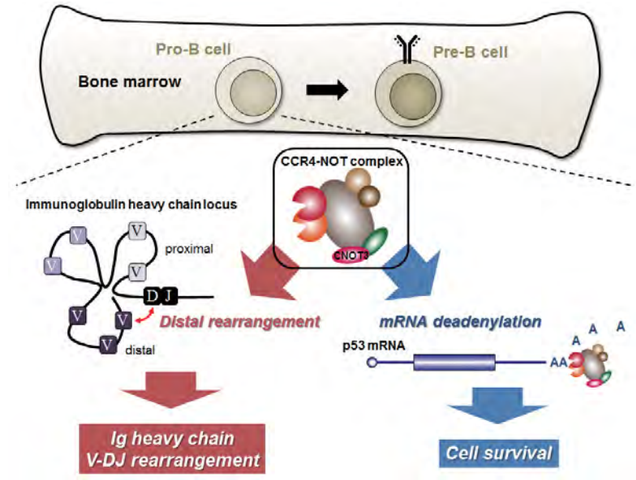


**Figure. CNOT3 subunit of the CCR4-NOT deadenylase complex is required for early B cell development**
**B cell-specific knockout of *Cnot3* results in a developmental block at the pro- to pre-B cell transition. CNOT3 regulates early B cell development by controlling the immunoglobulin heavy chain V-DJ earrangement and destabilizing *p53* mRNA.**

### Recent Publications


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# Malaria Immunology

## Cevayir Coban, MD/PhD

| | |
|---|---|
| Professor | Cevayir Coban |
| Postdoctoral Fellow | 1 |
| Research Assistant | 2 |
| Support Staff | 3 |

Our group is interested in how host interacts with pathogens. We try to understand host response to pathogens during acute and chronic phase of infection by using malaria infection as a model. Malaria, caused by *Plasmodium* parasites, costs millions of lives every year due to its complications such as cerebral malaria, and there is no fully potent drug and/or vaccine against this disease yet. Death from malaria occurs due to the organ-specific immunopathology caused by parasites; however, detailed understanding of this immunopathology remains unknown. Therefore, our recent focus is to understand how immunopathology is caused by these parasites at the tissue as well as cellular levels and transfer this information into treatment modalities. For example, we recently delineated the role of Lipocalin-2, a host protein with multiple cellular functions including controlling iron metabolism, during malaria infection (*Cell Host Microbe, 2012*). We concluded that Lipocalin 2 is one of the key molecules of host secreted against *Plasmodium* parasites. Our conclusions, therefore, could be easily transferred to the manipulation of other infectious diseases.

### Immunopathology of brain during malaria

One of the topics we work on is the pathology of cerebral malaria. Cerebral malaria is one of the deadliest complications of *P. falciparum* infection in humans. Early diagnosis of cerebral malaria is not easy due to non-specific symptoms, but is very important to initiate effective adjunct therapies which can save lives. Therefore, early, quick and cheap diagnosis of cerebral malaria has been the matter of investigation in patients and in animal models.

We've recently reported a new understanding of cerebral malaria pathogenesis by using cutting-edge imaging technologies such as ultra-high field MRI and multi-photon live imaging microscopy during experimental cerebral malaria. Deep investigation of brain by ultra-high field MRI showed that the olfactory bulb is physically and functionally damaged by *Plasmodium* parasites. Live multiphoton imaging of the olfactory bulb confirmed that the complex trabecular small capillaries comprising the olfactory bulb show parasite accumulation and cell occlusion followed by microbleeding, events associated with high fever and cytokine storm. With these findings, we've provided evidence that olfactory functional impairment (loss of smell) could be a valuable early diagnosis marker for cerebral malaria.

On the basis of these findings, we may have revealed one of the underlying mechanisms of CD8 T cell accumulation into brain vessels and tissue. Accordingly, astrocytes around olfactory glomeruli and vessels were activated during early stages of infection and released chemokine CCL21 that attracted a subpopulation of CD8 T cells (Fig. 1). Moreover, CCR7-deficient mice were able to significantly survive from experimental cerebral malaria. Although our studies have revealed that CCR7 is important for CD8α DC priming of CD8 T cells in spleen, however, it had no role for CD8 T cell recruitment into the brain. Instead, another chemokine receptor CXCR3, which was previously suspected to be a non-canonical receptor for CCL21 in the brain, is responsible for the recruitment of CD8 T cells. We have employed this new finding into a therapeutic application that, when two chemokine receptors, CCR7 and CXCR3, were targeted with an antibody even at the late stage of infection, could protect mice from cerebral malaria even with very low doses (Fig.1, *Cell Host Microbe*, 2014). These findings have promise as an easy application to be used in human cerebral malaria cases in the future.

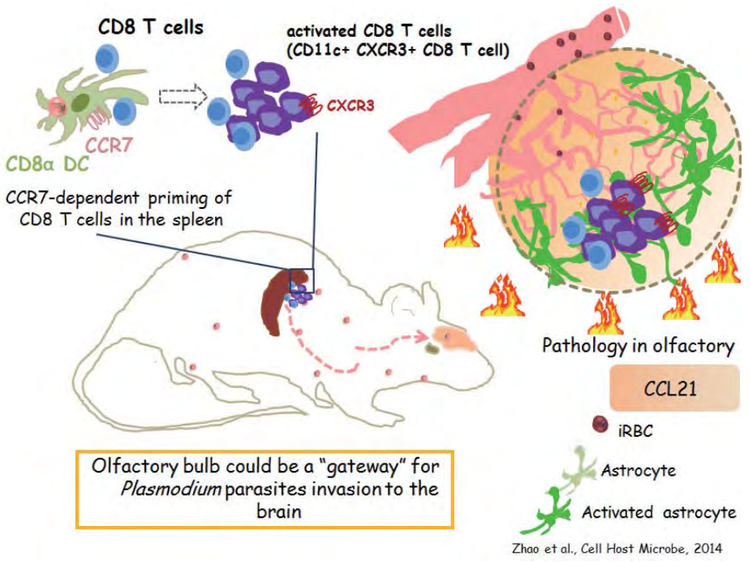


**Figure 1. Immunopathology in olfactory bulb during cerebral malaria**
**This study was performed by multi-disciplinary collaboration with a joint research team from Osaka University, Dokkyo Medical University, National Institute for Physiological Sciences, Mie University and Iwate Medical University.**

### Recent Publications


- Onishi M, et al. Hydroxypropyl-β-Cyclodextrin Spikes Local Inflammation That Induces Th2 Cell and T Follicular Helper Cell Responses to the Coadministered Antigen. J. Immunol.194:2673-82, 2015.
- Zhao H, et al. Olfactory Plays a Key Role in Spatiotemporal Pathogenesis of Cerebral Malaria. Cell Host & Microbe. 15:551-63, 2014.
- Kobiyama K, et al. Nonagonistic Dectin-1 ligand transforms CpG into a multitask nanoparticulate TLR9 agonist. Proc. Natl. Acad. Sci. USA. 111: 3086-91, 2014.
- Zhao H, et al. Lipocalin 2 bolsters innate and adaptive immune responses to blood-stage malaria infection by reinforcing host iron metabolism. Cell Host & Microbe. 12:705-16, 2012.
- Marichal T, et al. DNA released from dying host cells mediates aluminum adjuvant activity. Nat. Med. 17:996-1002, 2011.

# Vaccine Science

## Ken J. Ishii, MD/PhD

| | |
|---|---|
| Professor | **Ken J. Ishii** |
| Associate Professor | **Etsushi Kuroda** |
| Assistant Professor | **Taiki Aoshi**<br>**Kouji Kobiyama**<br>**Natsuko Kishishita**<br>**Takuya Yamamoto** |
| Postdoctoral Fellow | **2** |
| Research Assistant | **4** |
| Visiting Scientist | **6** |
| Support Staff | **5** |

The Primary goal of our laboratory is to understand the immunological mechanisms of the intra- and inter-cellular signaling pathways that mediate the immunogenicity of successful vaccines, as well as biological responses to adjuvants. Such knowledge will enable us to develop novel concepts, modalities and next generation immuno-preventive and/or therapeutic agents against infectious diseases, cancer and 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). In FY 2014-2015, we demonstrated that nucleic acids such as dsDNA or RNA:DNA hybrids can be found in the cytosol of cancer cells such as B cell lymphoma (Fig. 1, Koo CX et al. *J. Biol. Chem.* 2015, Shen YJ et al. *Cell Reports* 2015), potentially activating innate immunity.

**• 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 to identify that the 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). In FY 2014-2015, we coincidently found that hydroxypropyl-β-cyclodextrin (HP-β-CD), a common additive for many drugs, acts as a vaccine adjuvant by inducing host cell death, releasing dsDNA, and spiking local inflammation that induces Th2 Cell and T follicular helper cell responses to the coadministered antigen (Onishi M et al. *J. Immunol.* 2015).

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), 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) have not only revealed their critical role in driving the responses mediated by many current vaccines, but is 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 have been successful in developing a nucleic-acid-based adjuvant; humanized CpG-ODN for a travelers' malaria vaccine targeting a blood stage parasite antigen and initiated an investigator driven GCP Phase-I clinical trial during 2013 at Osaka University Hospital (Fig. 2). It was a big mile stone for myself as discovering K-type CpG ODN (K3) as a humanized CpG sequence took 15 years to be developed as GMP lot and finally administered into human. The results of which appear very promising will be released soon.

**• Development of the second-generation adjuvants:** As a second generation of CpG adjuvant, we generated a nano-size particle CpG ODN (K3) wrapped by a non-agonistic Dectin-1 ligand schizophyllan (SPG), namely K3-SPG. K3-SPG is a strong IFN-inducer as well as CTL inducer for immunotherapeutic applications (Kobiyama K et al. *PNAS* 2014). This K3-SPG has been nominated for a JST-supported grant with a pharmaceutical company in Japan. In addition, we found and invented a new immunotherapeutic way of CpG: a potent synergism between TLR9 and STING agonists. Together, they make an effective type-1 adjuvant and an anticancer agent. The synergistic effect between CpG ODN (K3) and STING-ligand cyclic GMP-AMP (cGAMP), culminating in NK cell IFN-γ (type-II IFN) production, is due to the concurrent effects of IL-12 and type-I IFNs, which are differentially regulated by IRF3/7, STING, and MyD88 (Temizoz B et al. *Eur. J. Immunol.*2015).

**• 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, Labour and Welfare). Cohort as well as retrospective analysis of human samples obtained from volunteers of vaccine clinical trials and patients of relevant immunological disorders are being conducted by four groups including our lab in IFReC and those in NIBIO. Preliminary results suggest serum miRNA may provide useful biomarkers to predict safety and immunogenicity of adjuvanted vaccines.

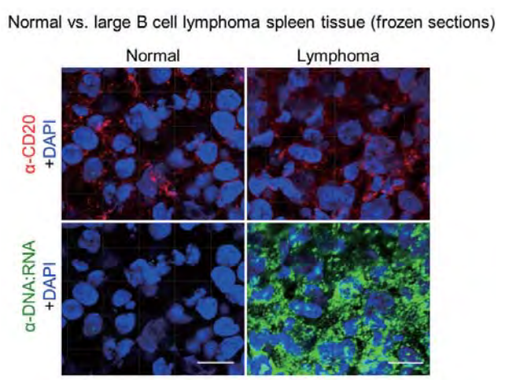


**Figure 1. Cytosolic RNA:DNA hybrid found in primary and cultured cancer cells regulated by RNA polymerase III (Koo CX et al J.B.C. 2015)**

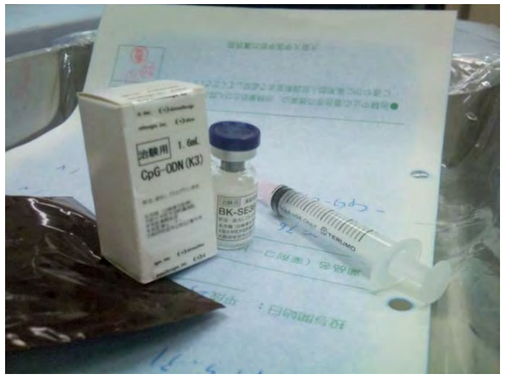


**Figure 2. First GMP lot of humanized CpG ODN "K3" administered in human clinical trial**

## Recent Publications


- Temizoz B, et al. TLR9 and STING agonists synergistically induce innate and adaptive type II IFN. Eur. J. Immunol. 45:1159-69, 2015.
- Onishi M, et al. Hydroxypropyl-β-Cyclodextrin Spikes Local Inflammation That Induces Th2 Cell and T Follicular Helper Cell Responses to the Coadministered Antigen. J. Immunol. 194:2673-82, 2015.
- Koo CX, et al. RNA Polymerase III Regulates Cytosolic RNA:DNA Hybrids and Intracellular MicroRNA Expression. J. Biol. Chem. 290:7463-73, 2015.
- Kobiyama K, et al. Nonagonistic Dectin-1 ligand transforms CpG into a multitask nanoparticulate TLR9 agonist. Proc. Natl. Acad. Sci. USA. 111: 3086-91, 2014.
- Desmet CJ, Ishii KJ. Nucleic acid sensing at the interface between innate and adaptive immunity in vaccination. Nat. Rev. Immunol. 12:479-91, 2012.

# Immune Regulation


## Tsuneyasu Kaisho, MD/PhD

■ Professor Tsuneyasu Kaisho


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

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

CD8α/103+CD11b- cDCs are characterized by the high ability to incorporate apoptotic or dead cells and crosspresent antigens to generate CD8 T cell responses. The cDCs contribute to antimicrobial or anti-tumor immunity. This was verified by the analysis on the XCR1-DTRvenus mice, in which DCs expressing a chemokine receptor, XCR1, i.e. CD103+CD11b- DCs, can be depleted upon injection of diphtheria toxin (C. Yamazaki et al. 2013, K. Shimizu et al. 2013).

XCR1-expressing DCs are globally detected not only in lymphoid tissues such as spleen or lymph nodes but also in peripheral tissues such as skin or intestine. However, it remains unknown whether or how these DCs are involved in maintaining the immune homeostasis. In order to clarify this issue, we have generated mutant mice, in which XCR1-expressing DCs are constitutively absent. We first generated the mutant mice, in which the gene for cre recombinase is knocked into the XCR1 gene locus. The mutant mice were further crossed with mutant mice, in which diphtheria toxin A subunit is designed to be expressed only when loxP-mediated deletion occurred. In the resultant mice, CD103+CD11b- DCs were ablated in spleen, lymph nodes and peripheral tissues such as lamina propria of intestine (Figure 1). The mice should be useful for clarifying the critical roles of XCR1-expressing DCs in immune homeostasis.

We have also generated XCR1-venus mice in which XCR1-expressing DCs can be detected as venus-expressing cells (C. Yamazaki et al. 2013). Analysis on the mice revealed that the T cell zone in the lymph node is compartmentalized into regions for CD8 T cell priming by XCR1-expressing DCs and for CD4 T cell priming. XCR1-expressing DCs in the lymph nodes consist of two types of DCs, i.e. migratory and resident DCs. In order to distinguish these two types of DCs, we have generated the mutant mice in which the XCR1 coding region was replaced by a gene encoding a photoconvertible fluorescent protein (Figure 2). Illuminating with violet-blue light turns green to red cells in the skin. The mice should be useful to track migratory and resident XCR1-expressing DCs.

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

pDC is characterized by the ability to produce large amounts of type I interferons (IFNs) in response to the signaling through TLR7 or TLR9, which can sense host- or microorganism-derived nucleic acids. This ability plays important roles in both protective immunity against viral infection and pathogenesis of certain autoimmune disorders such as SLE. We have found that an Ets family of transcription factor, Spi-B, expressed abundantly in pDC, is critical for pDC function (I. Sasaki et al. 2012).

Spi-B is expressed not only in pDC but also in various types of immune cells. Spi-B is expressed in a subset of intestinal epithelial cells, M cells, which function as gate-keeping cells in the intestine. Spi-B is crucial for development of M cells (T. Kanaya et al. 2012). Spi-B expression is induced in the medullary thymic epithelial cell (mTEC) upon stimulation with receptor activator of NF-κB ligand (RANKL). Spi-B then induced a RANKL signaling inhibitor, osteoprotegerin (OPG). This RANKL-Spi-B-OPG axis regulates development of mTEC, thereby limiting regulator T cell generation (N. Akiyama et al. 2014).

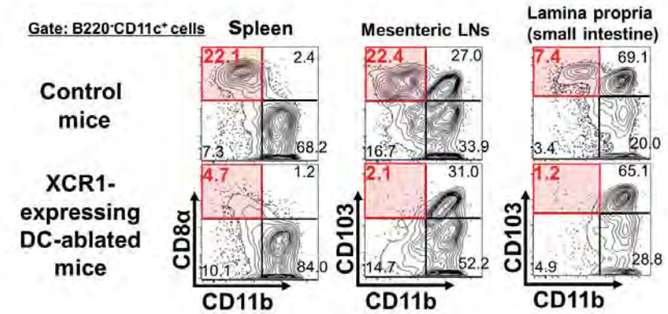


**Figure 1. DC population in XCR1-expressing DC-ablated mice**
We generated mutant mice, in which XCR1-expressing DCs are constitutively ablated. In the mice, CD103+CD11b- DCs are absent not only in lymphoid tissues but also in peripheral tissues.

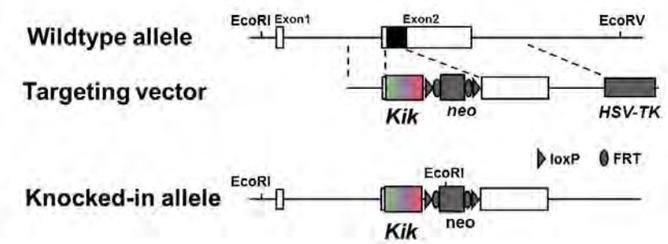


**Figure 2. Targeting strategy for generating mutant mice expressing a photochromic fluorescent protein in XCR1-expressing DCs**

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- Akiyama N, Shinzawa M, Miyauchi M, Yanai H, Tateishi R, Shimo Y, Ohshima D, Matsuo K, Sasaki I, Hoshino K, Wu G, Yagi S, Inoue J, Kaisho T, Akiyama T. Limitation of immune tolerance-inducing thymic epithelial cell development by Spi-B-mediated negative feedback regulation. J. Exp. Med. 211:2425-38, 2014.
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- Okuma A, Hoshino K, Ohba T, Fukushi S, Aiba S, Akira S, Ono M, Kaisho T, Muta T. Enhanced Apoptosis by Disruption of the STAT3-IκB-ζ Signaling Pathway in Epithelial Cells Induces Sjögren's Syndrome-like Autoimmune Disease. Immunity 38:450-60, 2013.
- 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.

# Immune Network

## Rikinari Hanayama, MD/PhD

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|---|---|
| Associate Professor | **Rikinari Hanayama** |
| Postdoctoral Fellow | 4 |
| Research Assistant | 1 |
| Support Staff | 1 |

Exosomes are small membrane vesicles of endosomal origin, composed of a lipid bilayer with inserted transmembrane proteins, enclosing cytosolic components derived from their producing cells. Over the past few years, there has been increasing evidence that exosomes play important roles in intercellular communication networks, enabling the conveyance of information and the exchange of proteins and lipids between their producing cells and target cells. Exosomes were also shown to carry mRNAs and microRNAs inside them, raising the possibility that exosomes transfer genetic information between cells. One of our current projects aims to characterize the communication networks between immune systems and other organs via exosomes.

In the central nervous system, exosomes can be released from all cell types including microglia, oligodendrocytes and neurons, and have been proposed to contribute to the physiology of the nervous system and to the neuron-glia communication. In particular, the findings that secretion of exosomes from neurons is promoted by depolarization and also by synaptic glutamatergic activity led us to hypothesize that neuronal exosomes may activate microglia to promote activity-dependent synaptic pruning. The creation of complex patterns of synaptic connectivity often requires the elimination of only a select subset of the connections initially established by neurons. The dynamic refinement of synaptic connections is essential not only for the appropriate wiring of neural circuits, but also for behavioral responses to a changing environment as well as for learning and memory. In the mammalian nervous system, synapse pruning events have been reported in various places such as retinotectal system, cerebellum, parasympathetic and sympathetic autonomic ganglia, and neuromuscular junctions. Recent studies have shown that glial cells have a central role in the pruning of synapses by specifically engulfing the degenerating neurites of inappropriate connections, but its regulatory mechanisms have been largely unknown.

To identify mediators of this process, we established an in vitro cell culture assay for the synapse elimination. Neuronal differentiation and synapse formation of PC12 cells were induced by culturing the cells with nerve growth factor (NGF) in a serum-free medium. To trigger synapse elimination, the NGF-containing medium was replaced with DMEM containing 10% FBS, and the neurites of PC12 cells degenerated within two days. Co-culturing with MG6 cells, a mouse microglial cell line, accelerated the removal of degenerating neurites of PC12 cells by phagocytosis. When MG6 cells were pre-incubated with exosomes secreted from the differentiated PC12 cells after depolarization, the removal was further accelerated by increasing the expression levels of complement component 3 in the MG6 cells. These results define a role for exosomes as a regulator of synapse elimination and clarify a novel mechanism whereby active synapses promote the pruning of inactive ones by stimulating microglial phagocytosis with exosomes.

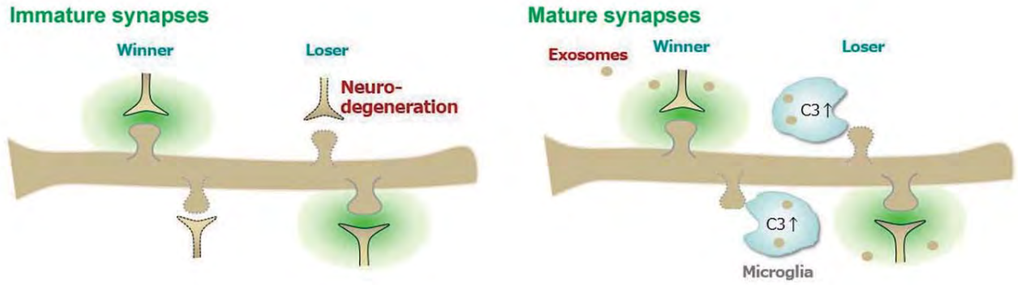


**Figure 1. Neuronal exosomes promote synaptic pruning by microglia**
A model proposed over two decades ago for how activity may drive synapse elimination suggested that strong synapses (winners), which are effective in driving postsynaptic responses, actively punish and eliminate nearby weaker synapses (losers). However, the entity of the "punishment" and the means whereby it promotes the synaptic pruning have not been identified. From our data, we propose a new model in which exosomes secreted from activated neurons act as the "punishment" signal for the weaker synapses (but preserving the stronger ones) by inducing the complement C3 in microglia for phagocytic clearance of the inappropriate synapses undergoing neurodegeneration.

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- Hanayama R. Autoimmune Diseases and the Role of MFG-E8. MFG-E8 and Inflammation. (Springer) 97-117, 2014.
- Toda S, Hanayama R, Nagata S. Two-step engulfment of apoptotic cells. Mol. Cell Biol. 32:118-25, 2012.
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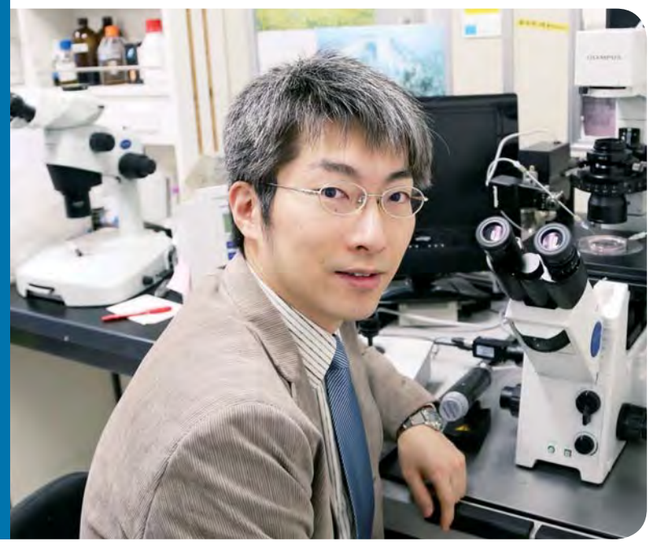
# Immunoparasitology



## Masahiro Yamamoto, PhD

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| Professor | **Masahiro Yamamoto** |
| Assistant Professor | **Miwa Sasai** |
| Research Assistant | **1** |
| Support Staff | **1** |

*Toxoplasma gondii* (*T. gondii*) is the causative agent of toxoplasmosis, a condition including life-threatening encephalitis, pneumonia and myocarditis in immuno-compromised individuals such as those suffering from acquired immunodeficiency syndrome and those being treated by chemotherapy. Furthermore, primary infection with this pathogen during pregnancy in humans and animals also leads to congenital diseases such as hydrocephalus and chorioretinitis in newborn children. *T. gondii* is an obligatory intracellular protozoan parasite and taxonomically belongs to the phylum Apicomplexa, which is defined by the presence of an apical complex including secretory organelles. Among them, the large bulb-shaped organelles rhoptries possess a number of proteins called ROPs, in which more than 40 members such as ROP5, ROP16, ROP18 and ROP38 harbor protein kinase domains. ROPs are secreted into the host cytoplasm during parasite invasion and eventually localize at the host nucleus or parasite-forming non-fusogenic vacuoles called parasitophorous vacuoles (PVs) to subvert and co-opt host cell functions.

Dense granules are another type of parasite secretory organelle that discharges GRA proteins (GRAs) into PVs that contain a network of elongated nanotubular structures. The membranes of nanotubules are connected by PV membranes, resulting in the formation of a large interface between host cell cytoplasm and parasite. Some GRAs, such as GRA3, 5, 7, 8, 10 and 14, have been shown to be located at the PV membranes. Conversely, GRA2, 4, 6, 9 and 12 are localized to the membrane of the nanotubule network. Among them, GRA2 and GRA6 play a central role in the formation and stabilization of the nanotubule network, respectively. In addition to being associated with the membranous interface between PVs and the host cytoplasm, two new GRA family members, GRA15 and GRA16, were recently shown to participate in the modulation of host cell functions. GRA15 is involved in NF-κB activation, which promotes the production of proinflammatory cytokines. The mode of action by which GRA15 activates NF-κB remains uncertain, however, it is dependent on a strong NF-κB activating signal transducer, TRAF6, but independent of the essential adaptors for Toll-like receptors, MyD88 and TRIF. GRA16 is secreted from dense granules and eventually exported to the host nucleus, where GRA16 interacts with the host deubiquitinase HAUSP and PP2A phosphatase, which regulate host cell cycle progression and the p53 tumor suppressor signaling pathway. Very recently, GRA24 is shown to modulate host immune responses by promoting p38 MAP kinase activation. Thus, GRAs, as well as ROPs, modulate host cell signaling pathways.

In this fiscal year, we report that the *T. gondii* polymorphic dense granule protein GRA6 regulates activation of the host transcription factor nuclear factor of activated T cells 4 (NFAT4). GRA6 overexpression robustly and selectively activated NFAT4 via calcium modulating ligand (CAMLG). Infection with wild-type, but not GRA6-deficient, parasites induced NFAT4 activation. Moreover, GRA6-deficient parasites failed to exhibit full virulence in local infection, and the treatment of wild-type mice with an NFAT inhibitor mitigated virulence of wild-type parasites. Notably, NFAT4-deficient mice displayed prolonged survival, decreased recruitment of $CD11b^+$ $Ly6G^+$ cells to the site of infection and impaired expression of chemokines such as Cxcl2 and Ccl2. In addition, infection with type I parasites culminated in significantly higher NFAT4 activation than type II parasites due to a polymorphism in the C-terminus of GRA6. Collectively, our data suggest that GRA6-dependent NFAT4 activation is required for *T. gondii* manipulation of host immune responses to maximize the parasite virulence in a strain-dependent manner.

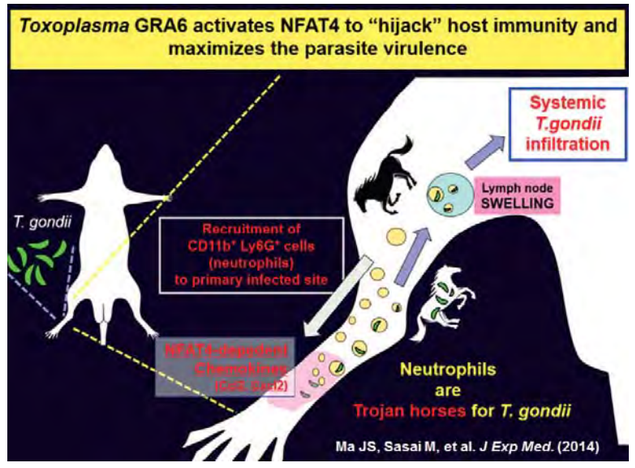


**Figure 1. A Toxoplasma virulence factor GRA6 specifically activates NFAT4 to recruit neutrophils to the infected sites to spread systemically**

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- Meunier E, Wallet P, Dreier RF, Costanzo S, Anton L, Rühl S, Dussurgey S, Dick MS, Kistner A, Rigard M, Degrandi D, Pfeffer K, Yamamoto M, Henry T, Broz P. Guanylate-binding proteins promote activation of the AIM2 inflammasome during infection with Francisella novicida. Nat. Immunol., 2015. in press.
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# Biochemistry and Immunology

## Shigekazu Nagata, PhD

| | |
|---|---|
| Professor | **Shigekazu Nagata** |
| Assistant Professor | **Jun Suzuki**<br>**Katsumori Segawa**<br>**Hiroshi Yamaguchi**<br>**Toshihiro Fujii** |
| Postdoctoral Fellow | **2** |
| Research Assistant | **4** |
| Visiting Scientist | **1** |
| Support Staff | **3** |

Apoptotic cells are swiftly engulfed by macrophages. If this process does not occur properly, materials released from dead cells activate the immune system leading to systemic lupus erythematosus-type autoimmune disease. Phospholipids in plasma membranes are asymmetrically distributed between inner and outer leaflets, and phosphatidylserine (PtdSer) is exclusively localized in the inner leaflet. The asymmetrical distribution of phospholipids is maintained by an ATP-dependent phospholipid translocase or flippase. When cells undergo apoptosis, the asymmetrical distribution of phospholipids is disrupted by scramblase, leading to PtdSer-exposure. The PtdSer exposed on dead cell surface is recognized by macrophages as an "eat me" signal. We are working on the molecular mechanism how PtdSer is exposed to the cell surface, and how macrophages recognize PtdSer for engulfment of dead cells. By establishing mice deficient in molecules involved in the PtdSer-exposure, and engulfment of apoptotic cells, we also study their physiological and pathological roles.

### Exposure of phosphatidylserine in apoptotic cells

We recently identified two membrane proteins (TMEM16F and Xkr8) as phospholipid scramblases, and a pair of proteins (ATP11C and CDC50A) as a flippase. TMEM16F, a protein with 8 transmembrane regions, requires $Ca^{2+}$ to support phospholipid scrambling, and plays an essential role in the PtdSer-exposure in activated platelets. Xkr8 carry 6 transmembrane regions, and caspases cleave off its C-terminal tail to promote the scramblase activity. ATP11C is a P4-type ATPase at plasma membrane, and CDC50A works as a chaperone to translocate ATP11C from endoplasmic reticulum to plasma membranes. ATP11C translocates PtdSer from outer to inner leaflets of plasma membranes in an ATP-dependent manner. When cells undergo apoptosis, ATP11C is inactivated by caspase-mediated cleavage. Thus, in addition to the caspase-mediated activation of scramblase, inactivation of flippase is required to expose PtdSer during apoptosis (Figure 1).

### Phosphatidylserine-dependent engulfment of apoptotic cells

Macrophages recognize PtdSer exposed on the surface of dead cells using specific receptors and opsonins. We found that mouse-resident peritoneal macrophages express a PtdSer receptor of Tim4, and a Tyrosine-kinase receptor of MerTK. Resident peritoneal macrophages efficiently engulf apoptotic cells in Tim4 and MerTK-dependent manner. *Tim4*-null macrophages exhibited reduced binding and engulfment of apoptotic cells, whereas *MerTK*-null macrophages efficiently bound apoptotic cells, but failed to engulf them. The incubation of wild-type peritoneal macrophages with apoptotic cells induced the rapid tyrosine phosphorylation of MerTK, which was not observed with *Tim4*-null macrophages. When mouse Ba/F3 cells were transformed with Tim4, apoptotic cells bound to the transformants, but were not engulfed. Transformation of Ba/F3 cells with MerTK had no effect on the binding or engulfment of apoptotic cells; however, Tim4/MerTK-transformants exhibited strong engulfment activity. These results indicate that the engulfment of apoptotic cells by resident peritoneal macrophages proceeds in two steps: binding to Tim4, a PtdSer-receptor, followed by MerTK-mediated cell engulfment (Figure 2).

### Enucleation and engulfment of pyrenocytes

In addition to apoptotic cells, pyrenocytes (nuclei expelled from erythroblasts) expose PtdSer, and engulfed by central macrophages in erythroblastic islands. We reconstituted the enucleation and engulfment of pyrenocytes with erythroblastic islands from phenylhydrazine-treated mouse spleens. We could show that as soon as pyrenocytes were separated from reticulocytes, pyrenocytes were engulfed by macrophages in a PtdSer-dependent manner. This process required MerTK, but not Tim4. We propose the following model for engulfment of pyrenocytes (Figure 3). Step 1; binding of erythroblasts to the central macrophages in erythroblastic islands through the interaction between integrin α4β1 on erythroblasts and Vcam1 on macrophages. Step 2; erythroblasts undergo enucleation, in which extracellular matrix promotes the separation of pyrenocytes from reticulocytes by providing shear stress, while it keeps the pyrenocytes in close proximity to the macrophage. Step 3; Pyrenocytes expose PtdSer, which is recognized by Protein S, a ligand for MerTK, in the serum. Apoptotic cells are then engulfed by macrophages in MerTK-dependent manner.

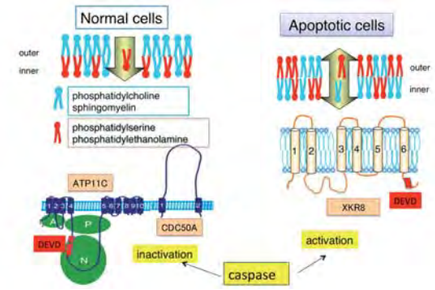


Figure 1. Regulation of PtdSer-exposure by flippase and scramblase

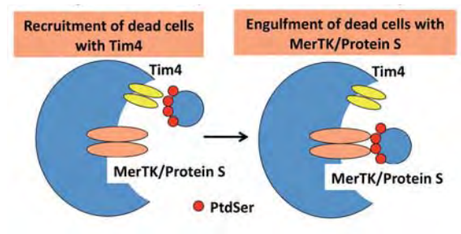


Figure 2. Two step-engulfment of apoptotic cells

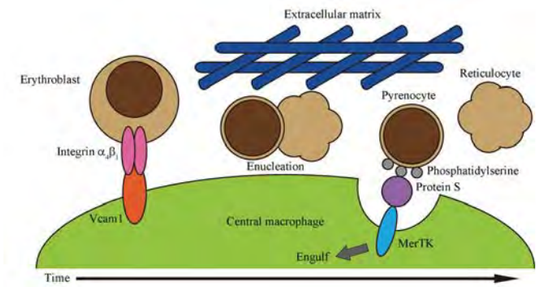


Figure 3. A model for enucleation and PtdSer-dependent engulfment of pyrenocytes

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# Single Molecule Imaging


## Toshio Yanagida, PhD

| | |
|---|---|
| Professor | **Toshio Yanagida** |
| Associate Professor | **Hideaki Fujita**<br>**Tomoyuki Yamaguchi**<br>**Yutaka Komai** |
| Postdoctoral Fellow | 1 |
| Research Assistant | 1 |
| Visiting Scientist | 2 |
| Support Staff | 1 |


### Counting activated T cells upon engagement with APC

To answer the question; is the activation of T cells deterministic in that a certain amount of stimuli above a threshold always results in the activation of T cells, or is the distribution of activation such that some cells are active while other cells are inactive against the same amount of stimuli? We counted the number of activated T cells upon engagement with APC by placing individual T cells on APC using a glass micropipette (Fig. 1A). Activation of the T cells was monitored by $Ca^{2+}$ indicator Rhod2 (Fig. 1B). When T cells from DO11.10 mouse were engaged with APC, 22% of T cells showed activation when OVA was present (Fig. 2A). However, 11% of T cells showed activation even when OVA was absent, indicating that activation of T cells is not deterministic (Fig. 2A). In addition, we found that the probability of activation increases when other T cells are attached to APC (Fig. 2B), indicating that T cell activation is influenced by APC status. These results indicate that activation of T cells may be more complex than previously thought where T cells have a broad activation threshold distribution and the status of APC has an influence on activation.

### Theoretical approach for understanding self/non-self discrimination by Treg cells

Immune system rapidly reacts against invasive pathogens, while it does not respond to self antigens. To react with a broad spectrum of foreign antigens, TCR recombination gives a high variety of specificity to an individual T cell. As consequence of this process, a substantial number of self-reactive T cells develop and a part of them survive even after depletion in the thymus. $Foxp3^+$ $CD4^+$ regulatory T cells (Treg), which consist of around 10% of $CD4^+$ T cells, play a crucial role to inhibit the activation of self-reactive T cells to maintain immunological tolerance. Although many mechanisms have been suggested for Treg-mediated suppression, it is still unclear how Treg cells robustly inhibit the activation of reactive T cells preserving potent reactivity of the T cells against foreign antigens.

With theoretical studies, we show that Treg enhance cell-interactions to stabilize the tolerant state. Theoretical modeling showed that T cell association to antigen-presenting cells (APC) is crucial to maintain stable unresponsiveness in addition to inhibition of activation-signaling when ligands for stimulation are limited and competed for among T cells. To inhibit T cell proliferation robustly in the presence of specific Treg even in the case where individual T cells behave in a stochastic way, Treg were required to inhibit two processes; dissociation and activation. Notably, the optimized model to discriminate reactivity under self and non-self conditions well reproduced the proliferation patterns of T cells under various conditions examined in in vitro experiments. Based on the prediction of the theoretical model, we found how to manipulate immune response in vivo. Transient reduction of T cell number enhanced the proliferation of antigen specific T cells in the draining lymph nodes.

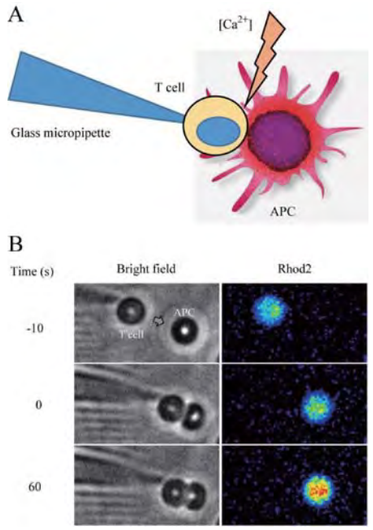


**Figure 1. Counting T cell activation upon engagement with APC**
(A) Rhod2 stained T cells were held with glass micro-needle and placed on APC and the fluorescence was monitored. (B) Bright field (left) and fluorescence (right) image of experimental procedure.

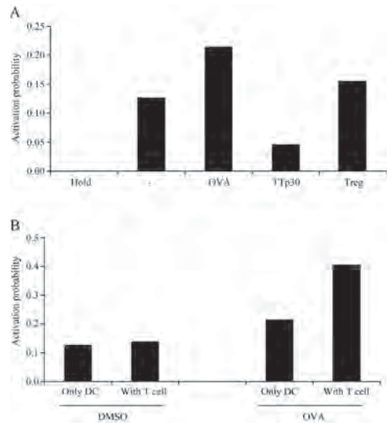


**Figure 2. Probability of $Ca^{2+}$ increase upon contact with APC**
(A) T cell from DO11.10 mouse was engaged with APC in the absence and presence of OVA. (B) Probability of $Ca^{2+}$ increase was compared between APC with or without other T cells.

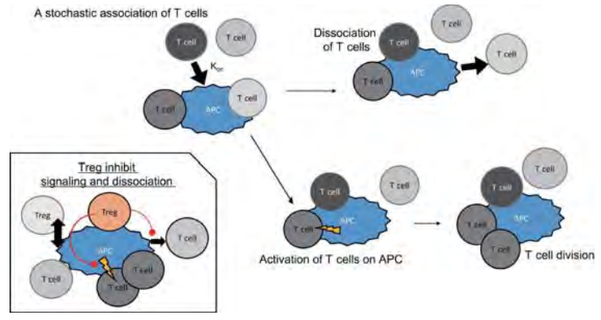


**Figure 3. Schematic illustration of simulation model**
**Treg inhibit dissociation and activation of T cells on APC.**

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# Biofunctional Imaging

## Yoshichika Yoshioka, PhD

| | |
|---|---|
| Professor | **Yoshichika Yoshioka** |
| Assistant Professor | **Chizuko Inui**<br>**Yuki Mori**<br>**Fuminori Sugihara** |
| Research Assistant | **1** |
| Visiting Scientist | **6** |
| Support Staff | **2** |

Our group has developed highly sensitive and specific in vivo visualization techniques with magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) to non-invasively visualize the dynamic immune responses. The technique could be used to obtain images and spectra of the same mouse repeatedly over time, and precise information, which is obscured by individual differences, could be obtained. Technical developments and refinements are important and necessary to obtain fine images and spectra (information).

MRI in particular offers a significant advantage in imaging deep regions with good spatial resolution and tissue contrast. A stronger magnetic field can be applied to increase the signal to noise ratio (SNR) of in vivo MRI. In addition, super-paramagnetic nanoparticles of iron oxide (SPIO), a contrast agent for MRI, improves MRI contrast to noise ratio (CNR) and detectability in the stronger magnetic fields by shortening the $T_2/T_2^*$ relaxation times. Combining high magnetic-field strength with high-sensitivity radio-frequency (RF) coils and optimal contrast agents will enable the visualization of cell populations and molecular events in vivo in both animals and humans.

Although immune cells may help to maintain a neural environment, the non-invasive visualization of immune cell dynamics in the central nervous system (CNS) at pathological as well as normal conditions is not easy with in vivo imaging techniques. Our group has succeeded in non-invasive in vivo visualization of the immune cells in the CNS and showed the recruitment of peripheral endogenous immune cells into the CNS even at the normal condition (Mori et al. Sci Rep 2014).

### Non-invasive detection of immune cells in live mouse brain at the single cell level

We used an 11.7 T high-field MRI scanner in combination with a high-sensitive coil and SPIO to detect and monitor peripheral immune cell migration into healthy and lipopolysaccharide (LPS)-treated mouse brains at the single-cell level without surgical invasion (Figure 1). Phagocytes were labeled in vivo by IV injection of SPIO (in vivo labeling). After administration of SPIO, $T_2$*-weighted MRI showed tiny and non-specific hypo intense spots (at 1 and 2 days) even in control mouse brain tissues. Almost all spots disappeared by 7 days (d, h). Significantly greater spot counts were found in LPS-treated brains at 1 and 2 days than in controls (i). These dark spots were endogenously labeled cells by SPIO. The existence of labeled macrophages in the brain parenchyma was confirmed histologically with brain tissues fixed just after MRI experiments (l, m, n).

### Non-invasive immune cell tracking in live mouse brain at the single cell level: Time-lapse MRI

Non-invasive real time single cell tracking in animals as well as in humans has been a challenging theme of in vivo imaging. We have tried to visualize the immune cell movements at deep regions by MRI. Figure 2 shows the result of our time-lapse MRI in a mouse brain. We labeled phagocytes (almost macrophages) by our in vivo labeling method with SPIO. The same mouse brain images were obtained with time from 12 to 48 h after SPIO administration. The time interval in this case was 20 min. The slice thickness is 300 μm. Figure 2 shows MR images of the same slice from 30 to 36 h post SPIO administration. Although many spots remained stationary like as in Figure 1 (e.g. blue circle), many small dark spots, that is macrophages, migrated along the visible sites of blood vessels (e.g. green arrowhead). Many other cells appeared and migrated to another location (e.g. yellow arrowhead). We succeeded in the non-invasive in vivo visualization of the immune cell movements in the CNS. Our time-lapse MRI movie is available at the following site. The moving cells we visualized are almost macrophages interacting with endothelial cells of blood vessels. (http://www.nature.com/srep/2014/141111/srep06997/extref/srep06997-s2.mov).

### Diverse information

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

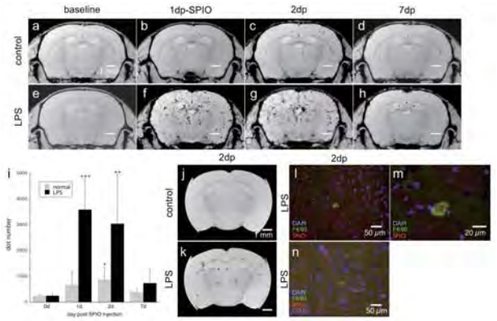


**Figure 1.**
**(a–h), Representative 300 μm thickness MRI of mouse brain at each time point: before (a, e), 1 day (b, f), 2 days (c, g), and 7 days (d, h) post SPIO administration. The upper row (a–d) is the same control mouse brain; the lower row (e–h) the same lipopolysaccharide (LPS)-treated mouse brain. The scale bar is 1 mm. (i), Quantification of spots in the whole brain at each time point. (j), (k), Ex vivo MRI after perfusion fixation. These images show hypo intense spots of the normal (j) and LPS-treated groups (k). (l), The histological image shows co-localization of fluorescent dye-cross-linked SPIO and F4/80⁺ cells in the brain of an LPS-treated mouse. SPIO itself and SPIO-labelled cells were rarely found in control normal brains (m). (n), SPIO-labelled cells were separated from blood vessels.**

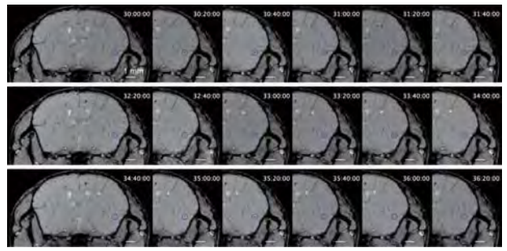

**Figure 2.**
**Representative 300 μm thickness MRI of the same position in the same mouse brain at different time points. MR images of from 30 to 36 h post SPIO administration are shown. These images show SPIO-labelled cells as $T_2$* hypo intense spots. Although many spots remained stationary (blue circle), many motile cells migrated along the visible sites of blood vessels (green arrowhead). Another cell appeared and migrated to another location (yellow arrowhead). Our movie is available at the following site.**
**(Link to our time-lapse MRI: http://www.nature.com/srep/2014/141111/srep06997/extref/srep06997-s2.mov).**

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- Mori Y, Chen T, Fujisawa T, Kobashi S, Ohno K, Yoshida S, Tago Y, Komai Y, Hata Y, Yoshioka Y. From cartoon to real time MRI: in vivo monitoring of phagocyte migration in mouse brain. Sci. Rep. 4:6997, 2014.
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# Immunology and Cell Biology

## Masaru Ishii, MD/PhD

| | |
|---|---|
| Professor | **Masaru Ishii** |
| Assistant Professor | **Junichi Kikuta**<br>**Keizo Nishikawa**<br>**Hiroki Mizuno**<br>**Szandor Simmons** |
| Postdoctoral Fellow | **1** |
| Research Assistant | **2** |
| Visiting Scientist | **1** |
| Support Staff | **2** |

The mission of our laboratory is to understand the fundamental principle controlling cellular dynamics in various kinds of tissues and organs in vivo, by means of our advanced imaging techniques. Recent advances in optical imaging technology have enabled us to visualize the dynamic nature of different cell types in a time-dependent manner, in addition to the spatial and structural information. We are now elucidating the dynamic systems in bone biology and immunology as well as biological science such as hematopoiesis and cell lineage commitment and other 'niches', cell dynamics during inflammation in adipose tissue, skin, lung, liver and intestines (see the Figure).

### 1. Intravital bone imaging revealed osteoclast dynamics in vivo

By using intravital multiphoton microscopy, our lab has originally elaborated the novel imaging system for visualizing inside the bones. We have first elucidated the in vivo behaviors of osteoclasts, bone-destroying special macrophages resident in bones, i.e., the migration and positioning of their precursor macrophages, their mode of bone-resorbing function in vivo and the functional and physical coupling with bone-reforming osteoblasts.

By utilizing this methodology, we showed that sphingosine-1-phosphate (S1P) controls the migratory behavior of osteoclast precursors in concert with various chemokines (Nature 2009; J Exp Med 2010). We also showed the substantial contribution of S1P-mediated migratory control of bone cells by S1P, by generating knockout mice deficient for endogenous S1P transporter (J Clin Invest 2012). Moreover, we demonstrated that vitamin D, which is well-known as a bone-protecting factor, significantly blocks bone destruction by modulating S1P-mediated migration control of osteoclast precursor monocytes (Proc Natl Acad Sci USA 2013). Based on a series of studies, we proposed a new concept in which the migration and positioning of osteoclast precursor monocytes on the sites to be resorbed are critical points of action in the regulation of bone destruction.

By improving bone imaging system, we 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.

### 2. DNA methylation regulates osteoclastogenesis

Metabolic reprogramming occurs in response to the cellular environment to mediate differentiation, but the fundamental mechanisms linking metabolic processes to differentiation programs remain to be elucidated. During osteoclast differentiation, a shift toward more oxidative metabolic processes occurs. We identified the *de novo* DNA methyltransferase 3a (Dnmt3a) as a transcription factor that couples these metabolic changes to osteoclast differentiation. We also found that RANKL induces this metabolic shift towards oxidative metabolism, which is accompanied by an increase in S-adenosylmethionine (SAM) production. We found that SAM-mediated DNA methylation by Dnmt3a regulates osteoclastogenesis via epigenetic repression of anti-osteoclastogenic genes. Dnmt3a-deficient osteoclast precursor cells do not differentiate efficiently into osteoclasts and that mice with an osteoclast-specific deficiency in Dnmt3a have elevated bone mass due to a smaller number of osteoclasts. Furthermore, inhibition of DNA methylation by theaflavin-3,3′ -digallate abrogated bone loss in models of osteoporosis. Thus, we revealed the role of epigenetic processes in the regulation of cellular metabolism and differentiation, which may provide the molecular basis for a new therapeutic strategy for a variety of bone disorders (Nat Med 2015).

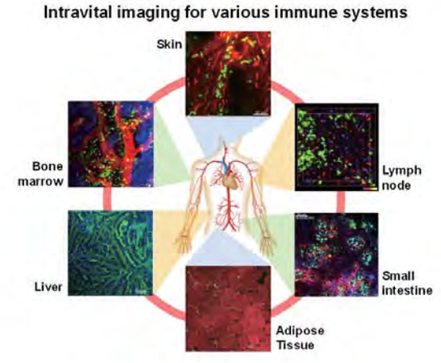


**Figure. Intravital imaging for various immune systems**
**Immune cells are high dynamic and interconnecting various tissues and organs, by forming a 'soft-wired' network. We are elucidating the basic principle controlling the dynamic nature of immune cells by visualizing in vivo behaviors with advanced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

### 3. Visualized macrophage dynamics and significance of S100A8 in obese fat

Chronic low-grade inflammation of adipose tissue plays a crucial role for the pathophysiology of obesity. Infiltration of several immune cells such as macrophages into adipose tissue was observed in obesity, although the initial factors triggering their migration have not been elucidated. By using intravital multiphoton imaging technique, we analyzed the detailed time-courses of inflammatory processes in adipose tissues under high-fat and high-sucrose (HF/HS) diet. Mobility of macrophages was shown to be activated just 5 days after HF/HS diet, when the distinct hypertrophy of adipocytes and the accumulation of macrophages have not still become prominent. Significant increase of S100A8 was detected in mature adipocyte fraction just 5 days after HF/HS diet. Recombinant S100A8 stimulated chemotactic migration both in vitro and in vivo, as well as induced pro-inflammatory molecules both macrophage and adipocytes, such as TNF-α and CCL2. Finally, a neutralizing antibody targeting S100A8 efficiently suppressed the HF/HS diet-induced initial inflammatory change, i.e., increased mobilization of adipose macrophages. In conclusion, time-lapse intravital multiphoton imaging of adipose tissues first identified the very early event exhibiting increased mobility of macrophages, which may be triggered by increased expression of S100A8 and resultant to progression of chronic inflammation *in situ* (Proc Natl Acad Sci USA 2015).

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- Nishikawa K, et al. Dnmt3a regulates osteoclast differentiation by coupling to an S-adenosyl methionine-producing metabolic pathway. Nat. Med. 21:281-7, 2015.
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# Nuclear Medicine

## Jun Hatazawa, MD/PhD

| | |
|---|---|
| Professor | Jun Hatazawa |
| Research Assistant | 3 |
| Support Staff | 1 |

Nuclear medicine is a field of great potential, for evaluating the in-vivo dynamic imaging of 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 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.

### Monitoring antiangiogenic therapy using $^{15}O$-$H_2O$ PET

In addition to these researches, we began the measurement of blood flow of non-small cell lung cancer (NSCLC) before and after chemotherapy with antiangiogenic agent bevacizumab (BEV), a humanized monoclonal antibody targeting circulating vascular endothelial growth factor, using $^{15}O$-$H_2O$ PET. BEV has been reported to affect tumor blood flow. However, the relationship between tumor blood flow change after BEV and the prognosis is unclear in patients with lung cancer. We found that mean tumor blood flow decreased within 1-2 days after administration of BEV. Individual differences in tumor blood flow change after BEV were large and large blood flow decrease was associated with rapidly progressing tumors. The addition of BEV was reported to increase overall and progression free survival compared with chemotherapy alone in advanced NSCLC. In the present study, the antiangeonenic therapy did not have benefit for patients with tumor blood flow decrease after BEV. In this patient subgroup BEV therapy should be reconsidered after first administration based on the PET study.

### Evaluation of pharmacokinetics using micro-dose PET

Micro-dose PET is a technique to investigate the whole body bio-distribution of $^{11}C$ or $^{18}F$ labeled drug using PET, which enable the in-vivo evaluation of the pharmacokinetics of each organ.

Donepezil is an acetylcholinesterase (AChE) inhibitor, which is used as a treatment drug for Alzheimer's disease. However, its pharmacokinetics in non-target organs other than the brain has not been clarified yet. We evaluated the time course of whole body distribution in rats using $^{11}C$-labeled donepezil hydrochloride (DNP) PET. We also evaluated the AchE activity in homogenized tissue solutions of the major organs using a fluorometric assay. As a result, high uptake in the adrenal gland, a non-target organ, was observed from an early stage after administration. The AChE activity was the third highest in the adrenal glands (following the small intestine and the stomach), indicating high activity of AChE in the adrenal glands. High accumulation of $^{11}C$-DNP in the adrenal glands suggested the risk of enhanced cholinergic synaptic transmission by the use of AChE inhibitors.

### Monitoring astrocytic metabolism using $^{11}C$-acetate PET in patients with multiple sclerosis

Activation of glial cells is a cardinal feature in multiple sclerosis (MS) pathology, and acetate has been reported to be selectively uptaken by astrocytes in the central nervous system. We investigated the efficacy of PET with $^{11}C$-acetate for MS diagnosis. The uptake of $^{11}C$-acetate was increased in both white and gray matter in MS patients compared to healthy volunteers. The number of MS lesions detected by MRI significantly correlated with the uptake of $^{11}C$-acetate in white and gray matter, which suggests that $^{11}C$-acetate PET can be a useful clinical examination for MS patients.

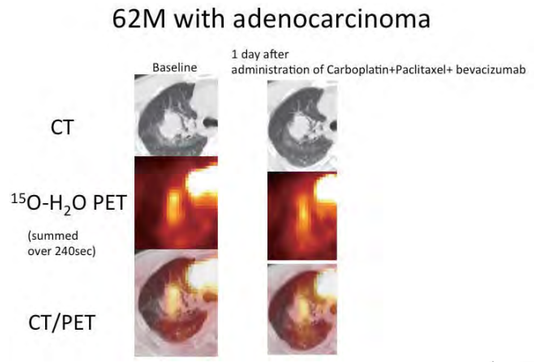



**Figure 1.**
**$^{15}O$-$H_2O$ PET/CT images of 62-year-old male with advanced adenocarcinoma of lung before/after administration of antiangiogenic agent bevacizumab (BEV). The tumor blood flow decreased 1 day after administration of BEV.**

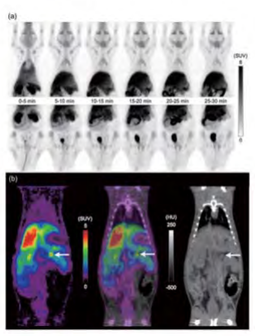


**Figure 2.**
**Whole body images after the administration of $^{11}C$-DNP: (a) dynamic maximum intensity projection images of PET, (b) coronal images of PET, CT, and PET/CT (20–40 min, arrow: left adrenal gland).**

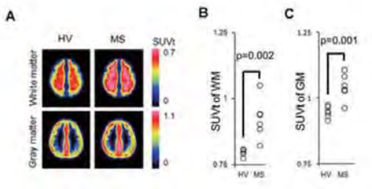



**Figure 3.**
**(A)Spatially normalized group mean images of $^{11}C$-acetate standardized uptake value relative to that in the bilateral thalami (SUVt) automatically segmented based on MRI. Volume of interest analysis summarizing the mean SUVt in white matter (B) and gray matter (C).**

## Recent Publications


- Hanaoka K, Watabe T, Naka S, Kanai Y, Ikeda H, Horitsugi G, Kato H, Isohashi K, Shimosegawa E, Hatazawa J. FBPA PET in boron neutron capture therapy for cancer: prediction of 10B concentration in the tumor and normal tissue in a rat xenograft model. EJNMMI Res. 4(1):70, eCollection, 2014.
- #Takata K, #Kato H, Shimosegawa E, Okuno T, Koda T, Sugimoto T, Mochizuki H, Hatazawa J, Nakatsuji Y. 11C-Acetate PET Imaging in Patients with Multiple Sclerosis. PLoS One. 9(11):e111598, 2014. # Equal contribution
- Watabe T, Naka S, Ikeda H, Horitsugi G, Kanai Y, Isohashi K, Ishibashi M, Kato H, Shimosegawa E, Watabe H, Hatazawa J. Distribution of intravenously administered acetylcholinesterase inhibitor and acetylcholinesterase activity in the adrenal gland: 11C-donepezil PET study in the normal rat. PLoS One. 9(9):e107427, 2014.
- Tamura M, Matsui H, Hirohara S, Kakiuchi K, Tanihara M, Takahashi N, Nakai K, Kanai Y, Watabe H, Hatazawa J. Selective accumulation of [$^{62}Zn$]-labeled glycoconjugated porphyrins as multi-functional positron emission tomography tracers in cancer cells. Bioorg Med Chem. 22(8):2563-70, 2014.
- Yamamoto S, Watabe H, Kanai Y, Kato K, Hatazawa J. Development of a high-resolution YSO gamma camera system that employs 0.8-mm pixels. Ann. Nucl. Med. (3):232-40, 2014.

# Chemical Imaging Techniques


## Kazuya Kikuchi, PhD

| | |
|---|---|
| Professor | **Kazuya Kikuchi** |
| Associate Professor | **Shin Mizukami** |
| Assistant Professor | **Yuichiro Hori** |
| | **Toshiyuki Kowada** |
| | **Yuko Kamikawa** |
| Support Staff | **1** |


### Activatable $^{19}$F MRI Nanoparticle Probes for the Detection of Reducing Environments

Magnetic Resonance Imaging (MRI) has received considerable interest because of its prominent properties such as deep tissue imaging and high spatial resolution. $^{19}$F MRI probes can detect biological phenomena such as cell dynamics, ion concentrations, and enzymatic activity without the endogenous background signals. We have developed a highly sensitive $^{19}$F MRI contrast agent comprised of a perfluoro[15]crown-5-ether (PFCE) core and a silica shell, termed FLAME (Matsushita, H. et al. Angew. Chem. Int. Ed. 53, 1008–1011, 2014). Although perfluorocarbon (PFC) encapsulated nanoparticles are of interest in $^{19}$F MRI imaging owing to their high sensitivity, activatable PFC nanoparticles have not been developed.

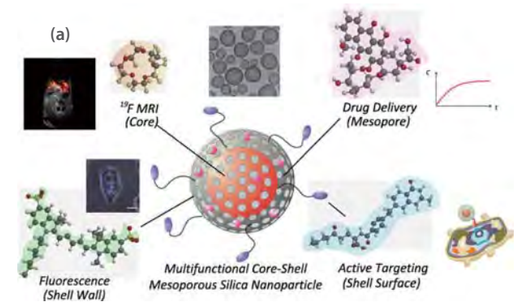
The facile surface modifications on FLAME enabled the introduction of the paramagnetic relaxation enhancement (PRE) effect of $Ln^{3+}$ complexes to create an OFF/ON switching ability. The PRE effect for transverse relaxation (*T2*) modulation is effective over short distances because of its $r^{-6}$ dependency, where *r* is the distance between nuclei observable by NMR spectroscopy and a paramagnetic center. On the basis of the PRE effect, we developed an activatable PFC-encapsulated nanoparticle probe, FLAME-SS-$Gd^{3+}$ (FSG) by introducing a reduction-responsive linker between FLAME and the surface-modified $Gd^{3+}$ complexes. When the disulfide of FSG was reduced, the $Gd^{3+}$ complexes were cleaved from the FLAME surface, resulting in the elongation of $T_2$ of the encapsulated PFCE and the increase of $^{19}$F NMR/MRI signal intensity.

We prepared three types of FSGs with different amount of surface $Gd^{3+}$ complexes. We found that addition of reducing agents made the $^{19}$F NMR peaks of FSGs sharper and taller as compared to those before the addition. The $T_2$ values of FSGs were also significantly increased upon addition of reducing agents. Calculations revealed that the ratio of fluorine atoms to $Gd^{3+}$ complexes per nanoparticle was more than approximately $5.0\times10^2$, resulting in the high signal amplification. FSGs are the first example of activatable $^{19}$F MRI nanoparticle probes and would be promising for further applications as in vivo imaging probes.

### Multifunctional Mesoporous Silica Nanoparticles for $^{19}$F Magnetic Resonance Imaging, Fluorescence Imaging, and Drug Delivery

Efficient delivery of drugs to diseased tissues is a major goal in the field of drug delivery in an effort to reduce adverse effects. Mesoporous silica nanoparticles (MSNs) are attractive drug carriers owing to their favorable properties such as extremely large surface areas, tunable pore sizes, and ease of functionalization via various synthetic approaches. To assess the drug efficacy and toxicity of drug carriers, it is essential to monitor the localization of the drug carrier. In particular, multimodal imaging techniques with near infrared (NIR) and magnetic resonance imaging (MRI) have gained attention because the combination of NIR and MRI provides detailed information regarding deep tissues and cell localization. Therefore, MSNs that can be traced via multiple imaging techniques are highly desired.

In this study, we developed a novel drug delivery carrier based on MSNs, mFLAME, which encapsulated highly sensitive $^{19}$F MRI contrast agents inside MSNs. The nanoparticles were labeled with fluorescent dyes and functionalized with small molecule-based ligands for active targeting, which enables both dual modal imaging (NIR/MRI) and drug delivery. By conjugating mFLAME with folate, the uptake of the nanoparticles into folate receptor positive-KB cells was successfully visualized using confocal laser scanning microscopy and $^{19}$F MR imaging. Furthermore, we demonstrated that drug-loaded mFLAMEs show efficient release capacities and cytotoxicity in KB cells after folate receptor-mediated uptake of the nanoparticles. Our results suggested that MSNs can serve as promising $^{19}$F MRI-traceable drug carriers for application in cancer therapy and bioimaging.



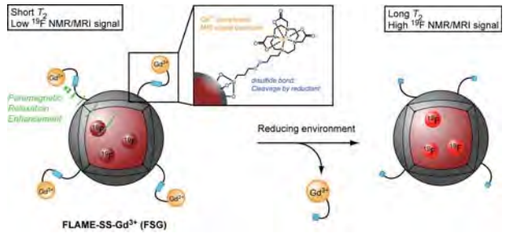




**Figure 1. Design of activatable $^{19}$F MRI probe, FLAME-SS-$Gd^{3+}$**

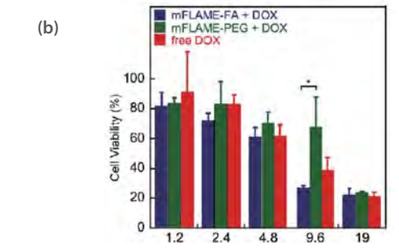



**Figure 2.**
**(a) Design of mFLAME (b) Concentration-dependent cell viability of folate receptor positive-KB cells treated with free DOX or DOX-loaded mFLAME for 1 day**

## Recent Publications


- Nakamura T, Matsushita H, Sugihara F, Yoshioka Y, Mizukami S, Kikuchi K. Activatable $^{19}$F MRI nanoparticle probes for the detection of reducing environments. Angew. Chem. Int. Ed. 54:1007–10, 2015.
- Nakamura T, Sugihara F, Matsushita H, Yoshioka Y, Mizukami S, Kikuchi K. Mesoporous silica nanoparticles for $^{19}$F magnetic resonance imaging, fluorescence imaging, and drug delivery. Chem. Sci. 6:1986–90, 2015.
- Okada S, Mizukami S, Sakata T, Matsumura M, Yoshioka Y, Kikuchi K. Ratiometric MRI sensors based on core-shell nanoparticles for quantitative pH imaging. Adv. Mater. 26:2989–92, 2014.
- Matsushita H, Mizukami S, Sugihara F, Nakanishi Y, Yoshioka Y, Kikuchi K. Multifunctional core-shell silica nanoparticles for highly sensitive $^{19}$F magnetic resonance imaging. Angew. Chem. Int. Ed. 53:1008–11, 2014.
- Hori Y, Norinobu T, Sato M, Arita K, Shirakawa M, Kikuchi K. Development of fluorogenic probes for quick no-wash live-cell imaging of intracellular proteins. J. Am. Chem. Soc. 135:12360–5, 2013.

# Biophotonics

## Nicholas Isaac Smith, PhD

| | |
|---|---|
| Associate Professor | **Nicholas Isaac Smith** |
| Assistant Professor | **Alison Jane Hobro** |
| Postdoctoral Fellow | **1** |

The biophotonics lab uses label-free imaging tools to study the dynamics of different cellular responses. We employ Raman spectroscopic imaging and analysis to observe these changes. The results show the chemical distribution inside cells, and can be used to track the redistribution of molecules in the cells. We are particularly interested in how label-free tools can provide new information to study disease progression and the immune response. Such new information can manifest as the appearance of new chemical components groups in the cell, or in how cell morphology changes during the response.

Unlike most types of biological imaging, which use labels, Raman imaging produces contrast based only on the inherent molecular contrast in the cell, where each vibrational bond in a molecule adds up to produce a final output signal composed of the whole ensemble of Raman-active bonds. This makes the method quite different to most of the tools employed by biologists. Rather than a specific target, we view ensemble changes in the cells, and these can be used to provide information on the cell type, activation status, or disease state.

After having built up custom imaging systems and analytic methods that are optimized for live-cell, high resolution and/or high-throughput measurements, depending on the application, we are now employing these techniques in a number of applications. Our first target was the diagnosis of malarial infection, where we used the properties of heme aggregation to acquire a spectral signature of the presence of malaria (Hobro et al. 2013). In collaboration with the Coban lab, this led to a method of disease diagnosis that could, in a mouse model of malaria, detect the presence of the parasite as early as one day following infection. For effective treatment, early detection is of paramount importance. We have now extended this analysis to include tracking of the reorganization of macrophages in response to the uptake of the malarial byproduct hemozoin. The macrophages were shown to undergo morphological changes with the formation of distinct spectral groups, and two types of hemozoin components were observed to be treated in different spatial locations inside the cell (Hobro et al. 2015). These results may help understand some of the adjuvant nature of the hemozoin particles, where slight differences in the stimulating particle may result in quite different responses in the immune system.

We created a custom imaging method based on two different but simultaneous label-free imaging modes that can provide complementary information on the cell response (Pavillon et al. 2014, Pavillon et al. 2013, Pavillon et al. 2015). This provides rapid and quantitative phase data, at speeds higher than what is possible with normal Raman imaging at the same time as the Raman data is acquired. The phase mode provides rapid quantitative spatial data, and the Raman mode provides chemical specificity. The additional mode allows the phase imaging to be used to map the morphology, while the Raman signature can be acquired from a single point, if necessary, rather than using a full Raman imaging mode. This is now allowing much higher throughput measurements of lymphocytes, and can allow Raman measurement in a cytometry-like mode while retaining the spatial information from the phase.

We also continued projects using nanoparticle enhancement of Raman signals. One typical difficulty in using nanoparticles in cells is the lack of control over the particle location. Leaving it up to chance as to whether a particle is in the region of interest or not adds significant limits to intracellular Raman imaging by nanoparticles. A notable finding was the use of laser light to fabricate gold nanostructures directly in the cell, including in locations where particles do not normally enter, such as nuclear structures, allowing enhanced molecular measurements from the fabricated structures (Smith et al. 2015)

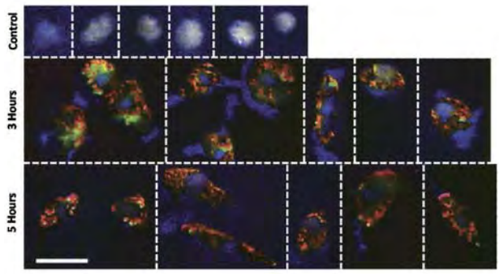



**Figure 1. Macrophage uptake of hemozoin crystals**
**The hemozoin occupies its own Raman spectral group (red channel) but interestingly, the presence of hemozoin at 3 and 5 hours forces other spectral components to emerge (green, yellow, blue) compared to the much more homogenous states in the control cells.**

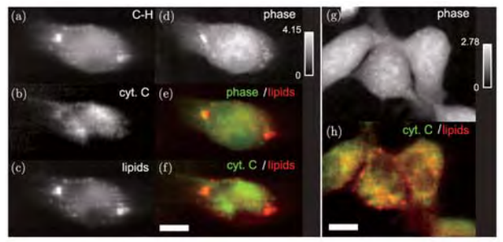



**Figure 2. Simultaneous quantitative phase and Raman imaging of macrophages**
**While based on different optical scattering processes, the phase images (d,g) look similar to the Raman data (a-c,e,f,h). This allows an increased reliance on the phase imaging mode, which can be orders of magnitude faster than the Raman, while chemical selectivity is still provided from the Raman data.**

## Recent Publications


- Hobro AJ, Pavillon N, Fujita K, Ozkan M, Coban C, Smith NI. Label-free Raman imaging of the macrophage response to the malaria pigment hemozoin. Analyst 140:2350-9, 2015.
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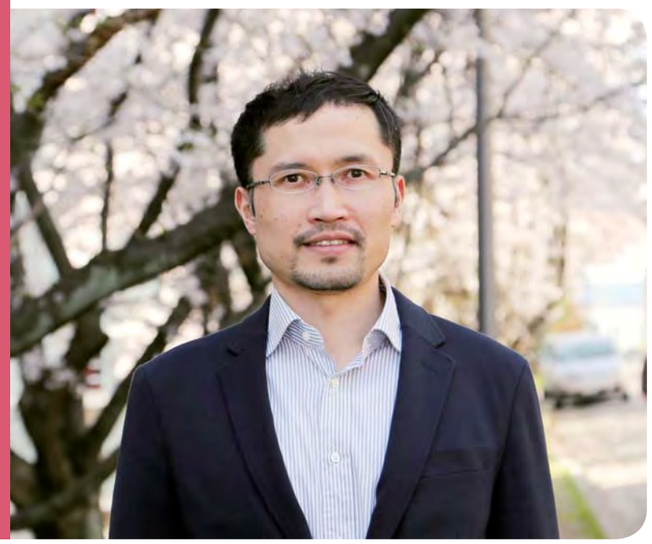
- Pissuwan D, Hobro AJ, Pavillon N, Smith NI. Distribution of label free cationic polymer-coated gold nanorods in live macrophage cells reveals formation groups of intracellular SERS signals of probe nanoparticles. RSC Adv. 4:5536-41, 2014.
- Pavillon N, Hobro AJ, Smith NI. Cell Optical Density and Molecular Composition Revealed by Simultaneous Multimodal Label-Free Imaging. Biophys. J. 105:1123-32, 2013.
- Hobro AJ, Standley DM, Ahmad S, Smith NI. Deconstructing RNA: optical measurement of composition and structure. Phys. Chem. Chem. Phys. 15: 13199-208, 2013.

# Immune Response Dynamics




## Kazuhiro Suzuki, MD/PhD

| | |
|---|---|
| Associate Professor | **Kazuhiro Suzuki** |
| Research Assistant | **1** |
| Support Staff | **2** |


Our body consists of multiple organ systems which mutually communicate to coordinate responses to external stimuli and maintain homeostasis of internal environments. Thus, to understand biological events occurring in complex organ systems in our body, it is important to reveal the interconnection among multiple organ systems rather than focus on an isolated organ system. Keeping this notion in mind, we have been investigating the communication between the nervous and immune systems since we set up the lab in 2011. As the proverb "Illness starts in mind." says, it has long been proposed that various aspects of immune responses are regulated by the nervous system. Indeed, the autonomic nervous system was shown to modulate the pathology of immune disorders, including rheumatoid arthritis and multiple sclerosis (Bellinger, et al. *Cell. Immunol.* 252: 27, 2008). Lymphoid organs are innervated by adrenergic, cholinergic and other neurons, and immune cells express corresponding neurotransmitter receptors, of which stimulation affects a broad range of immune cell activities, including proliferation, cytokine production and migration (Tracey. *Annu. Rev. Immunol.* 30: 313, 2012). However, little is known about how neuronal inputs are converted to the outputs from the immune system.

Precise trafficking and positioning of immune cells are essential for homeostasis of the immune system and induction of immune responses, most of which is orchestrated by a family of G protein-coupled receptors (GPCRs) that respond to chemoattractive molecules represented by chemokines. As well as chemokine receptors, many of the neurotransmitter receptors are also GPCRs. A recent study showed that different types of GPCRs form heteromeric complexes on the cell surface and cross-regulate their signals (Fribourg, et al. *Cell* 147: 1011, 2011). This observation prompted us to investigate the relationship between neurotransmitter and chemokine receptors. We found that $\beta_2$-adrenergic receptors, which are abundantly expressed on lymphocytes compared with other types of adrenergic receptors, form complexes with chemokine receptors CCR7 and CXCR4. Stimulation of $\beta_2$-adrenergic receptors on lymphocytes selectively enhanced the responsiveness of these chemokine receptors. Given these observations, we tested the role of $\beta_2$-adrenergic receptors in controlling lymphocyte trafficking and found that inputs through $\beta_2$-adrenergic receptors, a substantial part of which is provided by adrenergic nerves, inhibit lymphocyte egress from lymph nodes by augmenting retention-promoting signals mediated by CCR7 and CXCR4. Moreover, in mouse models of inflammatory diseases, including multiple sclerosis and allergic dermatitis, activation of $\beta_2$-adrenergic receptors inhibited lymph node egress of pathogenic lymphocytes and prevented their migration to target organs, leading to attenuated inflammation in the tissues (Nakai, et al. *J. Exp. Med.* 211: 2583, 2014).

These findings established the novel cellular and molecular basis by which adrenergic nerves control the immune system. Our study implies how stress or emotional changes are reflected on immune functions through adrenergic nerves and provides a rationale for developing therapeutic strategies against immune disorders by which control stress responses. At this moment, however, the mechanism for the crosstalk between $\beta_2$-adrenergic receptors and chemokine receptors is unclear. Additionally, the real picture of the interaction between adrenergic nerves and lymphocytes remains to be visualized. More important question is what is physiological significance of the adrenergic control of lymphocyte trafficking. We are going to address these questions in our future studies, and move a step closer to comprehensive understanding of immune regulation by the nervous system.

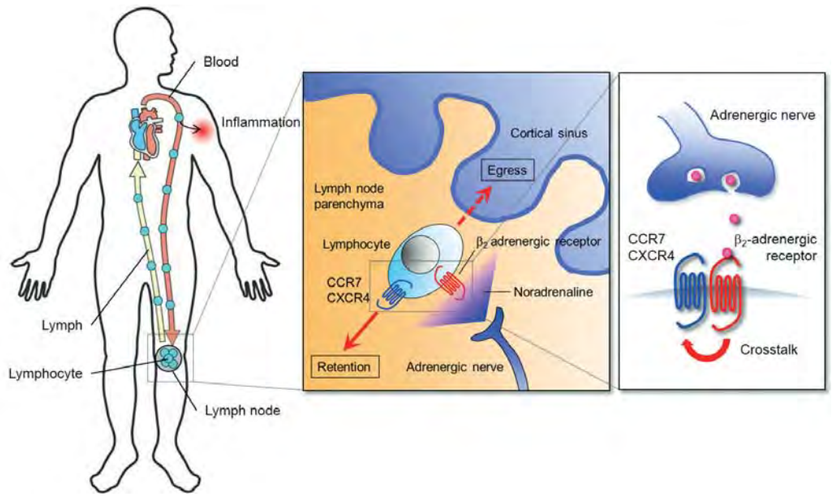



**Figure 1. Adrenergic control of lymphocyte dynamics**
**Activation of $\beta_2$-adrenergic receptors expressed on lymphocytes inhibits their egress from lymph nodes by enhancing retention-promoting signals mediated by chemokine receptors, CCR7 and CXCR4.**

## Recent Publications


- Nakai A, Hayano Y, Furuta F, Noda M, Suzuki K. Control of lymphocyte egress from lymph nodes through $\beta_2$-adrenergic receptors. J. Exp. Med. 211:2583-98, 2014.
- Gray EE, Friend S, Suzuki K, Phan TG, Cyster JG. Subcapsular sinus macrophage fragmentation and CD169$^+$ bleb acquisition by closely associated IL-17-committed innate-like lymphocytes. PLoS One. 7:e38258, 2012.
- Wang X, Cho B, Suzuki K, Xu Y, Green JA, An J, Cyster JG. Follicular dendritic cells help establish follicle identity and promote B cell retention in germinal centers. J. Exp. Med. 208:2497-2510, 2011.
- Green JA, Suzuki K, Cho B, Willison D, Palmer D, Allen CDC, Schmidt TH, Xu Y, Proia R, Coughlin SR, 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, Cyster JG. Identification of a motile IL-17-producing gamma delta T cell population in the dermis. J. Immunol. 186:6091-5, 2011.

# Brain-Immune Interaction

## Ben Seymour, MD/PhD

| | |
|---|---|
| Professor | **Ben Seymour** |
| Associate Professor | **Aya Nakae** |
| Assistant Professor | **Masaki Maruyama** |
| Support Staff | **1** |

We established a new lab at IFReC to study the complex interplay between the brain and immune systems. In particular, our lab aims to understand how peripheral inflammation influences cognition, how the pain and immune systems interact to mediate animal defence, and how the immune system might be involved in pathological states such as chronic pain.

### Cognitive NeuroImmunology Program

Peripheral injury and inflammation are known to cause changes in cognitive function, and this is referred to as sickness/illness behaviour. This is often presumed to be adaptive – stimulating recuperative behaviours that promote recovery. However, such behaviours generally reflect a reduction in motivation and action, so it remains unknown if these behaviours are genuinely and specifically adaptive (arising from an evolutionary selective pressure to modulate actions after injury), or instead reflect a non-specific or toxic effect on brain activity that just appears to be adaptive. Our research aims to answer this question using two different models of peripheral injury and inflammation: the lipopolysachharide (LPS) model, and the capsaicin model. We have been establishing a LPS task designed to look for selective strategic influence of systemic inflammation on decision-making, and (ii) a neurogenic inflammation task to identify whether there is an *cognitive* representation of injury i.e. the brain has an internal model of tissue damage which can be used for goal-orientated planning (and even immune modulation), and not just experience-dependent learning and responding. These experiments help us frame the new field of 'Cognitive Neuroimmunology'.

### Brain Networks Program

Together with colleagues at the Center for Information and Neural Networks (National Institute for Information and Communications Technology), we are studying the global brain changes that occur in chronic pain: both in human patients, and in animal models of pain and inflammation. With colleagues in the UK, we established an international collaboration to look at chronic back pain patients in both Cambridge and Osaka. This has allowed us to make a highly accurate biomarker for chronic pain based on functional brain network analysis (see figure). We established a small animal facility in collaboration with Yoshichika Yoshioka to look at comparable changes of pain in rodent models, and we aim to see whether we can modulate brain connectivity with techniques such as optogenetics. We have also established external collaborations to study brain network changes in animal models of inflammation, to get a better understanding of how peripheral immune mediators might modulate the brain to give rise to chronic illness behaviour.

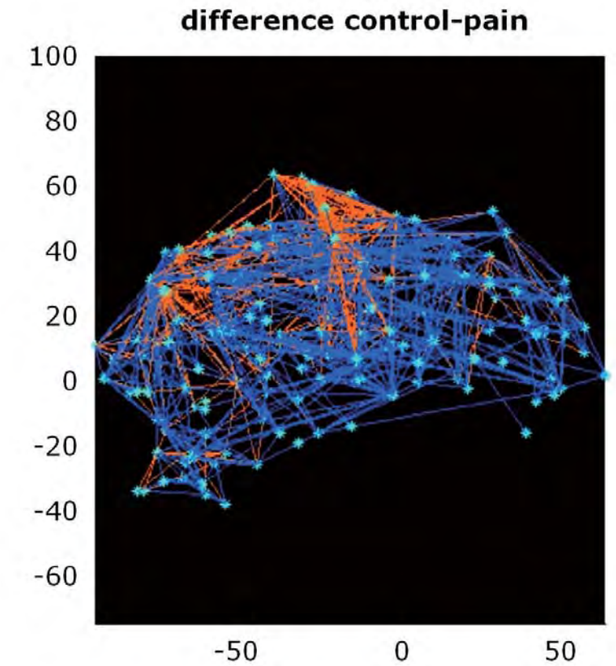



**Figure 1.**
**The image shows the difference between brain networks in chronic pain patients and healthy controls. The orange connections show greater connectivity in pain patients.**

## Recent Publications


- Winston J, Vlaev I, Seymour B, Chater N, Dolan R. Relative valuation of pain in human orbitofrontal cortex. J. Neurosci. 34:14526-35, 2014.
- Zhang S, Seymour B. Technology for Chronic Pain. Current Biology 24: 930-5, 2014.
- Lawson R, Seymour B, Loh E, Lutti A, Dolan R, Dayan P, Weiskopf N, Roiser J. The habenula encodes negative motivational value associated with primary punishment in humans. Proc. Natl. Acad. Sci. USA. 111:11858-63, 2014.
- Mano H, Seymour B. Pain – a distributed information network? PLoS Biol. 13: e1002037, 2014.
- Hosomi K, Seymour B, Saitoh Y. Modulating the pain network-neurostimulation for central poststroke pain. Nat. Rev. Neurol. 11:290-9, 2015.

# Information Systems

## Yutaka Hata, PhD

| | |
|---|---|
| Professor | Yutaka Hata |
| Associate Professor | Shin-ichiro Shima |
| | Shugo Yasuda |
| | Syoji Kobashi |
| Assistant Professor | Manabu Nii |
| Support Staff | 1 |

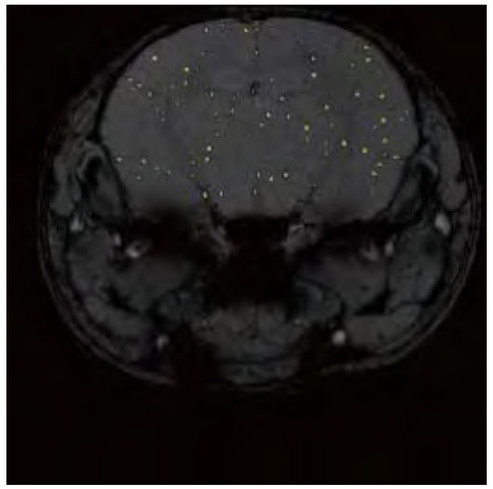


Figure 1. Automated detected macrophages in the mouse brain

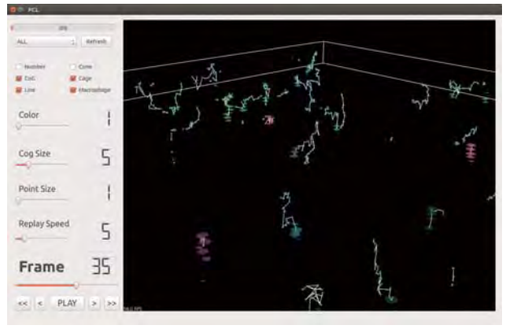



Figure 2. 4-D visualization software for macrophage cells' movement

### 1. Tracking a single macrophage in MRI images

Tracking single macrophage cells in vivo will be a powerful tool for immunology studies. State-of-the-art imaging using magnetic resonance imaging (MRI) enables us to acquire images of 3-D dynamic single macrophage cells in vivo. However, due to motion artifacts and magnetic field fluctuations, post-processing is required to observe macrophage cells. This research proposes an image analysis method for 11.7T animal MRI images of macrophages in the mouse brain. The method adjusts the motion artifacts by a rigid image registration technique, calibrates MR signal intensity fluctuation by using an optimization technique, and automatically detects macrophages. The method was applied to mouse brain MR images, and the results were validated by observers.

### 2. Observation results of macrophage in two–photon microphage

4-D visualizing multiple 2-D observation results of macrophage cells is an effective tool for immunology studies. Using two-photon microscopy and the technique proposed by Prof. Komai, a large volume of macrophage observation results are available. Our research develops a 4-D visualizing software for observation results from two-photon microscopy. We also propose an evolving cellar automaton based simulator to imitate the actual macrophage cells' movement.

### 3. A new Monte Carlo simulation technology for the chemotaxis of cells

We have developed a new Monte Carlo simulation technology for the chemotaxis of cells and applied the method to the traveling population wave of chemotactic bacteria in a micro channel. We have investigated the microscopic dynamics of cells and examined the effect of changing the sensitivity and modulation amplitude in a model response function of cell. The results obtained for this fundamental problem show the validity of the Monte Carlo method. Figure 1 shows the simulation result of the traveling population wave of bacterial cells in a micro channel.
There are three main contributions in this study.
(1)The connection between the microscopic dynamics of cells and macroscopic transports of chemical cues is specifically involved via the response function of cell. This feature is important to provide a solid mathematical and physical ground for the conventional macroscopic approaches from a microscopic point of view.
(2)The Monte Carlo method can be easily extended to the general multi-dimensional problems with complicated boundaries. This is useful especially in applying the method to the practical engineering and biological problems.
(3)The present Monte Carlo method can also directly incorporate various response functions, which may involve the memory of the cell. This allows us to clarify the microscopic mechanism for various complicated phenomena observed in the collective motions of chemotactic cells.

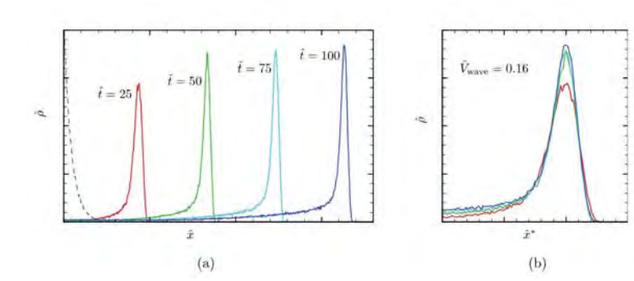



**Figure 3. Traveling population wave of chemotactic bacteria in a micro channel**
**(a) snap shots and (b) the super position of the snap shots**

## Recent Publications


- Mori Y, Chen T, Fujisawa T, Kobashi S, Ohno K, Yoshida S, Tago T, Komai Y, Hata Y, Yoshioka Y. From cartoon to real time MRI: in vivo monitoring of phagocyte migration in mouse brain. Sci. Rep. 6997, 2014.
- Kobashi S, Mori Y, Yoshioka Y, Hata Y. Image Alignment for Single-cell Imaging of Macrophage in the Mouse Brain Using 11.7T MRI. World Automation Congress, 2014.
- Nii M, Hayashi T, Takahama K, Nakashima T, Komai Y. A Macrophage Simulator based on Evolving Cellular Automata from Video Images. World Automation Congress, 2014.
- Yasuda S. A Monte Carlo simulation for kinetic chemotaxis models: an application to the traveling population wave. arXiv:1503.0899 (preprint).
- Hata Y, Nakajima H. A Survey of Intelligent Computing in Medical and Health Care System. IEICE Trans. Inf. & Syst. E97-D,9:2218-25, 2014.

# Systems Immunology

## Daron M. Standley, PhD

| | |
|---|---|
| Professor | **Daron M. Standley** |
| Associate Professor | **Kazutaka Katoh** |
| Postdoctoral Fellow | **3** |
| Visiting Scientist | **2** |
| Support Staff | **2** |

Our laboratory uses structural modeling in order to understand biological function, with an emphasis on antibody-antigen complexes and post-transcriptional regulation. We collaborate closely with a number of experimental groups and also develop software tools for general use. Some of our recent results are described below.

### 3D modeling of immune repertoires

Dynamic generation of antibodies and T-cell receptors (TCRs) is an essential part of the adaptive immune response, which protects our bodies from a wide range of pathogens as well as endogenous threats such as cancer and protein misfolding. At the same time, overproduction of these receptors can lead to autoimmune diseases. In order to understand antibody and TCR function at a molecular level, we have been developing tools for 3D modeling of the variable regions of antibodies. In a recent blind test of antibody structural modeling methods (AMA-II), our joint team with Astellas Pharma and the Institute for Protein Research (Shirai, *Proteins* (2014)) succeeded in submitting models with the lowest average error, a remarkable achievement considering our software is not specialized for antibodies but for general protein structure prediction. To facilitate fully automated 3D modeling of antibody variable regions, we next developed a pipeline called Kotai Antibody Builder (http://kotaiab.org/) that fully reproduces our team's performance in the AMA-II contest (Yamashita, et al. *Bioinformatics* (2014)). We are now extending Kotai Antibody Builder to enable high-throughput modeling of immune repertoires from single B-cell sequencing data (figure 1). We are also collaborating with a number of other groups developing therapeutic antibodies against viruses and cancers. Finally, we are using the same approach to determine the molecular mechanism of cross-reacting antibodies that cause autoimmunity.

### Post-transcriptional regulation of immune responses

Many cellular processes are regulated post-transcriptionally at the level of messenger RNA (mRNA), either by networks of RNA-binding proteins (RBPs) or micro-RNAs (miRNAs). One open problem in this area is to predict RBPs and their associated mRNA targets. Because of the complexity of the cellular environment, such work requires integration of in-cell experimental data (CLIP-seq, mass spectrometry) within a computational framework. In order to facilitate such studies, we have developed a core technology for predicting RNA binding sites on proteins (aaRNA: Li et al. *NAR* (2014)), which out-performs other established methods in three published benchmarks. The aaRNA server (http://sysimm.ifrec.osaka-u.ac.jp/aarna/) first identifies structural domains in the RBP sequence of interest, and then builds 3D models of each domain; from the models, sequence and structural features are extracted and used to score surface residues as potential RNA binding sites (figure 2). The resulting binding propensities can then be used in protein-RNA docking calculations or other downstream analyses. We have a number of ongoing collaborations where we have used such analysis to understand the molecular function of immune-related RBPs (Takemura et al *Nature Commun.* (2014), Mino et al. *Cell* (2015)).

### Mechanism of cooperativity in STIM1-mediated signal transduction

Intrinsically disordered domains (IDDs) have been reported to play important roles in signal transduction networks by introducing cooperativity into protein-protein interactions (PPIs). The $Ca^{2+}$-binding protein STIM1 undergoes an order-to-disorder transition upon a drop in [$Ca^{2+}$] in the ER, triggering extracellular $Ca^{2+}$ influx. This influx exhibits cooperativity with respect to the local ER $Ca^{2+}$ concentration, although the mechanism for the cooperativity is not known. We examined the response of the STIM1 EF-SAM domain to changes in $Ca^{2+}$ concentration using mathematical modeling based on in vitro experiments and found that the unfolding and dimerization are both cooperative with respect to $Ca^{2+}$ concentration, exhibiting Hill coefficients and half-maximal activation concentrations very close to the values observed in vivo for STIM1 redistribution and extracellular $Ca^{2+}$ influx. Moreover, our mathematical model of the dimerization reaction agrees quantitatively with in vitro measurements as well as previously published free energies of unfolding. A simple interpretation of these results is that $Ca^{2+}$ loss effectively acts as a denaturant, enabling cooperative dimerization and robust signal transduction (figure 3) (Furukawa et al. *J. Mol Biol* (2014)).

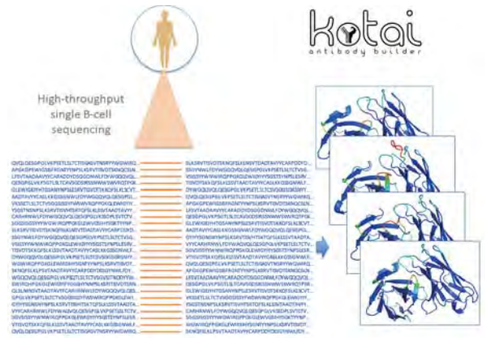



**Figure 1. Overall scheme for processing high-throughput, single B-cell sequencing data using Kotai Antibody Builder**

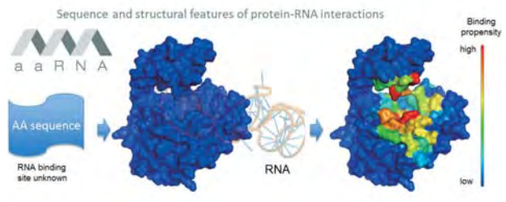



**Figure 2. Predicting RNA binding sites on a protein surface using aaRNA**

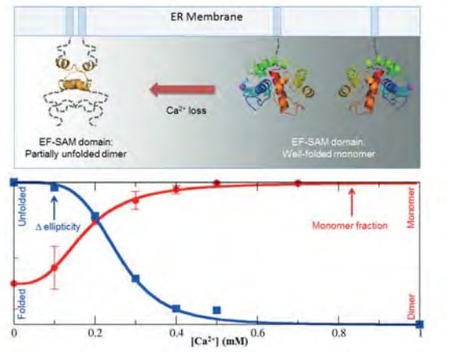



**Figure 3. Unfolding facilitates cooperative signal transduction by STIM1**

## Recent Publications


- Yamashita K. et al. Kotai Antibody Builder: automated high-resolution structural modeling of antibodies. Bioinformatics 30: 3279-80, 2014.
- Shirai H. et al. High-resolution modeling of antibody structures by a combination of bioinformatics, expert knowledge, and molecular simulations. Proteins 82:1624-35, 2014.
- Li S, Yamashita K, Amada KM, Standley DM. Quantifying sequence and structural features of protein-RNA interactions. Nucl. Acids Res. 42:10086-98, 2014.
- Katoh K, Standley DM. MAFFT: iterative refinement and additional methods. Methods Mol. Biol. 1079:131-46, 2014.
- Furukawa Y. et al. Intrinsic disorder mediates cooperative signal transduction in STIM1. J. Mol. Biol. 426:2082-97, 2014.

# Quantitative Immunology

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

## Mining Biological Networks

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

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

## Quantitative Approaches

Accurate quantification of biological responses is critical for understanding the dynamics of complex systems. Previously, we have developed a fluorescent protein reporter system for the quantitative monitoring of IFN-a6 (Kumagai et al. 2007). Now we are trying to increase the "dimension" of the observation in two ways: time and perturbation. Time lapse imaging of type I interferon expression under microscope will be combined with multiple fluorescent protein knock-in cells to monitor genes induced upon antiviral responses such as IL-6 and IL-10. We are also developing automated computational algorithms to extract important quantities to understand interferon regulation from such time lapse imaging data.

In spite of the importance of receptor molecule dynamics such as dimerization and clustering with downstream molecules, this process is still poorly understood. To address this problem, we are applying, in collaboration with RIKEN QBiC and other laboratories in IFReC, total internal reflection fluorescent microscopy (TIRFM) to monitor dynamics of single immune molecules. We have successfully monitored TLRs and their adaptors, and developed a novel algorithm to quantify the diffusion dynamics without bias. This highly quantitative technique can be used to describe the dynamics of the immune system's signaling pathways.

## Data Integration

The development of high-throughput ("omics") technologies has brought biology into the big data era and the need for approaches that integrate, summarize and extract relevant information that reveals the relation between biological components. We measure transcriptome and cistrome (transcription factor binding locations) levels under different experimental conditions and time, and integrate this information with protein-protein interaction data to obtain insight into signaling and gene regulatory immune networks.

We apply these methods to study the mechanisms behind several respiratory diseases, including chronic obstructive pulmonary disease (COPD) and silicosis (Diez et al. 2015). A common feature of these diseases is that inflammation and disease progression are irreversible even after removing exposure to the noxious components (tobacco smoke for COPD and silica dust for silicosis). Using a mouse model of silicosis we aim to uncover the regulatory pathways associated with irreversible inflammation.

## Mathematical Modeling

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

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

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

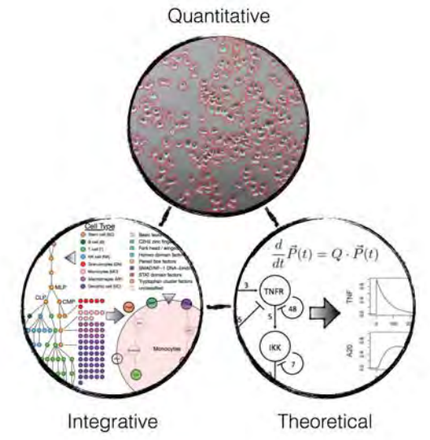
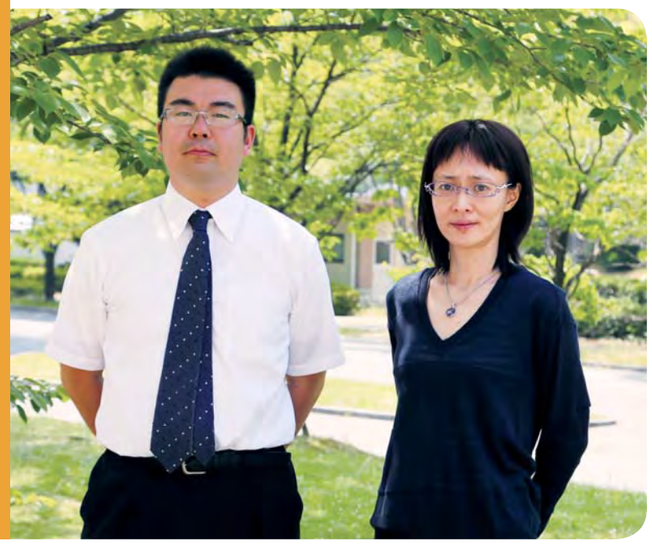



**Figure 1. The Quantitative Immunology Research Unit combines quantitative, integrative and theoretical approaches**

## Recent Publications


- Bahrini I, Song JH, Diez D, Hanayama R. Neuronal exosomes facilitate synaptic pruning by up-regulating complement factors in microglia. Sci. Rep. doi:10.1038/srep07989, 2015.
- Diez D, Agusti A, Wheelock CE. Network analysis in the investigation of chronic respiratory diseases. From basics to application. AJRCCM. doi:10.1164/rccm.201403-0421PP, 2014.
- Furukawa Y, et al. Intrinsic disorder mediates cooperative signal transduction in STIM1. J. Mol. Biol. doi:10.1016/j.jmb.2014.03.006, 2014.
- Patil A, Kumagai Y, Liang KC, Suzuki Y, Nakai K. Linking transcriptional changes over time in stimulated dendritic cells to identify gene networks activated during the innate immune response. PLoS computational biology doi:10.1371/journal.pcbi.1003323, 2013.
- 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. 84: 062903, 2011.

# Next Generation Optical Immune-imaging




- Associate Professor **Noriko Takegahara**
- Assistant Professor **Kazuaki Tokunaga**

**(Visiting academic staff)**


This unit was started on November 1st in 2013. Our aim is to understand the complex dynamic mechanisms of cell fusion.

Polyploidy, in which a cell has more than the diploid complement of chromosomes, is a widespread physiological phenomenon observed especially in plants, fungi, and insects. Although it is less common in mammals, polyploidization occurs in selected tissues including the placenta, liver, heart, skeletal muscle and bone marrow during normal development and aging. Cell fusion is one of mechanisms of generation of polyploidy. Myeloid cells such as macrophages and osteoclasts have a pronounced potential of cell fusion during development. Especially for osteoclasts, polyploidization via cell fusion is thought to be necessary to acquire sufficient bone-resorbing activity. However, neither molecular mechanisms underlying cell fusion nor physiological significance of polyplodization via cell fusion are fully understood. Our aim is to try to make advances in understanding the cellular and molecular mechanisms underlying cell fusion. We are mainly focusing on cell fusion of myeloid cells.

## An approach to find fusion competent cells

Polyploidy is a hallmark of mature osteoclasts. When myeloid precursors receive signals mediated by the osteoclasts differentiation factor RANKL, which is mainly produced by osteoblasts, they commit to becoming pre-osteoclasts, and ultimately differentiate into multinucleated osteoclasts via cell fusion. Myeloid precursors need to pass multiple steps to become mature multinucleated cells and one of the steps is to become "fusion competent cells". However the biology of cells which are committed to be fusion competent is largely unknown. To address this issue we focused on relationship between cell proliferation and osteoclast differentiation. The fluorescent ubiquitylation-based cell cycle indicator (Fucci) is a powerful tool for studying coordination of the cell cycle with other developmental processes. In 2014, using monocytes derived from Fucci transgenic mice, we investigated whether cell-cycle progression has an impact on cell fusion during osteoclastogenesis, and if so, how and to what extent the cell cycle regulates the cell fusion of osteoclasts. We found that RANKL stimulation induced a unique cell population of which ploidy was increased by the mechanism of cell-cycle progression and had the potential of cell fusion. These observations revealed an unexpected cell population which obtain a permission of cell fusion.

## Exploration of fusion master molecule(s)

Given that the ploidy-increased cells obtain fusion competence, they should express molecules required for cell fusion. The ploidy-increased cells were able to be detected not only in pre-osteoclast but also in multinucleated giant cells (MGCs). Those are formed by cell fusion of macrophages in response to foreign bodies at the site of implantation. By isolating the "ploidy-increased" cell population, we performed gene screening and identified a transmembrane molecule (molecule-X). In 2014, we examined the biological function of molecule-X using molecular and cellular biological methods, genetic methods and optical methods such as flow cytometry and microscopy. Overexpression of molecule-X enhanced multinucleation, while knocked down molecule-X inhibited multinucleation of osteoclasts and MGCs. By tagging a fluorescent protein at the C-terminal region of molecule-X, we found that molecule-X localized at the site and time of cell fusion. In addition, gene knockout mice of this molecule exhibited osteopetrotic phenotype, suggesting the involvement of molecule-X in osteoclast function in vivo. To clarify mechanisms underlying cell fusion, further analysis of this molecule is currently in progress.

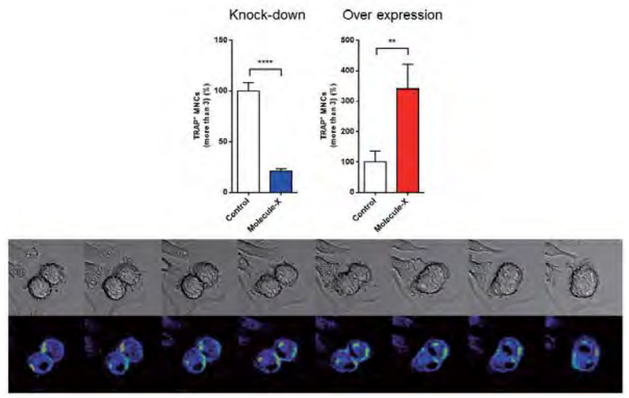
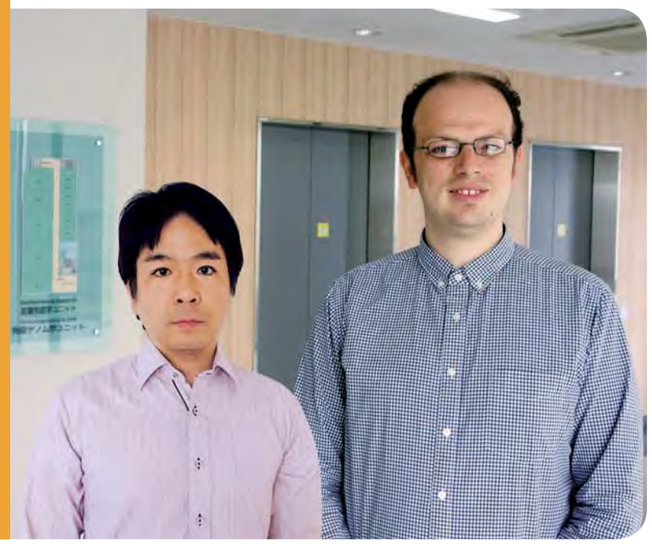



**Figure.**
**Top: Knocked down of molecule-X inhibited formation of multinucleated osteoclasts while overexpression of molecule-X enhanced formation of multinucleated osteoclasts.**
**Bottom: Fluorescent protein-tagged molecule-X localized at the site time of cell-fusion.**

## Recent Publications


- Tokunaga K, Saitoh N, Goldberg IG, Sakamoto C, Yasuda Y, Yoshida Y, Yamanaka S, Nakao M. Computational image analysis of colony and nuclear morphology to evaluate human induced pluripotent stem cells. Sci. Rep. 4: 6996, 2014.
- Kim H, Kim T, Jeong BC, Cho IT, Han D, Takegahara N, Negishi-Koga T, Takayanagi H, Lee JH, Sul JY, Prasad V, Lee SH, Choi Y. Tmem64 modulates calcium signaling during RANKL-mediated osteoclast differentiation. Cell Metab. 17:249-60, 2013.
- Hirosue A, Ishihara A, Tokunaga K, Watanabe T, Saitoh N, Chandra T, Narita M, Shinohara M, Nakao M. Quantitative assessment of higher-order chromatin structure of the INK4/ARF locus in human senescent cells. Aging Cell 11:553-6, 2012.
- Kuwajima T, Yoshida Y, Takegahara N, Petros TJ, Kumanogoh A, Jessell TM, Sakurai T, Mason C. Optic chiasm presentation of Semaphorin6D in the context of Plexin-A1 and Nr-CAM promotes retinal axon midline crossing. Neuron 74:676-90, 2012.
- Takegahara N, Kang S, Nojima S, Takamatsu H, Okuno T, Kikutani H, Toyofuku T, Kumanogoh A. Integral roles of a guanine nucleotide exchange factor, FARP2, in osteoclast podosome rearrangements. FASEB J. 24:4782-92, 2010.

# Immuno-Genomics



- Assistant Professor **Alexis Vandenbon**
- Visiting Academic Staff **Hiromasa Morikawa**
- Research Assistant **1**
- Visiting Scientist **1**

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

### Identification of key factors for inducing functionally stable regulatory T cells

Regulatory T cells (Tregs) are essential for immune homeostasis and can suppress excessive immune reactions harmful to the host. Our analysis of DNA hypomethylation in Tregs revealed that Treg-specific hypomethylation was closely associated with Treg-specific gene induction. On the other hand, Foxp3 binding was clearly associated with repressed genes only in activated Tregs (Morikawa et al., *PNAS,* 2014). These and other results support the concept of Treg-specific transcriptional regulation being controlled by two distinct and complementary mechanisms, involving Foxp3 activity and Treg-specific DNA methylation. Both mechanisms remain poorly understood.

In a follow-up study, we aim to identify new regulators that play a role in defining functionally stable Tregs. In brief, we inferred a gene co-expression network based on a large collection of Treg-derived gene expression data. Network analysis revealed several candidate genes of importance, which are frequently co-expressed with a set of Treg-specific genes. For one candidate gene, additional experimental validation experiments showed that its expression is associated with hypomethylation of Treg-defining genomic loci, and that it has elevated expression in activated Tregs and tumor-associated Tregs. We are further investigating the use of this gene as a surface marker for Tregs, and as a target for tumor immunity.

### Development of a database for gene co-expression in the immune system

Biological processes within cells - from metabolism to the response to a pathogen - are controlled by signal transduction and other biological networks. Study of gene co-expression can help us to understand higher-order properties of biological systems (e.g. co-expression networks), but can also be used for estimating the functions of genes. We have been developing the Immunology Gene co-Expression (iGenEx) database of gene co-expression in various cell types of the immune system. At present, our database (http://sysimm.ifrec.osaka-u.ac.jp/iGenEx/ ; still under development), contains gene expression correlation data for 24 cell types, based on 3,434 mouse microarray samples. iGenEx can be used for inspecting cell type-specific gene expression, and correlation of expression between gene pairs, as well as more complex analyses. One example is the prediction of cell type-specific candidate regulator genes, as mentioned above for Tregs. Currently, we are planning to add human samples to the database, as well as additional functions.

### Analysis of the regulation of gene expression on the epigenetic, transcriptional, and post-transcriptional level

In close collaboration with immunology laboratories, we are using various "omics" approaches for studying regulation of gene expression in response to various immune stimuli.

Several of our studies revealed links between transcription factors (TFs) and epigenetic regulation, such as between Irf4 and Jmjd3 (Satoh et al., *Nature Immunology,* 2010), and between Jdp2 and inhibition of histone acetylation at the Atf3 promoter (Maruyama et al., *Immunity,* 2012). More recently, we used bioinformatics analyses to help elucidate the role of Akirin2 in recruiting the SWI/SNF complex to NF-κB target promoters (Tartey et al., *EMBO J.,* 2014).

In a more large-scale analysis, we are using ChIP-seq data for studying the changes in chromatin structure in dendritic cells upon LPS stimulation on a genome-wide scale. LPS stimulation induces acetylation and methylation of lysine residues of histones at the promoters of key induced genes. Integration of this data with TF factor binding data allowed us to generate hypotheses regarding the ordering of epigenetic changes and the regulatory mechanism underlying them.

Regarding post-transcriptional regulation of gene expression, we are collaborating in a genome-wide analysis of RNA degradation rates and their changes after immune stimulus. Through integrative analysis of gene expression and RNA sequences, we also contributed to the identification of the targets of the RNase Regnase-1 and the 3' UTR stem-loop structure which it recognizes (Uehata et al., *Cell,* 2013; Mino et al., *Cell,* 2015).

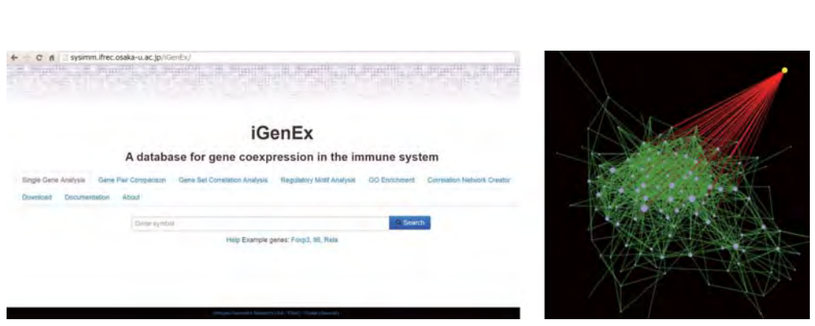


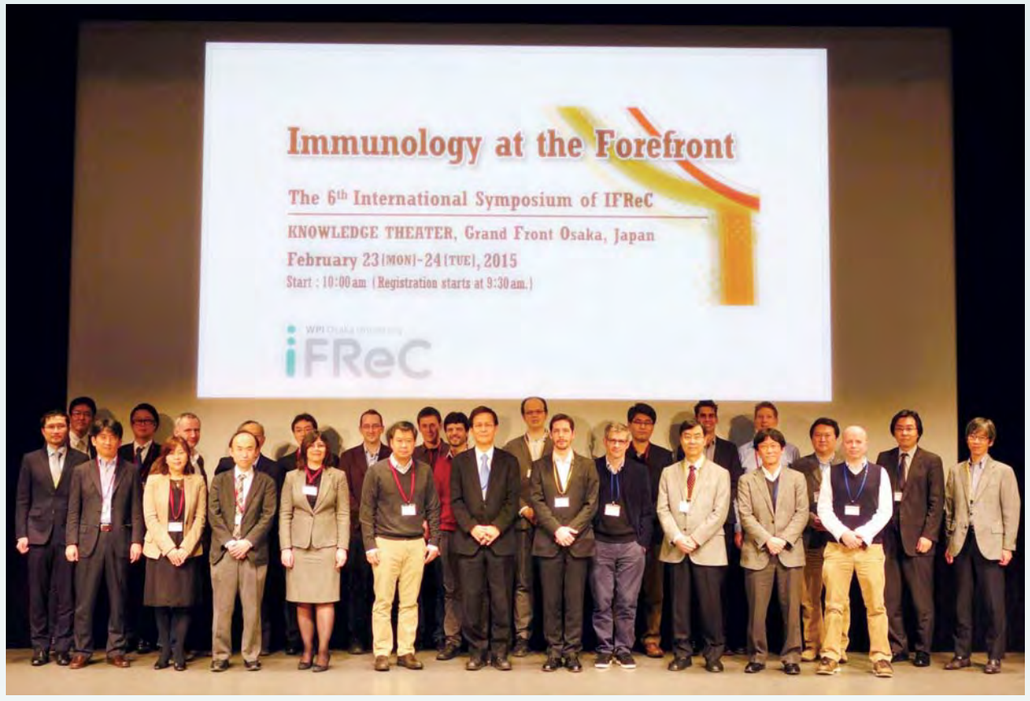

**Figure 1. The iGenEx database**
**(Left) Screenshot of the top page of iGenEx. (Right) Visualization of a co-expression network for a set of Treg-specific genes. Nodes represent genes, and edges indicate co-expressed genes in Tregs. Blue nodes: Treg-specific genes. Yellow node: a new candidate gene of importance in Tregs.**

## Recent Publications


- Ito Y, et al. Detection of T cell responses to a ubiquitous cellular protein in autoimmune disease. Science 346:363-8, 2014.
- Vandenbon A, Teraguchi S, Takeuchi O, Suzuki Y, Standley DM. Dynamics of enhancers in myeloid antigen presenting cells upon LPS stimulation. BMC Genomics 15:S4, 2014.
- Tartey S, et al. Akirin 2 is critical for inducing inflammatory genes by bridging IκB-ζ and the SWI / SNF complex. EMBO J. 33:2332-48, 2014.
- Morikawa H, et al. Differential roles of epigenetic changes and Foxp3 expression in regulatory T cell-specific transcriptional regulation. Proc. Natl. Acad. Sci. USA. 111:5289-94, 2014.
- Uehata T, et al. Malt1-induced cleavage of regnase-1 in CD4(+) helper T cells regulates immune activation. Cell 153:1036-49, 2013.

# Symposia & Seminars

# The 6th International Symposium of IFReC : Immunology at the Forefront




This symposium provided a forum for the newest developments in wide-ranging areas of immunology. Seventeen leading scientists from institutions around the world presented their recent achievements.

**Date :** February 23-24, 2015

**Venue :** KNOWLEDGE THEATER, Grand Front Osaka


**Feb. 23**

| Speaker | Title |
|---|---|
| **Yumiko Imai**<br>Akita University, Japan | Dynamic nuclear interactions between influenza virus and its host |
| **Yukinori Okada**<br>Tokyo Medical and Dental University, Japan | Human genetics contribute to disease biology, clinical medicine, and drug discovery |
| **Magnus Rattray**<br>University of Manchester, UK | Insights into transcription dynamics and gene regulation from non-parametric modelling |
| **Gabriel D. Victora**<br>Whitehead Institute for Biomedical Research, USA | Cellular and clonal dynamics in germinal centers |
| **Kenji Kabashima**<br>Kyoto University, Japan | Perivascular leukocyte clusters are essential for efficient effector T cell activation in the skin |
| **Paola Di Meglio**<br>MRC National Institute for Medical Research, UK | A tale of mice and men: the Aryl hydrocarbon Receptor (AhR) as inflammatory brake in psoriasis |
| **Markus Feuerer**<br>German Cancer Research Center (DKFZ) | Immune control maintained by specialized regulatory cells |
| **Daniel Gray**<br>The Walter and Eliza Hall Institute of Medical Research, Australia | How apoptosis controls regulatory T cell differentiation and homeostasis in steady-state and disease |
| **Hiroyoshi Nishikawa**<br>Osaka University, Japan | Regulatory T cells in tumor immunity |

**Feb. 24**

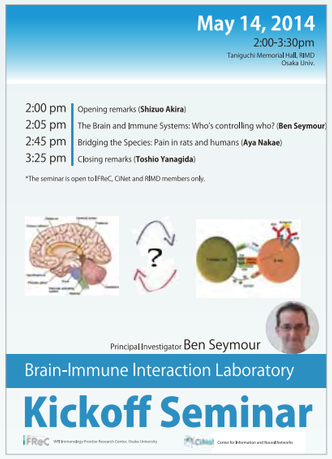
| Speaker | Title |
|---|---|
| **Ryu Okumura**<br>Osaka University, Japan | Lypd8 maintains gut homeostasis by segregating commensal bacteria and colonic epithelia |
| **Takashi Satoh**<br>Osaka University, Japan | The physiological role and differentiation mechanism of various M2 macrophages |
| **Sho Yamasaki**<br>Kyushu University, Japan | Regulation of immune responses through C-type lectin receptors |
| **Joseph C. Sun**<br>Memorial Sloan Kettering Cancer Center, USA | The RAG recombinase dictates functional heterogeneity and cellular fitness in natural killer cells |
| **Hisashi Arase**<br>Osaka University, Japan | Cellular misfolded proteins rescued from protein degradation by MHC class II molecules are targets for autoantibodies in autoimmune diseases |
| **Yeonseok Chung**<br>Seoul National University, Korea | Cross-regulation of atherosclerosis and autoimmunity |
| **Neil Harrison**<br>Brighton and Sussex Medical School, UK | Sickness behaviors: Imaging the effects of peripheral inflammation on human brain structure and function |
| **Ben Seymour**<br>Osaka University, Japan | Pain: A behavioral system for human defense |

## Brain-Immune Interaction Laboratory Kickoff Seminar

The Brain-Immune Interaction Laboratory Kickoff Seminar was held in commemoration of the establishment of the new laboratory of IFReC. The participants from IFReC, CiNet, and RIMD listened with interest to two researchers' talk about new research field of IFReC.

**Date :** May 14, 2014
**Venue :** Taniguchi Memorial Hall, Osaka University

| Speaker | Program |
|---|---|
| Shizuo Akira | Opening remarks |
| Ben Seymour | The brain and immune systems : Who's controlling who? |
| Aya Nakae | Bridging the species : Pain in rats and humans |
| Toshio Yanagida | Closing remarks |




## Cancer Immunotherapy Forum

IFReC and Bristol-Myers K.K co-organized Cancer Immunotherapy Forum. All the sessions were facilitated by Shimon Sakaguchi and Hiroyoshi Nishikawa.

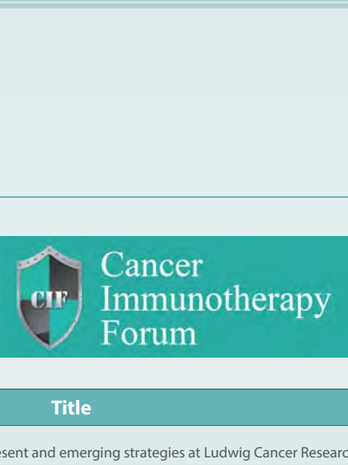
**Date :** November 21, 2014
**Venue :** Rihga Royal Hotel Osaka

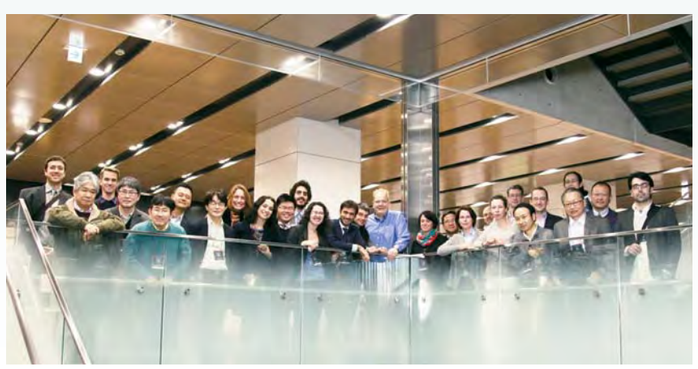
| Speaker | Title |
|---|---|
| **Gerd Ritter**<br>Developmental Research Director, Ludwig Cancer Center, USA | Cancer immunotherapy : Past, present and emerging strategies at Ludwig Cancer Research |
| **Jill O'Donnell-Tormey**<br>Chief Executive Officer and Director of Scientific Affairs, Cancer Research Institute, USA | Cancer immunotherapy : A not-for-profit vantage point |
| **Carl H. June**<br>Richard W. Vague Professor in Immunotherapy, Perelman School of Medicine University of Pennsylvania, USA | Designing CAR T cells for cancer therapy |
| **Guido Kroemer**<br>Professor, University of Paris Descartes, France | A hallmark of successful cancer therapies : Reinstatement of immunosurveillance |
| **Glenn Dranoff**<br>Professor, Department of Medicine, Harvard Medical School, USA | Mechanisms of protective tumor immunity |

## The 1st CiNet Conference : New Direction in Pain Neuroscience

**Date :** December 2-5, 2014
**Venue :** The Center for Information and Neural Networks (CiNet), Osaka, Japan

This conference chaired by Ben Seymour (PI, Brain-Immune Interaction Lab of IFReC) highlighted some of the most innovative new ideas, and created a free space for lively and creative discussion. Topics covered computational theories of the pain system, network and connectivity models of chronic pain, novel neuroimaging methods, the role of the immune system in pain, etc. On December 2, the audience had a special lecture from Seiji Ogawa, who is the inventor of functional MRI.

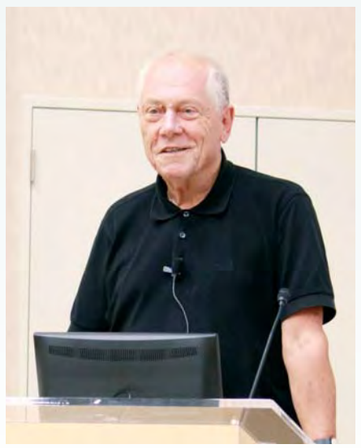


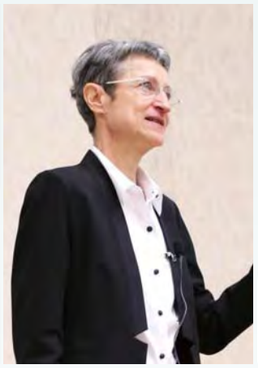



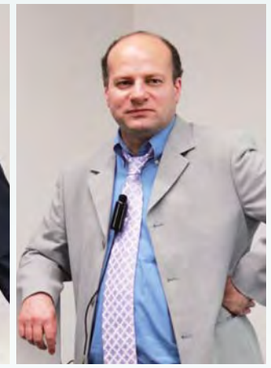
# IFReC Seminars

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

| Date | Speaker | Affiliation | Title |
|---|---|---|---|
| Apr. 10 | **Sylviane Muller** | CNRS, Institute of Molecular and Cellular Biology, Immunopathology and Therapeutic Chemistry, Strasbourg University, France | Chaperone-mediated autophagy as a target of therapeutic P140 peptide used in lupus |
| Jun. 5 | **Junichi Nabekura** | National Institute for Physiological Sciences, Japan | Remodeling of cortical synapses: glia-neuron interaction |
| Jun. 13 | **Barry Ripley** | Laboratory of Immune Regulation, IFReC, Japan | New insights into the control of cytokine production in vivo.Relevance to autoimmune disease and therapeutic targets |
| Jun. 23 | **Paul Horton** | Computational Biology Research Center, AIST, Japan | Predicting protein translocation to the mitochondria and subsequent protease processing from sequence |
| Aug. 25 | **Fritz Melchers** | Max Planck Fellow, Germany / IFReC, Japan | Oncogenic transformations of B-lineage cells |
| Sep. 12 | **Yair Reisner** | Weizmann Institute of Science, Israel | Novel perforin positive regulatory DCs in metabolic syndrome and autoimmunity |
| Oct. 28 | **Yasutaka Okabe** | Yale University School of Medicine, USA | Functional specialization of tissue-resident macrophages |
| Nov. 6 | **Gaetan Burgio** | Macquarie University, Australia<br>John Curtin School of Medical Research, Australian National University | Host response to malaria, a voyage into the genetic mechanisms |
| Nov. 13 | **Thomas Marichal** | Cellular and Molecular Immunology, University of Liege, Belgium | Recent advances in type 2 immunity: Damage-associated host DNA and protective immunoglobulin E |



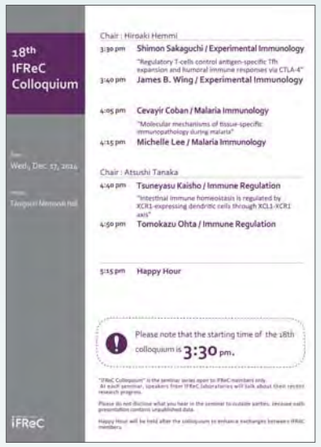


# IFReC Colloquia

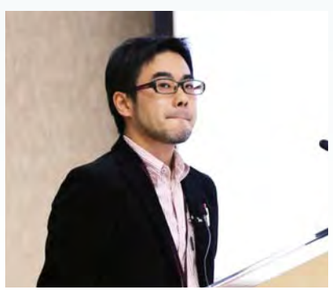
IFReC colloquia are a series of discussion meetings for IFReC members, held once every other month. At each colloquium, three speakers from IFReC laboratories give talks about their latest research progress followed by intensive discussion. After the colloquium, a small social gathering is held to further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

**Date :** **15th Colloquium : April 13, 2014**
**16th Colloquium : June 11, 2014**
**17th Colloquium : August 27, 2014**
**18th Colloquium : December 17, 2014**
**19th Colloquium : February 4, 2015**

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




| | Speaker | Title |
|---|---|---|
| 15th | **Kenta Maruyama** (Host Defense) | Identification of the novel therapeutic target for bone destructive diseases; Beyond RANKL inhibition |
| | **Ryu Okumura** (Mucosal Immunology) | Regulation of gut homeostasis by a molecule selectively expressed in intestinal epithelia |
| 16th | **Alison Hobro** (Biophotonics) | Taking Raman microscopy from cells to tissue imaging |
| | **Kouyuki Hirayasu** (Immunochemistry) | Immune sensing system for bacterially degraded immunoglobulin via activating receptor DIR |
| | **Akiko Nakai** (Immune Response Dynamics) | Adrenergic control of lymphocyte trafficking and inflammation |
| 17th | **Yoshiko Murakami** (Immunoglycobiology) | Inherited GPI deficiencies and the overlapping diseases |
| | **Manabu Nii** (Information Systems) | Computer-aided visualization of two-photon microscopy observation |
| 18th | **Tomokazu Ohta** (Immune Regulation) | Intestinal immune homeostasis is regulated by XCR1-expressing dendritic cells through XCL1-XCR1 axis |
| | **James B. Wing** (Experimental Immunology) | Regulatory T-cells control antigen-specific Tfh expansion and humoral immune responses via CTLA-4 |
| | **Michelle Lee** (Malaria Immunology) | Molecular mechanisms of tissue-specific immunopathology during malaria |
| 19th | **Keiko Matsunaga** (Nuclear Medicine) | Monitoring response to antiangiogenic therapy of non-small cell lung cancer using $^{15}$O-water and PET |
| | **Shuhei Sakakibara** (Molecular Immunology) | Generation and selection of disease-related autoreactive B cells in systemic lupus erythematosus patients |
| | **Kazuya Masuda** (Immune Regulation) | Arid5a stabilizes Stat3 mRNA by counteracting Regnase-1 at the post-transcriptional level to promote Th17 development |



# Events

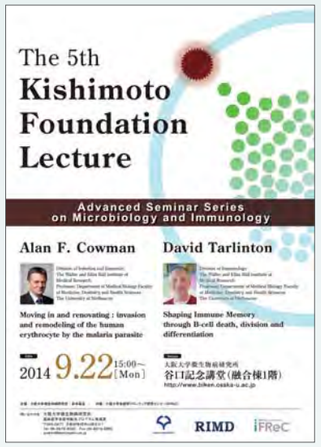
## The 2nd Immunology Frontier : B to B Seminar

This seminar was organized by Atsushi Kumanogoh (Osaka University Graduate School of Medicine / IFReC) and other IFReC PIs.

**Date :** July 17, 2014
**Venue :** Icho kaikan, Osaka University
**Speaker :** Kenji Kabashima (Kyoto University)
Koji Yasutomo (Tokushima University)

## The 5th Kishimoto Foundation Lecture

**Date :** September 22, 2014
**Venue :** Taniguchi Memorial Hall, Osaka University
**Host :** Research Institute for Microbial Diseases & IFReC




**Alan F. Cowman**

Walter and Eliza Hall institute of Medical Research / University of Melbourne
"Moving in and renovating: Invasion and remodeling of the human erythrocyte by the malaria parasite"

**David Tarlinton**

Walter and Eliza Hall institute of Medical Research / University of Melbourne
"Shaping Immune Memory through B-cell death, division and differentiation"

# The 4th NIF Winter School on Advanced Immunology

The fourth Winter School on Advanced Immunology was jointly organized with Singapore Immunology Network (SIgN). Fifty young researchers, who were competitively selected from 180 applicants, and 16 world leading immunologists got together in Singapore on 18-23 January 2015. Three young IFReC researchers participated in the school. The participants shared intriguing insights and findings in immunology, discussed new ideas and forged friendships that will fuel networking and future collaborations.

**Date :** **January 18-23, 2015**

**Venue :** **Grand Copthorne Waterfront Hotel, Singapore**


| Lecturer | Title |
|---|---|
| **Shizuo Akira**<br>IFReC, Japan | Regnase-1, a ribonuclease involved in the control of immune responses |
| **Facundo Batista**<br>London Research Institute, UK | Dynamic imaging of lymphocyte activation - from single molecule to living tissue |
| **Burkhard Becher**<br>University of Zurich, Switzerland | Cytokine networks in autoimmunity : How helper T cells instruct macrophages |
| **Yasmine Belkaid**<br>National Institute of Allergy and Infectious Diseases, USA | Consequences of host microbiota interaction for tissue specific immunity |
| **Frank Carbone**<br>The University of Melbourne, Australia | Formation and function of tissue-resident memory T cells |
| **James Di Santo**<br>Institut Pasteur, France | Staying innate: Transcription factor maintenance of innate lymphoid cell identity |
| **Nicholas Gascoigne**<br>National University of Singapore, Singapore | T cell development |
| **Tomohiro Kurosaki**<br>IFReC, Japan | Cellular and molecular basis for humoral memory responses |
| **Ana-Maria Lennon-Duménil**<br>INSERM / Institut Curie, France | Coordinating cell migration and cell function : The example of dendritic cells |
| **Kenneth Murphy**<br>Howard Hughes Medical Institute, USA | Transcriptional basis of DC diversification |
| **Shalin Naik**<br>Walter and Eliza Hall Institute of Medical Research, Australia | Haematopoiesis at the single cell level |
| **Evan Newell**<br>Singapore Immunology Network, Singapore | High dimensional analysis of human T cell phenotype, function and antigen specificity using mass cytometry |
| **Laurent Rénia**<br>Singapore Immunology Network, Singapore | Malaria vaccination: Hopes and hurdles |
| **Shimon Sakaguchi**<br>IFReC, Japan | Control of immune responses by regulatory T cells |
| **Mark Smyth**<br>QIMR Berghofer Medical Research Institute, Australia | The age of combination immunotherapy |
| **Kiyoshi Takeda**<br>IFReC, Japan | Regulation of gut homeostasis : Implication of the pathogenesis of inflammatory bowel diseases |

# Immunology Lecture Series


The Immunology Lecture Series was initiated as staff development to provide fundamental knowledge of immunology to IFReC research support staff such as technicians, secretaries and administrative staff. A young researcher of IFReC is invited as a speaker to give a talk about the basics of their research up to cutting-edge research in an easy-to-understand manner. The lecture is open to all Osaka University members and held in the evening so that the participants can attend after work. In the lecture, participants can interact with the speaker, asking questions in an informal setting. Initiated in December 2013, the series was held eight times until the end of FY2014 and the questionnaire results from the participants show a high level of satisfaction throughout the series. The average number of participants is 46. These events serve as effective measures to develop IFReC support staff as members of a WPI center, to publicize IFReC in Osaka University as well as to give educational value to the young speakers selected from IFReC researchers.

**Venue：　Biken Hall, Osaka University**


| Date | Speaker | Title |
|---|---|---|
| **2014** | | |
| Apr. 23 | **Tomoyuki Yamaguchi**<br>Associate Professor | Self and Nonself<br>—How the immune system selects the correct response |
| Jun. 26 | **Daisuke Sugiyama**<br>Graduate Student | Young researchers on cancer immunotherapies<br>—New options in cancer therapies |
| Aug. 29 | **Rikinari Hanayama**<br>Associate Professor | The death of cells and autoimmune disorders |
| Nov. 6 | **Kazuya Masuda**<br>Assistant Professor | What is facing the frontier of immunology?<br>—Bacteria and immunity, fighting viruses, regenerative medicine and immunity |
| Dec. 9 | **Jun Sakanoue**<br>Associate Professor | The birth of immunology<br>—Those who came before |
| **2015** | | |
| Jan. 29 | **Daisuke Sugiyama**<br>Graduate Student | Cancer immunotherapy options and potential |
| Mar. 12 | **Masanori Matsumoto**<br>Assistant Professor | Vaccines and immune memory<br>—Does the influenza vaccine work or not? |

## Seminar on Harassment : Current situation and prevention of academic and power harassment

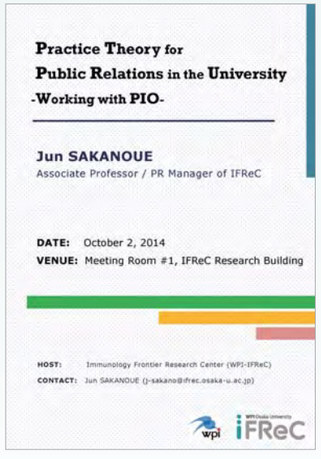
In order to understand and avoid harassment at work, Prof. Zako presented the current situation at Osaka University with examples actually arising in Osaka University. He suggested solutions to prevent harassment to an audience of 23 people from IFReC and other departments of Osaka University.

**Date :** July 3, 2014
**Venue :** Meeting Room 1, IFReC Research Building
**Lecturer :** Masaru Zako (Counselor, Harassment Counseling Offices / Emeritus Professor of Osaka University)


## Seminar on Public Relations

The PIO (Public Information Officers) in research institutes are responsible for issuing press releases, answering queries from the media. In this seminar, Dr. Sakanoue (RPMO, IFReC) explained how researchers and staff in the university should work with PIOs.

**Date :** October 2, 2014
**Venue :** Meeting Room 1, IFReC Research building




**Reference**
Working with Public Information Officers,
Dennis Meredith, North Carolina: Glyphus, 2010

## Japanese Lessons

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

We offer two lecture-style classes, Class A: Beginner to Elementary and Class B: Pre-intermediate to Intermediate. Class members are expected to learn hiragana, katakana, some kanji and basic phrases necessary for daily conversation.

During the course, special events were arranged to give the members an experience of Japanese culture.

In FY2014, international staff and Japanese volunteers enjoyed cooking Onigiri (rice ball), Okonomiyaki (savory pancake), Gyouza (dumpling) and Chirashi sushi (unrolled sushi). Participants learnt new vocabulary and practiced speaking Japanese under the supervision of a professional Japanese teacher. It was also a good opportunity for participants to communicate with staff from other laboratories.

At the end of the course, the feedback received from participants indicated that most of them realized significant improvement in their Japanese proficiency.


# Outreach Activities

May 27, 2014
Onigiri



July 31, 2014
Okonomiyaki


February 26, 2015
Chirashi Sushi

# Science Cafe

The series of Science Cafes is a long lasting IFReC outreach activity to promote communication among researchers and the general public. It also enhances people's understanding of immunology researches and the researchers involved in them. About 140 people in total participated in our Science Cafes in FY2014.

A unique attempt was achieved in the 14th Science Cafe on the Edge. Guest speakers of the 6th IFReC international symposium kindly joined the cafe as guests. They explained basic knowledge of their research fields in English with the help of simultaneous translation.

## Science Cafe Event at 2014 Icho Festival

### [The Cleaner in Our Body - Various functions of the macrophage]

Date : May 3, 2014

Venue : Biken Hall, Osaka University

Guest : Rikinari Hanayama (Immune Network, IFReC)

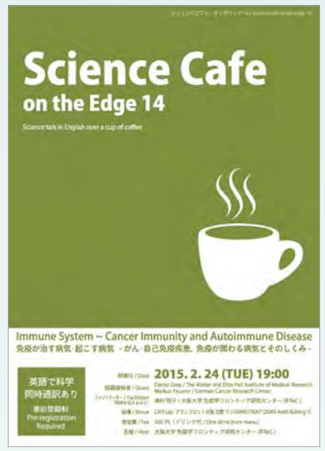

## Science Cafe on the Edge 14

### [Immune System - Cancer immunity and autoimmune disease]

Date : February 24, 2015

Venue : CAFE Lab, Grand Front Osaka

Guest : Daniel Gray (The Walter and Eliza Hall Institute of Medical Research, Australia)
Markus Feuerer (German Cancer Research Center)



## Super Science High School Student Fair 2014

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

The 2014 annual SSH symposium was held in Yokohama and more than 200 schools held booths with posters to present their researches. WPI institutes held a collaborative booth and introduced the research activities by each institute using posters, booklets and demonstrations.

Aya Nakae (Brain-Immune Interaction, IFReC) delivered a presentation at Researchers' Mini Live Talk. She introduced her research interests and her career as a basic researcher with the work experience of medical doctor. Her talk inspired the student audience, especially those who wish to enter medical departments in universities, to understand the difference and importance of medical treatment and medical science.

**Date :** August 6-7, 2014

**Venue :** PACIFICO Yokohama, Kanagawa

**Host :** MEXT, JST

**Support :** Boards of Education (Kanagawa prefecture , Yokohama city)



## Student Visit - Gunma Prefectural Takasaki High School

IFReC welcomed students from Gunma Prefectural Takasaki High School. They enjoyed a tour of BIKEN museum and the IFReC research building as well as talking with researchers, Masato Okada (Department of Oncogene Research, RIMD ; a graduate of the high school ) and Yoshichika Yoshioka (Biofunctional Imaging, IFReC). Yoshioka showed them bioimaging pictures and movies of immune cells in specific tissues taken by MRI, and explained how the latest MRI technology can be useful for revealing immune functions in our body.

One of the students said: "I was excited to know about high level researches in immunology. "

**Date :** September 4, 2014

## The 4th Annual WPI Joint Symposium

An annual WPI joint symposium is held to introduce WPI research achievements to mainly high school students, as well as to provide them with a good opportunity to know about world top level science. It aims to encourage the student's interest in science.

The 4th WPI joint symposium entitled "Science: a bridge to your future" was held in Tokyo. The program included talks by WPI scientists, presentations by high school students, and booth/poster exhibition.

IFReC opened a booth introducing its research activities to encourage students to consider entering Osaka University and immunology research as a career at IFReC. Yuki Mori (Biofunctional Imaging, IFReC) joined the symposium and presented his research with a poster and an iPad. Booth visitors excitedly watched high definition movies of immune cells by MRI on the iPad, and enjoyed communication with Mori.

**Date :** December 13, 2014
**Venue :** Yurakucho Asahi Hall, Tokyo
**Host :** Kavli Institute for the Physics and Mathematics of the Universe
**Cohost :** WPI Institutes
**Support :** MEXT, JSPS, Boards of Education (Tokyo, Kanagawa, Chiba, Saitama, Ibaraki)




### Speakers

- Dan Ohtan Wang (Assistant Professor, iCeMS, Kyoto University)
- Aleksandar Tsekov Staykov (Assistant Professor, I²CNER, Kyushu University)
- Hitoshi Murayama (Director, Kavli IPMU, The University of Tokyo)

### Student Presentations

Oral

Hiroo Gakuen Senior High School (Tokyo)
Ichikawa Gakuen Ichikawa Senior High School (Chiba)
Tokyo Metropolitan Toyama High School (Tokyo)

Poster

Seven high schools selected from the Kanto region


## AAAS 2015 Annual Meeting

The American Association for the Advancement of Science (AAAS) is the biggest international non-profit organization advancing science in the world, and its mission is to "advance science and serve society". The AAAS Annual Meeting assembles diverse participants, including scientists, families, science policymakers, and the media etc. The AAAS 2015 Annual Meeting offered more than 160 symposia, lectures, seminars, poster presentations and exhibitions, with the theme of Innovations, Information, and Imaging.

WPI institutes held a collaborative booth to introduce the WPI program and the institutes' activities using posters and booklets. More than 360 participants visited the booth and gained interest in WPI program and world leading researches in Japan.

**Date :** February 12-26, 2015
**Venue :** San Jose McEnery Convention Center, USA




# Research Projects

# Support Program for Fusion Researches

One of the goals of World Premier International Research Center Initiative (WPI) program is to generate novel research fields through fusion of existing research fields. IFReC aims to create innovative immunology fields by combining with imaging and bioinformatics technologies. In order to promote this challenge, we launched the following two programs.

**Research Support Program for Combined Research Fields** was established in FY2009 to financially support research projects, whose members consist of researchers from different groups/backgrounds. This program effectively encourages interaction and fusion among different groups. The projects are selected by screening proposals submitted by applicants. So far, 25 projects have received financial support from IFReC and some of them have reached the publication stage.

**Dual Mentor Program** focuses on graduate students or young post-doctoral fellows engaging in interdisciplinary projects under the supervision of two PIs from different disciplines. Financial support is given to the recipients and their primary mentor for three years. Financial support and/or other types of incentives are also given to the secondary mentor if necessary. This program was introduced as a platform to foster young pioneers in the fusion field and to further promote interdisciplinary research at IFReC.

A total of 10 Combined Research Program projects and one Dual Mentor Program project were in progress in FY2014.

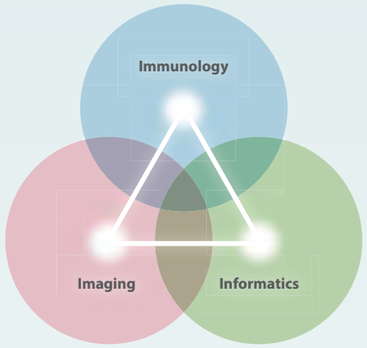
| Project Leader | Collaborators | Project Title |
|---|---|---|
| **2012** | | |
| Masahiro Yamamoto | D. Standley<br>E-M Frickel | Trilateral analysis of interferon-γ-mediated cellular innate immunity against Toxoplasma gondii |
| Masako Kohyama | C. Coban K. Suzuki<br>F. Sugihara T. Aoshi | Role of tissue macrophage in malaria infection, and their developmental control by parasite metabolite |
| Barry Ripley | D. Standley<br>G. Kurisu | Role of Arid5A in the selective control of IL-6 mRNA stability and development of TH17 cells |
| Fuminori Sugihara | R. Hanayama K. Kikuchi<br>M. Kohyama S. Satoh<br>S. Akira | In vivo imaging of germinal center development in mouse spleen using MRI |
| Tomoyuki Yamaguchi | H. Fujita S. Sakaguchi<br>T. Watanabe H. Machiyama<br>C. Furusawa S. Esaki | Imaging analysis of immune activation and regulation |
| Dual Mentor Program<br>Takeshi Yoshida | R. Hanayama<br>K. Suzuki | Visualizing the dynamics of exosomes during various immune responses in vivo |
| **2013** | | |
| Diego Diez | R. Hanayama | The dynamics of novel signaling networks of macrophages exposed to pathogens |
| Yutaro Kumagai | J. Kozuka S. Teraguchi<br>N.Trost | Visualizing information processing of immune cells via combination of fluorescent reporter and single molecule imaging |
| Naganari Ohkura | A. Vandenbon S. Nakamura<br>S. Yamazaki S. Kato<br>M. Hashimoto | Development of an epigenome-based computational classification system for the treatment of autoimmune diseases |
| Kazuhiro Suzuki | Y. Baba | Visualizing activation of germinal center B cells using genetically encoded calcium indicators |
| Alexis Vandenbon | S. Sakaguchi H. Morikawa<br>N. Ohkura | Identification of key factors for inducing functionally stable regulatory T cells |

Project leader from the groups of <Immunology>, <Imaging>, <Informatics>

# Evaluation Workshop for Research Support Program

All the Combined Research Program and Dual Mentor Program recipients presented their on-going research in front of IFReC members. The IFReC PIs served as evaluators in the workshop. The result of the evaluation, including scores and comments, was provided to the recipients.

**Date :** October 15, 2014
**Venue :** Taniguchi Memorial Hall, Osaka University



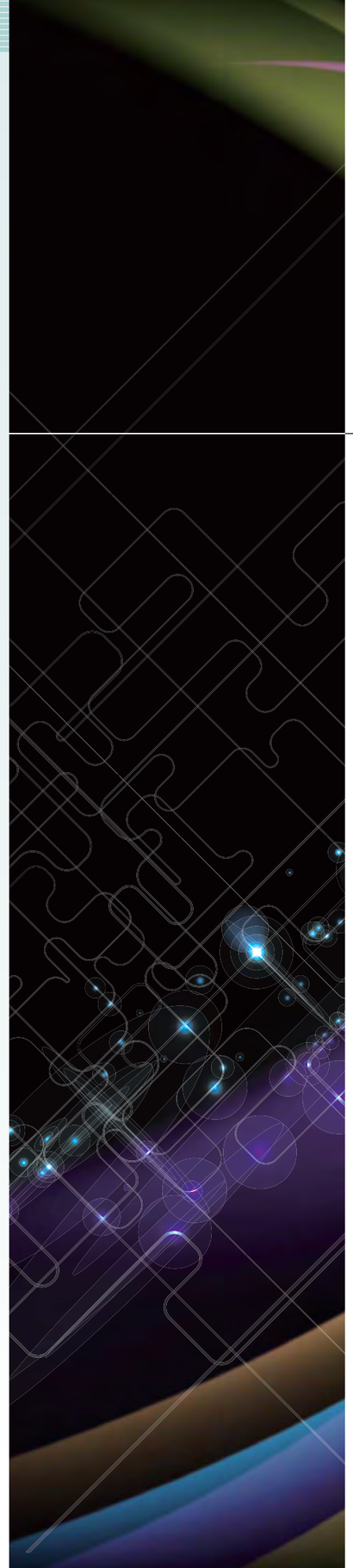


# Young Scientist Support Program for Research Abroad

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. Seven researchers used this support program in FY2014.

Young Scientist Support Program for Research Abroad 2014

| Name | Country | Conferences attended |
|---|---|---|
| **Kazuya Masuda** | Germany | Interleukin 6 Biology-Pathophysiology-Therapy MidTerm Conference of ICIS |
| **Yuki Mori** | USA | World Automation Congress 2014 |
| **Alison Jane Hobro** | Germany / Poland | ICORS2014 / SPEC2014 |
| **Szandor Simmons** | Germany | 44th Annual Meeting of German Society for Immunology |
| **David Millrine** | Australia | ICIS2014 |
| **Kazuya Masuda** | Australia | ICIS2014 |
| **Soyoung Lee** | USA | 2014 ACR/ARHP Annual Meeting |
| **Hui Jin** | USA | Keystone Symposia on Autoimmunity and Tolerance |



# Data

# Common Facilities

IFReC and its parent institution, the Research Institute for Microbial Diseases (RIMD) are located on the same site, constituting a large research complex. It contains the Core Instrumentation Facility, the Animal Resource Center and the Network Administration Office, all of which are jointly operated by IFReC and RIMD. The Core Instrumentation Facility is equipped with various highly advanced instruments and skilled technicians provide in-house services to IFReC and RIMD researchers. The Animal Resource Center consists of three buildings for specific pathogen-free (SPF) animals and the live immuno-imaging facility. With a large animal-breeding capacity facility in IFReC, researchers are able to choose animal rooms suitable for their experiment purpose.

Using these common facilities, IFReC researchers are able to effectively and smoothly carry out their experiments to promote their world-leading research at IFReC.

IFReC–RIMD Research Complex at Suita Campus of Osaka University


Photo : S. Higashiyama

1. IFReC Research Building
2. Integrated Life Science Building
3. Research Institute for Microbial Diseases, RIMD
4. Genome Information Research Center, RIMD
5. Cutting-edge Research Building for Infectious Diseases
6. Animal Resource Center for Infectious Diseases

## Animal Resource Center for Infectious Diseases

- Specific pathogen-free (SPF) animal facility
- Sperm /embryo freezing and preservation
- In vitro fertilization and embryo transplantation
- Intracytoplasmic sperm injection
- Transgenic and knock-out animals
- Genome editing in experimental animals

## Live Immuno-Imaging Facility

- SPF animal facility with high-performance 11.7T MRI & two- photon microscope

## Network Administration Office

- Provision and maintenance of network infrastructures:
  LAN system and servers (web, mail, mailing lists etc.)

## Core Instrumentation Facility

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

Members of the Core Instrumentation Facility

## Kishimoto Foundation Fellowships

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

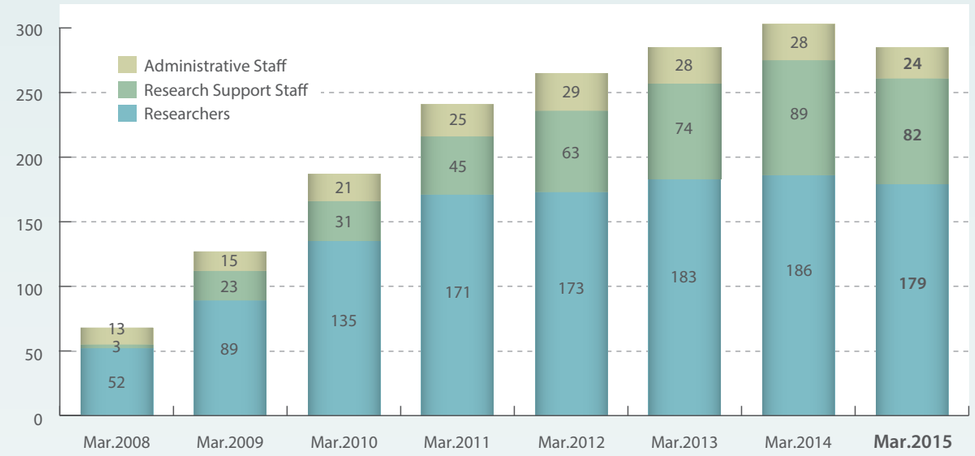
The Kishimoto Foundation was established in 2008 in honor of Tadamitsu Kishimoto, who, during the 1980s and 90s, elucidated the function of interleukin-6 (IL-6), a key molecule for stimulating immune responses. He later developed the anti-IL6 receptor-based therapy, tocilizumab, to treat immune disorders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

### FY2014 Kishimoto Fellowship Recipients

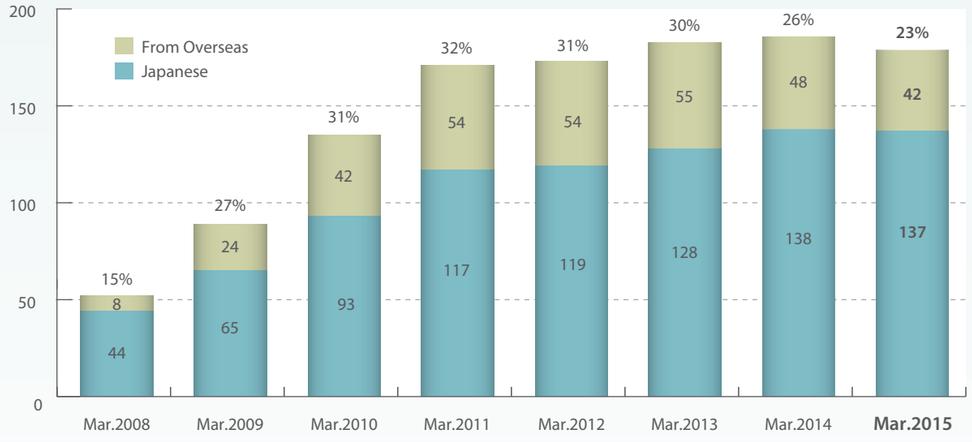
| Position of Recipient | Name (initials) | Nationality | Host researcher | Period |
|---|---|---|---|---|
| Specially Appointed Researcher | I. B. | Tunisia | Hanayama | Jan. 16, 2012 - Mar. 31, 2015 |
| Specially Appointed Researcher | P. D. | India | Kishimoto | Nov. 1, 2012 - Oct. 31, 2015 |
| Specially Appointed Researcher | J. H. | China | Sakaguchi | Dec. 1, 2013 - Nov. 30, 2014 |
| Specially Appointed Researcher | D. M. | Britain | Kishimoto | Dec. 1, 2013 - Mar. 31, 2016 |
| Specially Appointed Researcher | Y. R. | China | K. Ishii | Apr. 1, 2014 - Mar. 31, 2017 |
| Specially Appointed Researcher | H. J. | China | Arase | May 1, 2014 - Apr. 30, 2015 |
| Research Fellow | H. H. | Jordan | Kishimoto | Jun. 8, 2014 - Aug. 9, 2014 |
| Specially Appointed Researcher | R. K. | Slovenia | Akira | Nov. 16, 2014 - Nov. 15, 2015 |
| Specially Appointed Researcher | H. L. | Taiwan | Coban | Dec. 16, 2014 - Dec. 15, 2015 |

## Members

### Number of IFReC Staff




### Percentage of International Researchers



# Major Awards

## Shimon Sakaguchi The Gairdner International Award

The Gairdner Foundation announced on March 25 that Shimon Sakaguchi (Deputy Director of IFReC) had been awarded the Canada Gairdner International Award.

The Canada Gairdner International Award is one of the most prestigious awards in biomedical sciences. Previous awardees include Kimishige Ishizaka, Susumu Tonegawa (Immunology), and Shinya Yamanaka (Regenerative medicine). Shizuo Akira (Director of IFReC) was also awarded the Gairdner Award for his groundbreaking discoveries in the field of innate immunity in 2011.

The Gairdner Foundation commented "Prof. Sakaguchi is awarded for his discovery of regulatory T cells, characterization of their role in immunity and application to the treatment of autoimmune diseases and cancer." The foundation provides $100,000 (CDN) to each awardee, and will hold the Gairdner National Program, a lecture series given by Gairdner Award winners on October 26-30, 2015 in Canada.

Prof. Sakaguchi stated "… I have recognized that science is an international endeavor, and I am happy to be able to pursue what I love."

Shimon Sakaguchi (R) and President Toshio Hirano (L) at the press conference

## Shizuo Akira The Member of the Japan Academy

The Japan Academy (Nippon Gakushi-in) is an organization that accords special recognition to researchers with the most eminent records of academic and scientific achievement. The Academy's primary purpose is to carry out programs that contribute to the advancement of academic pursuit.
(Website of the Japan Academy)

日本学士院
The Japan Academy

## Toshio Yanagida Honorary Member of the Physical Society of Japan

Yanagida was chosen for the JPS Honorary Fellow by his outstanding achievements in the studies of fundamental structure of biological system through the developments of single molecular measurements techniques. The previous honorees for JPS Fellow include Hideki Yukawa, Makoto Kobayashi, Toshihide Masukawa, and Yoichiro Nambu.

## Ken Ishii Osaka Science Prize, Fellow of the International Society for Vaccines


## Atsushi Kumanogoh Elected Membership, American Society for Clinical Investigation (ASCI)

## Masaru Ishii JSPS Award

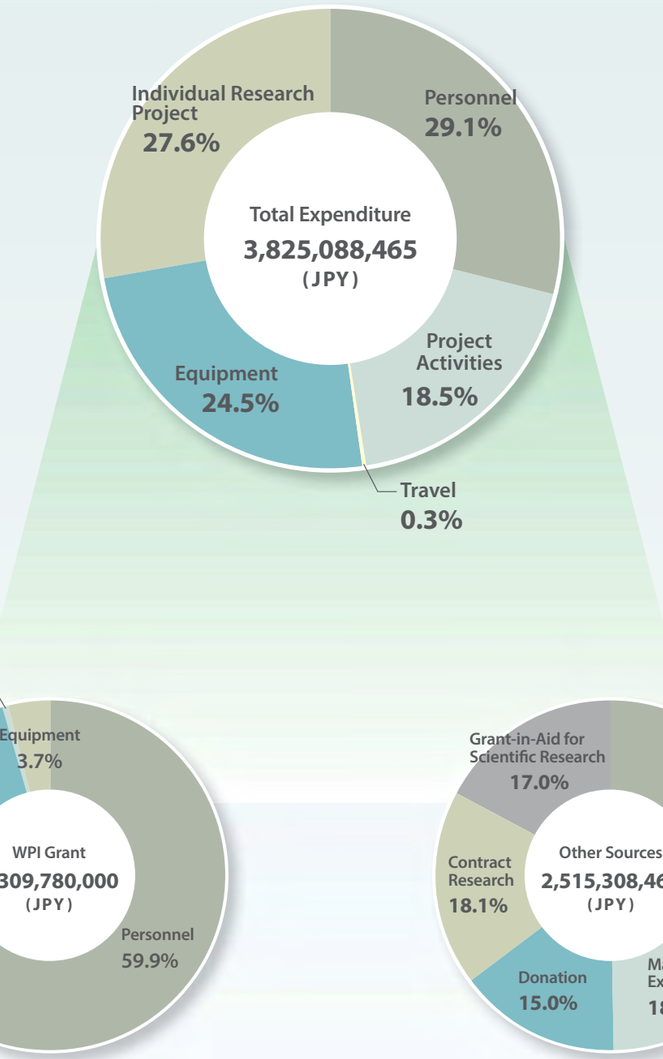
## Takashi Satoh Young Investigator Award, Japanese Society for Immunology

## Kazutaka Katoh Young Scientist Initiative Award, Society of Evolutionary Studies, Japan

In FY 2014, many IFReC researchers were awarded Osaka University Presidential Awards for Achievement and/or the Presidential Awards for Encouragement, for their recent acquisition of research grants.

## Finance

Break down of total expenditure at IFReC

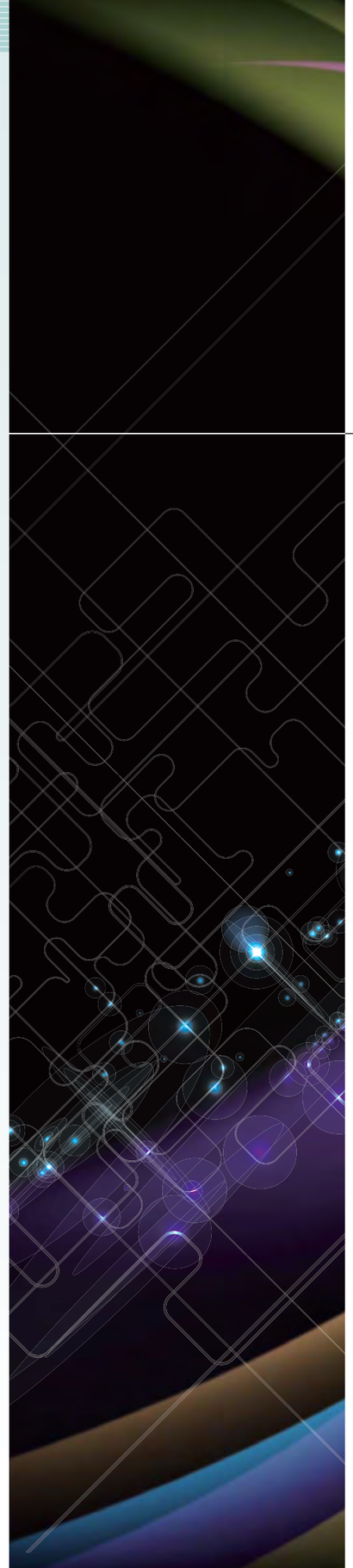










# Research Outputs

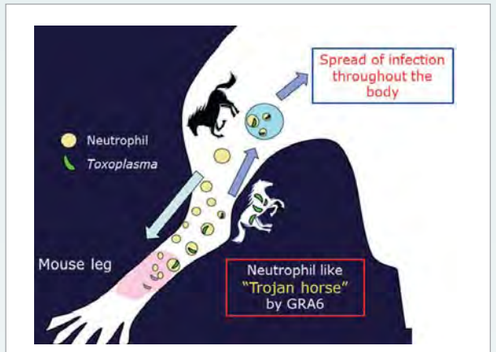
# Selected Articles

## Selective and strain-specific NFAT4 activation by the *Toxoplasma gondii* polymorphic dense granule protein GRA6

***J Exp Med.*** 211 : 2013-32, 2014.

Ji Su Ma, Miwa Sasai, Jun Ohshima, Youngae Lee, Hironori Bando, Kiyoshi Takeda, Masahiro Yamamoto

*Toxoplasma gondii* infection results in co-option and subversion of host cellular signaling pathways. This process involves discharge of *T. gondii* effector molecules from parasite secretory organelles such as rhoptries and dense granules.
Masahiro Yamamoto and his group reported that the *T. gondii* polymorphic dense granule protein GRA6 regulates activation of the host transcription factor nuclear factor of activated T cells 4 (NFAT4). Their data suggest that GRA6-dependent NFAT4 activation is required for *T. gondii* manipulation of host immune responses to maximize the parasite virulence in a strain-dependent manner.

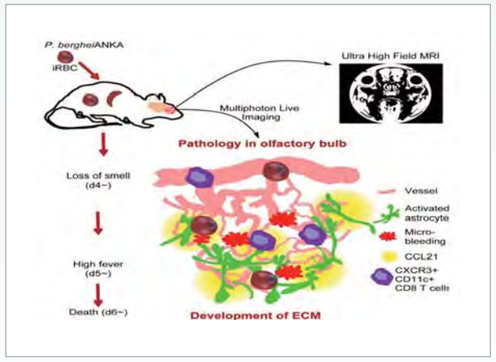



## Olfactory plays a key role in spatiotemporal pathogenesis of cerebral malaria

***Cell Host & Mirobe.*** 15:551-63, 2014.

Zhao H, Aoshi T, Kawai S, Mori Y, Konishi A, Ozkan M, Fujita Y, Haseda Y, Shimizu M, Kohyama M, Kobiyama K, Eto K, Nabekura J, Horii T, Ishino T, Yuda M, Hemmi H, Kaisho T, Akira S, Kinoshita M, Tohyama K, Yoshioka Y, Ishii KJ, Coban C

Coban and her group showed by ultra-high-field MRI and multiphoton microscopy that the olfactory bulb is physically and functionally damaged (loss of smell) by Plasmodium parasites during ECM. The trabecular small capillaries comprising the olfactory bulb show parasite accumulation and cell occlusion followed by microbleeding, events associated with high fever and cytokine storm. Specifically, the olfactory upregulates chemokine CCL21, and loss or functional blockade of its receptors CCR7 and CXCR3 results in decreased CD8 T cell activation and recruitment, respectively, as well as prolonged survival. Thus, early detection of olfaction loss and blockade of pathological cell recruitment may offer potential therapeutic strategies for ECM.



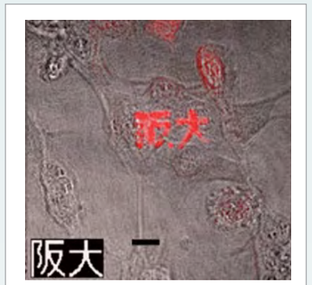

**Olfactory bulb is the Achilles' heel during experimental cerebral Malaria**

## Laser-targeted photofabrication of gold nanoparticles inside cells

***Nat Commun.*** 5 : 5144, 2014.

Nicholas I. Smith, Kentaro Mochizuki, Hirohiko Niioka, Satoshi Ichikawa, Nicolas Pavillon, Alison J. Hobro, Jun Ando, Katsumasa Fujita, Yutaro Kumagai

Smith and his group showed that by infusing gold ion solution, focused laser light-induced photoreduction allows in-situ fabrication of gold nanoparticles at precise locations. The resulting particles are pure gold nanocrystals, distributed throughout the laser focus at sizes ranging from 2 to 20 nm, and remain in place even after removing the gold solution.
They demonstrate the spatial control by scanning a laser beam to write characters in gold inside a cell. Plasmonically enhanced molecular signals are then detected from nanoparticles, allowing their use as nano-chemical probes at targeted locations inside the cell, with intracellular molecular feedback. Such light-based control of the intracellular particle generation reaction also offers avenues for in-situ plasmonic device creation in organic targets, and may eventually link optical and electron microscopy.



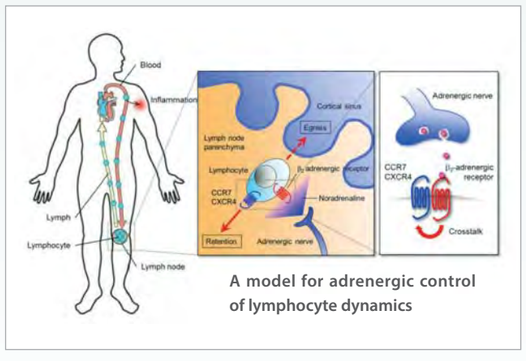

**Microscopic imaging of photofabricated characters of gold particles inside a cell (Scale bar, 12 micro meters). The red characters mean "Osaka University" in kanji characters.**

## Control of lymphocyte egress from lymph nodes through β2-adrenergic receptors

***J Exp Med.*** 211 : 2583-98, 2014.

Akiko Nakai, Yuki Hayano, Fumika Furuta, Masaki Noda, Kazuhiro Suzuki

Kazuhiro Suzuki and his group revealed that β2-adrenergic receptors (β2ARs) expressed on lymphocytes regulate their egress from lymph nodes by altering the responsiveness of chemokine receptors CCR7 and CXCR4. In mouse models of inflammation, signals though β2ARs were shown to inhibit trafficking of pathogenic lymphocytes and reduce their numbers recruited into inflamed tissues.




**A model for adrenergic control of lymphocyte dynamics**

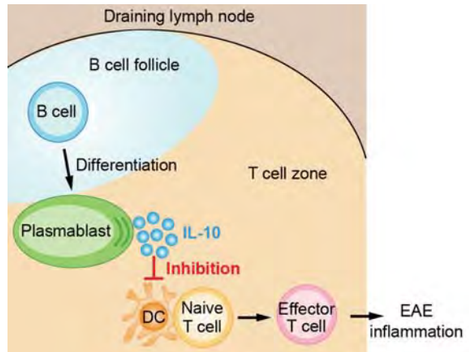
# Selected Articles

## Interleukin-10-producing plasmablasts exert regulatory function in autoimmune inflammation

***Immunity*** 41 : 1040-51, 2014.

Masanori Matsumoto, Akemi Baba, Takafumi Yokota, Hiroyoshi Nishikawa, Yasuyuki Ohkawa, Hisako Kayama, Axel Kallies, Stephen L. Nutt, Shimon Sakaguchi, Kiyoshi Takeda, Tomohiro Kurosaki, Yoshihiro Baba

Kurosaki, Baba and their group found that plasmablasts in the draining lymph nodes (dLNs), but not splenic B lineage cells, predominantly expressed IL-10 during experimental autoimmune encephalomyelitis (EAE). These plasmablasts were generated only during EAE inflammation. Mice lacking plasmablasts by genetic ablation of the transcription factors Blimp1 or IRF4 in B lineage cells developed an exacerbated EAE.

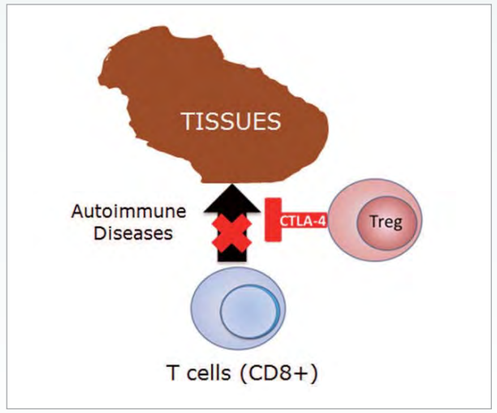



## Detection of self-reactive CD8+ T cells with an anergic phenotype in healthy individuals

***Science*** 346 : 1536-40, 2014.

Yuka Maeda, Hiroyoshi Nishikawa, Daisuke Sugiyama, Danbee Ha, Masahide Hamaguchi, Takuro Saito, Megumi Nishioka, James B. Wing, Dennis Adeegbe, Ichiro Katayama, Shimon Sakaguchi

Shimon Sakaguchi and his group found Treg can render self-reactive human CD8+ T cells anergic (i.e., hypoproliferative and cytokine hypoproducing upon antigen restimulation) in vitro, likely by controlling the costimulatory function of antigen-presenting cells. Anergic T cells were naïve in phenotype, lower than activated T cells in T cell receptor affinity for cognate antigen, and expressed several coinhibitory molecules, including cytotoxic T lymphocyte–associated antigen-4 (CTLA-4).



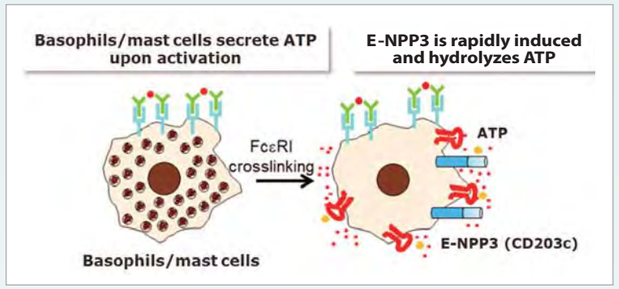

**Tregs render self-reactive human CD8+ T cells anergic**

## The ectoenzyme E-NPP3 negatively regulates ATP-dependent chronic allergic responses by basophils and mast cells

***Immunity*** 42 : 279–93, 2015.

Shih Han Tsai, Makoto Kinoshita, Takashi Kusu, Hisako Kayama, Ryu Okumura, Kayo Ikeda, Yosuke Shimada, Akira Takeda, Soichiro Yoshikawa, Kazushige Obata-Ninomiya, Yosuke Kurashima, Shintaro Sato, Eiji Umemoto, Hiroshi Kiyono, Hajime Karasuyama, Kiyoshi Takeda

Kiyoshi Takeda and his group showed that ectonucleotide pyrophosphatase-phosphodiesterase 3 (E-NPP3), also known as CD203c, rapidly induced by FcεRI crosslinking, negatively regulated chronic allergic inflammation. Basophil and mast cell numbers increased in Enpp3-/- mice with augmented serum ATP concentrations. Enpp3-/- mice were highly sensitive to chronic allergic pathologies, which was reduced by ATP blockade. FcεRI crosslinking induced ATP secretion from basophils and mast cells, and ATP activated both cells. ATP clearance was impaired in Enpp3-/- cells. Enpp3-/-P2rx7-/- mice showed decreased responses to FcεRI crosslinking. Thus, ATP released by FcεRI crosslinking stimulates basophils and mast cells for further activation causing allergic inflammation. E-NPP3 decreases ATP concentration and suppresses basophil and mast cell activity.



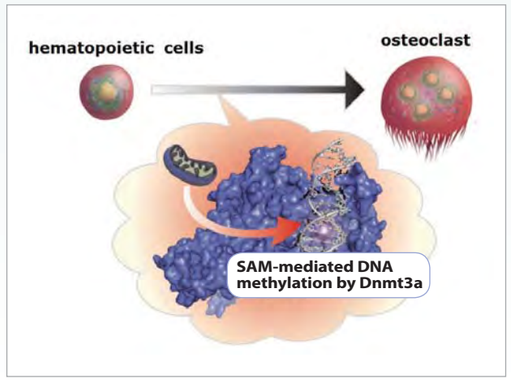

## DNA methyltransferase 3a regulates osteoclast differentiation by coupling to an S-adenosylmethionine–producing metabolic pathway

***Nat Med.*** 21:281-7, 2015.

Keizo Nishikawa, Yoriko Iwamoto, Yasuhiro Kobayashi, Fumiki Katsuoka, Shin-ichi Kawaguchi, Tadayuki Tsujita, Takashi Nakamura, Shigeaki Kato, Masayuki Yamamoto, Hiroshi Takayanagi, Masaru Ishii

Masaru Ishii and his group identified the de novo DNA methyltransferase Dnmt3a to be a transcription factor that couples metabolic changes to osteoclast differentiation. Receptor activator of nuclear factor-κB ligand (RANKL) is an essential cytokine for osteoclastogenesis that induces a metabolic shift toward oxidative metabolic processes, accompanied by an increase in S-adenosyl methionine (SAM) production.

They found that SAM-mediated DNA methylation by Dnmt3a regulates osteoclastogenesis viaepigenetic repression of the anti-osteoclastogenic gene and that Dnmt3a-deficient osteoclastprecursor cells do not undergo osteoclast differentiation efficiently.



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*The data were acquired using WEB of SCIENCE™ on April 30. 2015, and sorted by alphabetical order of the first authors.

# Lectures by PIs

| Lecturers | Meeting | Country | Date |
|---|---|---|---|
| **Shigekazu Nagata** | Henry Kunkel Lecture 2014 | USA | Apr. 3 |
| **Masaru Ishii** | Annual Meeting of Korean Society of Osteoporosis | Korea | Apr. 4 |
| **Shizuo Akira** | Distinguished Ludwig Lecture Series | Switzerland | Apr. 24 |
| **Ken J. Ishii** | 2nd International Molecular Immunology & Immunogenetics Congress (MIMIC-II 2014) | Turkey | Apr. 27 |
| **Cevayir Coban** | 2nd International Molecular Immunology & Immunogenetics Congress (MIMIC-II 2014) | Turkey | Apr. 29 |
| **Ken J. Ishii** | WHO Meetings of Stakeholders for Selected Health R&D Demonstration Project | Switzerland | May 7 |
| **Tadamitsu Kishimoto** | Mid-Term Conference of ICIS | Germany | May 14 |
| **Shizuo Akira** | Lerner Lecture | USA | May 19 |
| **Taroh Kinoshita** | 36th Congress of the Japanese Society on Thrombosis and Hemostasis | Japan | May 20 |
| **Takashi Saito** | EMBO Conference | Italy | May 20 |
| **Nicholas Isaac Smith** | META 14, the 5th International Conference on Metamaterials, Photonic Crystals and Plasmonics | Singapore | May 22 |
| **Shizuo Akira** | 1st EMBO Conference Series " Cellular Signalling & Cancer Therapy" | Croatia | May 26 |
| **Yoshichika Yoshioka** | 29th Annual Meeting of the Japan Biomagnetism and Bioelectromagnetics Society | Japan | May 29 |
| **Masaru Ishii** | 22nd International Symposium on Molecular Cell Biology of Macrophages | Japan | Jun. 3 |
| **Hisashi Arase** | 31th Annual Meeting of the Infectious Diseases Society in Obstetrics and Gynecology | Japan | Jun. 8 |
| **Kiyoshi Takeda** | 1st KI-OU Joint Symposium on Immunology | Sweden | Jun. 10 |
| **Shizuo Akira** | 1st KI-OU Joint Symposium on Immunology | Sweden | Jun. 10 |
| **Masaru Ishii** | 1st KI-OU Joint Symposium on Immunology | Sweden | Jun. 10 |
| **Tadamitsu Kishimoto** | Uehara Memorial Foundation Symposium 2014 | Japan | Jun. 17 |
| **Shizuo Akira** | 5th International Conference on Osteoimmunology:Interactions of the Immune and Skeletal Systems | Greece | Jun. 18 |
| **Tadamitsu Kishimoto** | 79th Annual Meeting of the Japanese Society of Interferon & Cytokine Research | Japan | Jun. 19 |
| **Ben Seymour** | Japan Society for the Study of Pain | Japan | Jun. 20 |
| **Kazuhiro Suzuki** | 9th IMS-JSI International Symposium on Immunology | Japan | Jun. 27 |
| **Takashi Saito** | FASEB Science Research Conference | USA | Jun. 30 |
| **Masahiro Yamamoto** | Institute for Genetic Medicine Research Congress 2014 | Japan | Jul. 3 |
| **Cevayir Coban** | University of Tokyo, Dept. of Animal Resource Sciences | Japan | Jul. 10 |
| **Hisashi Arase** | Annual Meeting of Shizuoka Rheumatism Network | Japan | Jul. 12 |
| **Takashi Saito** | 37th Naito Conference | Japan | Jul. 17 |
| **Kazuhiro Suzuki** | 37th Naito Conference | Japan | Jul. 17 |
| **Ben Seymour** | Memory and Awareness in Anesthesia 09 | Japan | Jul. 22 |
| **Masahiro Yamamoto** | 16th Immunology Summer School 2014 | Japan | Jul. 28 |
| **Hisashi Arase** | 21st Rheumatology Seminar | Japan | Jul. 31 |
| **Tomohiro Kurosaki** | JSI Summer School | Japan | Jul. 31 |
| **Ken J. Ishii** | 2nd International Immunological Memory and Vaccine Forum (IIMVF) | USA | Aug. 8 |
| **Hisashi Arase** | Taishan Academic Forum on Cancer & Immune Signaling Pathways and First Session Stem Cell Immunology Qilu International Forum | China | Aug. 10 |
| **Taroh Kinoshita** | 33rd Japanese Carbohydrate Symposium | Japan | Aug. 11 |
| **Taroh Kinoshita** | 51st Complement Symposium | Japan | Aug. 23 |
| **Tomohiro Kurosaki** | 2nd Symposium of International Immunological Memory and Vaccine Forum | USA | Aug. 26 |
| **Jun Hatazawa** | XI Congress of FWNMB in Cancun | Mexico | Aug. 28 |
| **Takashi Saito** | Cold Spring Harbor Asia Symposium | China | Sep. 3 |
| **Taroh Kinoshita** | Invited Seminar at Bioinformatics Institute, A*STAR | Singapore | Sep. 4 |
| **Toshio Yanagida** | 20th International Workshop on "Single Molecule Spectroscopy and Ultra Sensitive Analysis in the Life Sciences" | Germany | Sep. 4 |
| **Nicholas Isaac Smith** | 23rd Annual Meeting of the Bioimaging Society of Japan | Japan | Sep. 5 |
| **Tadamitsu Kishimoto** | Society for Regulatory Science of Medical Products | Japan | Sep. 5 |
| **Hisashi Arase** | Meet the Expert | Japan | Sep. 9 |
| **Tomohiro Kurosaki** | 5th International Congress on Cell Membranes and Oxidative Stress: Focus on Calcium Signaling and TRP Channels | Turkey | Sep. 10 |
| **Ben Seymour** | Japan Neuroscience | Japan | Sep. 11 |
| **Hisashi Arase** | 57th Japanese Society of Laboratory Medicine at Kinki Section | Japan | Sep. 20 |
| **Yutaka Hata** | 4th International Symposium in Computational Medical and Health Technology | Taiwan | Sep. 21 |
| **Tomohiro Kurosaki** | France-Japan Immunology Meeting | France | Sep. 23 |
| **Kiyoshi Takeda** | 13th Awaji International Forum on Infection and Immunity | Japan | Sep. 23 |
| **Tadamitsu Kishimoto** | 13th Awaji International Forum on Infection and Immunity | Japan | Sep. 25 |
| **Tomohiro Kurosaki** | 42nd Annual Meeting of the Japan Society for Clinical Immunology | Japan | Sep. 25 |
| **Cevayir Coban** | 13th Awaji International Forum on Infection and Immunity | Japan | Sep. 26 |
| **Masahiro Yamamoto** | 13th Awaji International Forum on Infection and Immunity | Japan | Sep. 26 |
| **Daron M. Standley** | Biophysical Society of Japan | Japan | Sep. 26 |
| **Kazuya Kikuchi** | Labeling and Nanoscopy | Germany | Sep. 26 |
| **Tadamitsu Kishimoto** | Novo Nordisk Innovation Summit 2014 | Japan | Oct. 1 |
| **Hisashi Arase** | Novo Nordisk Innovation Summit 2014 | Japan | Oct. 2 |
| **Takashi Saito** | Novo Nordisk Innovation Summit 2014 | Japan | Oct. 2 |
| **Ken J. Ishii** | Keystone Symposia on Molecular and Cellular Biology – The Modes of Action of Vaccine Adjuvants | USA | Oct. 9 |
| **Toshio Yanagida** | 2014 IEEE Photonics Conference | USA | Oct.13 |
| **Masahiro Yamamoto** | 87th Annual Meeting of the Japanese Biochemical Society | Japan | Oct.15 |
| **Hisashi Arase** | 2014 NHRI/IBMS Joint International Conference on Inflammation & Disease | Taiwan | Oct. 16 |
| **Tsuneyasu Kaisho** | 87th Annual Meeting of the Japanese Biochemical Society | Japan | Oct.16 |
| **Rikinari Hanayama** | 87th Annual Meeting of the Japanese Biochemical Society | Japan | Oct. 17 |
| **Kiyoshi Takeda** | 1st International Symposium on Mucosal Immunity and Vaccine Development 2014 | Japan | Oct. 20 |
| **Ken J. Ishii** | 1st International Symposium on Mucosal Immunity and Vaccine Development 2014 | Japan | Oct. 20 |
| **Hisashi Arase** | France-Japan Immunology Meeting | France | Oct. 23 |
| **Takashi Saito** | France-Japan Immunology Meeting | France | Oct. 23 |
| **Kazuhiro Suzuki** | France-Japan Immunology Meeting | France | Oct. 23 |
| **Ken J. Ishii** | 8th Vaccine & ISV Congress for International Society of Vaccine | USA | Oct. 27 |
| **Shizuo Akira** | Cytokines Down Under in 2014 : Second Annual Meeting of the International Cytokine and Interferon Society (ISIC) | Australia | Oct. 29 |
| **Tadamitsu Kishimoto** | Fourth International Conference on Regulatory T Cells and Th Subsets and Clinical Application in Human Diseases | China | Nov. 3 |
| **Shizuo Akira** | Fourth International Conference on Regulatory T Cells and Th Subsets and Clinical Application in Human Diseases | China | Nov. 4 |
| **Masaru Ishii** | 2014 CSHA Conference on Bone and Cartilage: from Development to Human Diseases | China | Nov. 4 |
| **Ken J. Ishii** | 2014 Fall Conference of the Korean Association of Immunologists | Korea | Nov. 6 |
| **Tadamitsu Kishimoto** | 57th Annual Meeting of the Japan Thyroid Association | Japan | Nov. 13 |
| **Hisashi Arase** | 2014 Forum Global Network for Infectious Disease Research at Chiba University | Japan | Nov. 15 |
| **Masaru Ishii** | Japan-Germany Cancer Workshop | Germany | Nov. 15 |
| **Toshio Yanagida** | 58th Symposium of the Japanese Society of Microscopy | Japan | Nov. 16 |
| **Toshio Yanagida** | NICT Open House 2014 | Japan | Nov. 27 |
| **Tomohiro Kurosaki** | International Seminar Series. Institute for Basic Science (IBS) | Korea | Dec. 3 |
| **Hisashi Arase** | 12th Japan Consortium for Glycobiology and Glycotechnology Symposium | Japan | Dec. 4 |
| **Tadamitsu Kishimoto** | 27th Annual Meeting of the Japan Society for Biological Therapy | Japan | Dec. 4 |
| **Tsuneyasu Kaisho** | 50th Annual Meeting of the Society for Hypertension Related Disease Model Research | Japan | Dec. 5 |
| **Jun Hatazawa** | International Workshop on Frontier of Science and Technology 2014 | China | Dec. 6 |
| **Tomohiro Kurosaki** | 43rd Annual Meeting of Japanese Society for Immunology | Japan | Dec.10 |
| **Nicholas Isaac Smith** | Japan-Singapore Workshop on Nanophotonics, Plasmonics, and Metamaterials | Singapore | Dec. 12 |
| **Toshio Yanagida** | Initiative for High-Dimensional Date-Driven Science through Deepening of Sparse Modeling | Japan | Dec. 16 |
| **Jun Hatazawa** | Lecture in TSNM2014 | Thailand | Dec. 19 |
| **Kiyoshi Takeda** | 2nd Hengstberger Symposium on "Microbial Sensors in the B lymphocyte Response" | Germany | Jan. 7 |
| **Daron M. Standley** | Antibody Design, Modeling, and Applications | Japan | Jan. 14 |
| **Nicholas Isaac Smith** | Opto Osaka 2015 | Japan | Jan. 14 |
| **Hisashi Arase** | 2015 Chiba Allergy Clinical Conference | Japan | Jan. 21 |
| **Ken J. Ishii** | NIAID 17th International Conference on Emerging Infectious Diseases (EID) | Taiwan | Jan. 28 |
| **Takashi Saito** | Fourth Bizan Immunology Symposium | Japan | Jan. 29 |
| **Hisashi Arase** | Fourth Bizan Immunology Symposium | Japan | Jan. 30 |
| **Tsuneyasu Kaisho** | 3rd Homeostatic Inflammation International Symposium | Japan | Jan. 31 |
| **Jun Hatazawa** | Symposium on Integrative Brain Imaging Center, NCNP | Japan | Feb. 5 |
| **Hisashi Arase** | 22th Autoantibody and Autoimmune Symposium | Japan | Feb. 7 |
| **Tadamitsu Kishimoto** | 150th Anniversary of Okayama University Medical School | Japan | Feb. 14 |
| **Tomohiro Kurosaki** | 150th Anniversary of Okayama University Medical School | Japan | Feb. 14 |
| **Masahiro Yamamoto** | International Research Center for Infectious Diseases, Joint Research Symposium for Young Researchers | Japan | Feb. 18 |
| **Toshio Yanagida** | Osaka University Center for Advanced Medical Engineering and Informatics 10th Anniversary Symposium | Japan | Mar. 2 |
| **Ken J. Ishii** | Lecture of Department of Mol. Biol. and Genetics, Life Sciences and Technologies Research Center Bogazici University | Turkey | Mar. 2 |
| **Taroh Kinoshita** | Gordon Research Conference on Glycobiology | Italy | Mar. 4 |
| **Daron M. Standley** | Analysis and Prediction of Protein Assembly Structures by Bioinformatics | Japan | Mar. 6 |
| **Ken J. Ishii** | Academia Sinica ABRC Lecture | Taiwan | Mar. 9 |
| **Ken J. Ishii** | Regulatory Affairs Professionals Society (RAPS) Taiwan Chapter, National Tsung Hua University | Taiwan | Mar. 11 |
| **Hisashi Arase** | 2015 Annual Meeting of Atopy Research Center (ARC) | Japan | Mar. 12 |
| **Masaru Ishii** | Advances in Targeted Therapies Meeting 2015 | France | Mar. 18 |
| **Jun Hatazawa** | Lecture in BSNM 2015 | Bangladesh | Mar. 20 |
| **Toshio Yanagida** | ImPACT Advanced Information Society Infrastructure Linking Quantum Artificial Brains in Quantum Network 1st General Meeting | Japan | Mar. 26 |
| **Rikinari Hanayama** | Invited Lecture at Taipei Medical University | Taiwan | Mar. 26 |

# Access Map

## SUITA CAMPUS Osaka University


### Access from the nearest station

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

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

**By Hankyu Bus**
Route 1 :
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.
Route 2 :
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.



QBiC
QUANTITATIVE BIOLOGY CENTER

**ADDRESS** 2F IFReC Research Building, Osaka University
3-1 Yamadaoka, Suita, 565-0871, Osaka, Japan
**TEL** General Affairs Section : +81-6-6879-4275
Accounting Section : +81-6-6879-4917
Research Planning & Management Office : +81-6-6879-4777
**FAX** +81-6-6879-4272
**E-mail** ifrec-office@ifrec.osaka-u.ac.jp