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WPI Immunology Frontier Research Center FY2015

Osaka University

World Premier International
Research Center

Osaka University
Immunology Frontier Research Center

Annual Report of IFReC FY 2015

Osaka University

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Contents

Message from the Director

As the Director of the Immunology Frontier Research Center (WPI-IFReC) at Osaka University, I am very pleased to present the IFReC annual report for fiscal 2015.

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

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

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

In FY2015, as an approach to nurturing young researchers, the seventh International Symposium of IFReC "Immunology at the Forefront" was effectively organized in combination with the fifth NIF Winter School by IFReC and Singapore Immunology Network. The symposium and the school pride themselves 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 to evolving into a world top immunology research center.

Shizuo Akira

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

Looking back on IFReC's activities over the years

Jun Sakanoue (Research Planning and Management Office, IFReC)

Since the establishment of IFReC in 2007, there have been 1090 science articles published by IFReC researchers (as of March, 2016). About 10% of these articles have appeared in "High Impact Journals" such as Science, Nature, Cell and their affiliates (1). The impact factor of a journal is a measure reflecting the number of citations to recent articles published in that journal, and is used to reflect the relative importance of a journal within its field.

Here, I evaluate the quality of the articles by IFReC researchers using percentiles of top papers. A percentile defines a fraction or subset of papers in a research field that has received a minimum number of citations. For example, if the value of the 10% percentile is 20, a paper in the same research field in the same year must receive at least 20 citations to belong to that percentile (2). The percentile in each research field gives a more equitable evaluation compared to citation numbers, because IFReC researchers have such diversity.

Table 1 shows the numbers of articles that appear in the percentiles of top papers in the three major research fields for IFReC; Immunology, Cell Biology, and Biochemistry & Molecular Biology. Data for the whole of Osaka University is also shown for comparison. Table 2 shows the percentage of these top papers among all papers.

Tabel 1 Number of Top 1% & Top 10% Articles

	Field	Osaka University	IFReC
1%	Immunology	48	34
	Cell Biology	35	8
	Biochemistry & Molecular Biology	41	5
10%	Immunology	219	138
	Cell Biology	211	38
	Biochemistry & Molecular Biology	339	46

Tabel 2 Percentage of Top 1% & Top 10% Articles

	Field	Osaka University	IFReC
1%	Immunology	3.8%	8.9%
	Cell Biology	2.1%	6.8%
	Biochemistry & Molecular Biology	1.1%	2.5%
10%	Immunology	17.5%	36.0%
	Cell Biology	12.4%	32.2%
	Biochemistry & Molecular Biology	9.4%	23.1%

The top papers by the whole of Osaka University appeared at a high level except for the top 10% in Biochemistry & Molecular Biology (9.4%), whereas the 23.1% level produced in this field by IFReC was more than double. Further, the value of 8.9% for the top 1% in Immunology represents an almost nine fold compilation of top papers in immunology fields at IFReC.

All the top 1% papers are listed in the final table (P.4). The highest score by the percentile analysis is recorded by the Daron Standley group (Katoh and Standley. Mol. Biol. Evol., 2013). 0.01% in a percentile means an unusually high value in the subject area.

Table 3 shows the academic contribution of IFReC to Osaka University in the three research fields.

Tabel 3 Contribution of IFReC to Osaka University

	Osaka University	IFReC	Outside of IFReC
Article Number in Immunology, Cell Biology, Biochemistry & Molecular Biology	4897	530	4061
Citation Impact	**22.2**	**52.9**	**17.7**
h-index	114	69	93

As well as the number and percentage of top papers in Tables 1 and 2, the average citation number per article (Citation Impact) of IFReC is much higher than that of Osaka University as a whole or other departments at the university (outside of IFReC). The academic contribution IFReC makes to Osaka University is reaffirmed through this objective analysis.

The percentage for international co-authored papers in these three research fields by Osaka University and IFReC were 29.5% and 43.0%, respectively (3). It is believed that international papers provide a high level of research. The high research level attracts higher international cooperation, and consequently improves the performance of internationalization in IFReC. The maintenance of a high academic research level is an indispensable element for the "Internationalization of the Institute", one of the goals of the WPI project.

References

(1) Annual Report of IFReC FY2014

(2) InCites™ by Thomson Reuters

(3) Web of Science™ by Thomson Reuters

All the Top 1% Articles by IFReC Researchers Since 2008

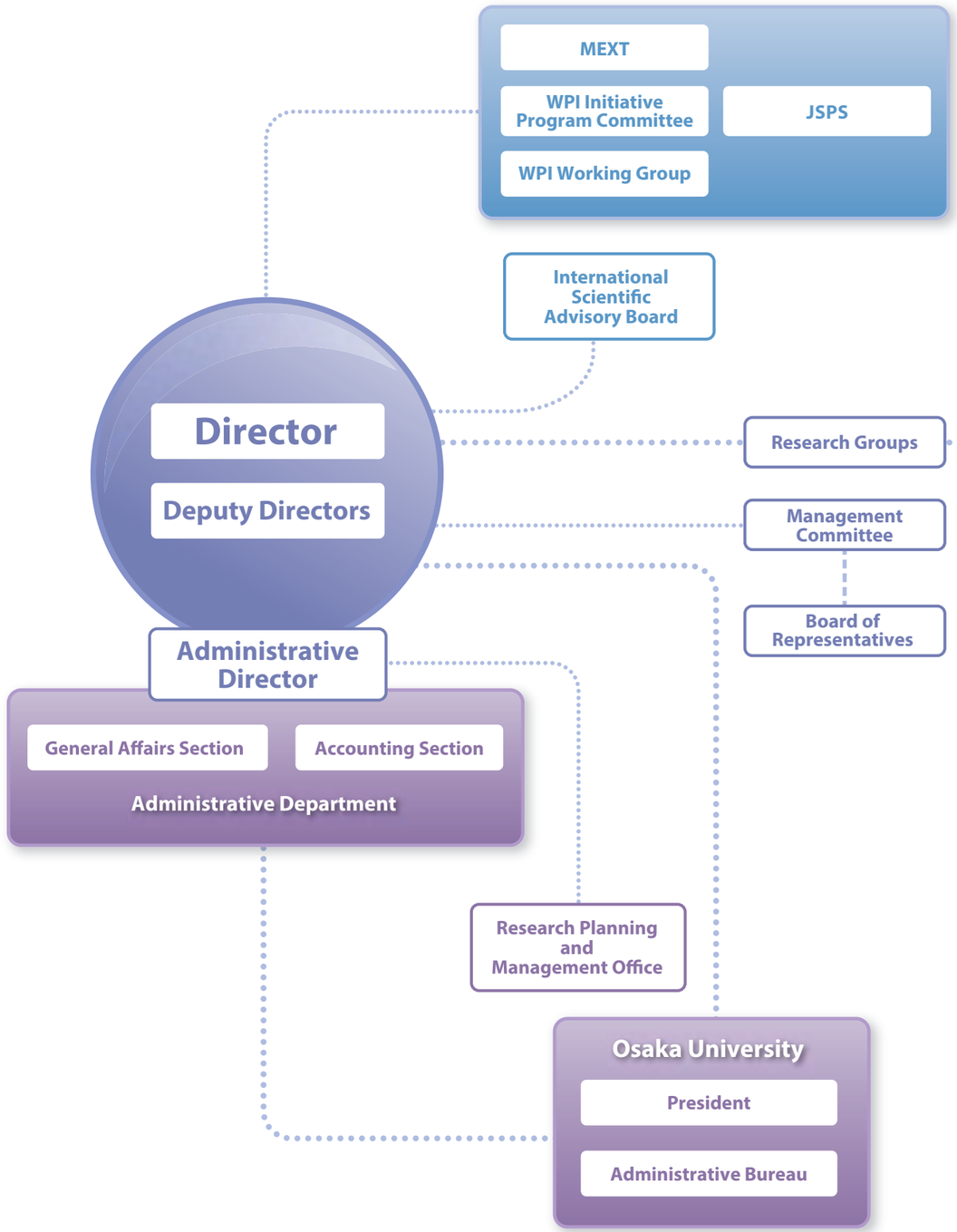
No.	Article	Percentile in Subject Area
1	Katoh, Kazutaka & Standley, Daron M. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Molecular Biology and Evolution 30, 772-780 (2013).	0.01
2	Ivanov, Ivaylo I. et al. Induction of Intestinal Th17 Cells by Segmented Filamentous Bacteria. Cell 139, 485-498 (2009).	0.01
3	Wing, Kajsa et al. CTLA-4 control over Foxp3(+) regulatory T cell function. Science 322, 271-275 (2008).	0.02
4	Forrest, Alistair R. R. et al. A promoter-level mammalian expression atlas. Nature 507, 462-470 (2014).	0.02
5	Takeuchi, Osamu & Akira, Shizuo. Pattern Recognition Receptors and Inflammation. Cell 140, 805-820 (2010).	0.03
6	Miyara, Makoto et al. Functional Delineation and Differentiation Dynamics of Human CD4(+) T Cells Expressing the FoxP3 Transcription Factor. Immunity 30, 899-911 (2009).	0.03
7	Kawai, Taro &; Akira, Shizuo. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nature Immunology 11, 373-384 (2010).	0.04
8	Kawai, Taro & Akira, Shizuo. Toll-like Receptors and Their Crosstalk with Other Innate Receptors in Infection and Immunity. Immunity 34, 637-650 (2011).	0.04
9	Atarashi, Koji et al. T-reg induction by a rationally selected mixture of Clostridia strains from the human microbiota. Nature 500, 232-236 (2013).	0.04
10	Klionsky, Daniel J. et al. Guidelines for the use and interpretation of assays for monitoring autophagy. Autophagy 8, 445-544 (2012).	0.05
11	Abbas, Abul K. et al. Regulatory T cells: recommendations to simplify the nomenclature. Nature Immunology 14, 307-308 (2013).	0.06
12	Kato, Hiroki et al. Length-dependent recognition of double-stranded ribonucleic acids by retinoic acid-inducible gene-I and melanoma differentiation-associated gene 5. Journal of Experimental Medicine 205, 1601-1610 (2008).	0.07
13	Hibar, Derrek P. et al. Common genetic variants influence human subcortical brain structures. Nature 520, 224-229 (2015).	0.11
14	Saitoh, Tatsuya et al. Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1 beta production. Nature 456, 264-268 (2008).	0.15
15	Atarashi, Koji et al. ATP drives lamina propria T(H)17 cell differentiation. Nature 455, 808-812 (2008).	0.15
16	Kumar, Himanshu et al. Toll-like receptors and innate immunity. Biochemical and Biophysical Research Communications 388, 621-625 (2009).	0.20
17	Misawa, Takuma et al. Microtubule-driven spatial arrangement of mitochondria promotes activation of the NLRP3 inflammasome. Nature Immunology 14, 454-460 (2013).	0.20
18	Deretic, Vojo et al. Autophagy in infection, inflammation and immunity. Nature Reviews Immunology 13, 722-737 (2013).	0.22
19	Satoh, Takashi et al. The Jmjd3-Irf4 axis regulates M2 macrophage polarization and host responses against helminth infection. Nature Immunology 11, 936-944 (2010).	0.23
20	Oka, Takafumi et al. Mitochondrial DNA that escapes from autophagy causes inflammation and heart failure. Nature 485, 251-255 (2012).	0.23
21	Uematsu, Satoshi et al. Regulation of humoral and cellular gut immunity by lamina propria dendritic cells expressing Toll-like receptor 5. Nature Immunology 9, 769-776 (2008).	0.24
22	Ohkura, Naganari et al. T Cell Receptor Stimulation-induced Epigenetic Changes and Foxp3 Expression are Independent and Complementary Events Required for Treg Cell Development. Immunity 37, 785-799 (2012).	0.26
23	Sakaguchi, Shimon et al. The plasticity and stability of regulatory T cells. Nature Reviews Immunology 13, 461-467 (2013).	0.29
24	Sakaguchi, Shimon et al. FOXP3(+) regulatory T cells in the human immune system. Nature Reviews Immunology 10, 490-500 (2010).	0.33
25	Nishikawa, Hiroyoshi & Sakaguchi, Shimon. Regulatory T cells in tumor immunity. International Journal of Cancer 127, 759-767 (2010).	0.35
26	Saijo, Shinobu et al. Dectin-2 Recognition of alpha-Mannans and Induction of Th17 Cell Differentiation Is Essential for Host Defense against Candida albicans. Immunity 32, 681-691 (2010).	0.35
27	Yang, Huan et al. A critical cysteine is required for HMGB1 binding to Toll-like receptor 4 and activation of macrophage cytokine release. PNAS 107, 11942-11947 (2010).	0.35
28	Ueno, Masaki et al. Layer V cortical neurons require microglial support for survival during postnatal development. Nature Neuroscience 16, 543-551 (2013).	0.35
29	Clarke, Michael et al. Genome of Acanthamoeba castellanii highlights extensive lateral gene transfer and early evolution of tyrosine kinase signaling. Genome Biology 14, R11 (2013).	0.36
30	Satoh, Takashi et al. LGP2 is a positive regulator of RIG-I- and MDA5-mediated antiviral responses. PNAS 107, 1512-1517 (2010).	0.37
31	Saitoh, Tatsuya et al. Neutrophil Extracellular Traps Mediate a Host Defense Response to Human Immunodeficiency Virus-1. Cell Host & Microbe 12, 109-116 (2012).	0.37
32	Ishii, Ken J. et al. TANK-binding kinase-1 delineates innate and adaptive immune responses to DNA vaccines. Nature 451, 725-729 (2008).	0.40
33	Tokunaga, Fuminori et al. Involvement of linear polyubiquitylation of NEMO in NF-kappa B activation. Nature Cell Biology 11, 123-132 (2009).	0.41
34	Dorner, Marcus et al. Completion of the entire hepatitis C virus life cycle in genetically humanized mice. Nature 501, 237-241 (2013).	0.41
35	Matsunaga, Kohichi et al. Two Beclin 1-binding proteins, Atg14L and Rubicon, reciprocally regulate autophagy at different stages. Nature Cell Biology 11, 385-396 (2009).	0.44
36	Lesina, Marina et al. Stat3/Socs3 Activation by IL-6 Transsignaling Promotes Progression of Pancreatic Intraepithelial Neoplasia and Development of Pancreatic Cancer. Cancer Cell 19, 456-469 (2011).	0.48
37	Oldenburg, Marina et al. TLR13 Recognizes Bacterial 23S rRNA Devoid of Erythromycin Resistance-Forming Modification. Science 337, 1111-1115 (2012).	0.51
38	Schubert, Desiree et al. Autosomal dominant immune dysregulation syndrome in humans with CTLA4 mutations. Nature Medicine 20, 1410-1416 (2014).	0.52
39	Yanai, Hideyuki et al. HMGB proteins function as universal sentinels for nucleic-acid-mediated innate immune responses. Nature 462, 99-103 (2009).	0.55
40	Marichal, Thomas et al. DNA released from dying host cells mediates aluminum adjuvant activity. Nature Medicine 17, 996-1002 (2011).	0.57
41	Goto, Yoshiyuki et al. Innate lymphoid cells regulate intestinal epithelial cell glycosylation. Science 345, 1254009 (2014).	0.61
42	Ishikawa, Eri et al. Direct recognition of the mycobacterial glycolipid, trehalose dimycolate, by C-type lectin Mincle. Journal of Experimental Medicine 206, 2879-2888 (2009).	0.63
43	Yokosuka, Tadashi et al. Programmed cell death 1 forms negative costimulatory microclusters that directly inhibit T cell receptor signaling by recruiting phosphatase SHP2. Journal of Experimental Medicine 209, 1201-1217 (2012).	0.67
44	Ohkura, Naganari et al. Development and Maintenance of Regulatory T cells. Immunity 38, 414-423 (2013).	0.70
45	Fukuda, Akihisa et al. Stat3 and MMP7 Contribute to Pancreatic Ductal Adenocarcinoma Initiation and Progression. Cancer Cell 19, 441-455 (2011).	0.73
46	Nishikawa, Hiroyoshi & Sakaguchi, Shimon. Regulatory T cells in cancer immunotherapy. Current Opinion In Immunology 27, 42376 (2014).	0.77
47	Wing, Kajsa & Sakaguchi, Shimon. Regulatory T cells exert checks and balances on self tolerance and autoimmunity. Nature Immunology 11, 42564 (2010).	0.78
48	Yamasaki, Sho et al. Mincle is an ITAM-coupled activating receptor that senses damaged cells. Nature Immunology 9, 1179-1188 (2008).	0.79
49	Kitano, Masahiro et al. Bcl6 Protein Expression Shapes Pre-Germinal Center B Cell Dynamics and Follicular Helper T Cell Heterogeneity. Immunity 34, 961-972 (2011).	0.79
50	Sugiyama, Daisuke et al. Anti-CCR4 mAb selectively depletes effector-type FoxP3(+)CD4(+) regulatory T cells, evoking antitumor immune responses in humans. PNAS 110, 17945-17950 (2013).	0.79
51	Satoh, Takashi et al. Critical role of Trib1 in differentiation of tissue-resident M2-like macrophages. Nature 495, 524-528 (2013).	0.80
52	Nishikawa, Keizo et al. DNA methyltransferase 3a regulates osteoclast differentiation by coupling to an S-adenosylmethionine-producing metabolic pathway. Nature Medicine 21, 281-287 (2015).	0.82
53	Katoh, Kazutaka & Frith, Martin C. Adding unaligned sequences into an existing alignment using MAFFT and LAST. Bioinformatics 28, 3144-3146 (2012).	0.90
54	Saitoh, Tatsuya et al. Atg9a controls dsDNA-driven dynamic translocation of STING and the innate immune response. PNAS 106, 20842-20846 (2009).	0.95
55	Shichita, Takashi et al. Peroxiredoxin family proteins are key initiators of post-ischemic inflammation in the brain. Nature Medicine 18, 911-917 (2012).	0.98
56	Nguyen, Nam Trung et al. Aryl hydrocarbon receptor negatively regulates dendritic cell immunogenicity via a kynurenine-dependent mechanism. PNAS 107, 19961-19966 (2010).	1.00
57	Haldar, Malay et al. Heme-Mediated SPI-C Induction Promotes Monocyte Differentiation into Iron-Recycling Macrophages. Cell 156, 1223-1234 (2014).	1.00

InCites dataset updated 2016-02-26.

TOP 1% Articles: 57 - 5.4 times as many as average (based on theoretical value)
TOP 10% Articles: 289 - 2.7 times as many as average (based on theoretical value)

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
Immunoparasitology	Masahiro Yamamoto
Biochemistry and Immunology	Shigekazu Nagata

Imaging Group

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

Informatics Group

Information Systems	Yutaka Hata
Systems Immunology	Daron M. Standley

Units for Combined Research Fields

Quantitative Immunology	Yutaro Kumagai
	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 Institutes of Biomedical Innovation, Health and Nutrition

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 Centre, The University of Auckland, New Zealand

Committee and Advisory Board for IFReC

As one of the nine centers selected for the World Premier International Research Center Initiative (WPI), IFReC has been the subject of evaluations including site visits and follow-ups by the WPI Program Committee.
In close cooperation with the Program Directors, the Program Officer and working group members, the WPI Program Committee conducts follow-up activities on progress being made by the WPI institutes including IFReC, with an eye to developing them into "highly visible research centers".

WPI Program Committee

Program Director

As of FY2015

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

Deputy Program Director

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

Program Committee Members

Toshiaki Ikoma	Special Adviser, Canon Inc., Japan
[Chairperson] Hiroo Imura	Honorary Chairman, Foundation for Biomedical Research and Innovation, Japan Former President, Kyoto University, Japan
Hiroto Ishida	President Emeritus, Kanazawa Gakuin University, Japan
Tsutomu Kimura	Advisor, National Institution for Academic Degrees and University Evaluation, Japan
Makoto Kobayashi	Director, Research Center for Science Systems, Japan Society for the Promotion of Science Nobel Laureate in Physics (2008)
Kiyoshi Kurokawa	Adjunct Professor, National Graduate Institute for Policy Studies, Japan
Ryozo Nagai	President, Jichi Medical University, Japan
Michiharu Nakamura	Counselor to the President, JST, Japan
Ryoji Noyori	Director-General, Center for Research and Development Strategy, JST, Japan Nobel Laureate in Chemistry (2001)
Shinichiro Ohgaki	President, Japan Water Research Center
Robert Aymar	Senior Advisor, Atomic Energy Authority, France
Rita Cowell	Distinguished Professor, University of Maryland, USA
Richard Dasher	Consulting Professor, Stanford University, USA
Ian Halliday	Professor Emeritus, The University of Edinburgh, UK
Chuan Poh LIM	Chairman, Agency for Science, Technology and Research, Singapore

Working Group Leader and Assigned Members

As of FY2015

[Program Officer] Takehiko Sasazuki	Institute for Advanced Study, Kyushu University, Japan
Hiroshi Kiyono	Institute of Medical Science, The University of Tokyo, Japan
Nagahiro Minato	Kyoto University, Japan
Kazuhiko Yamamoto	The University of Tokyo, Japan
Günter J. Hämmerling	German Cancer Research Center DKFZ, Germany
Hisataka Kobayashi	National Cancer Institute, NIH, USA
Philippe Kourilsky	The Institute of Pasteur, France

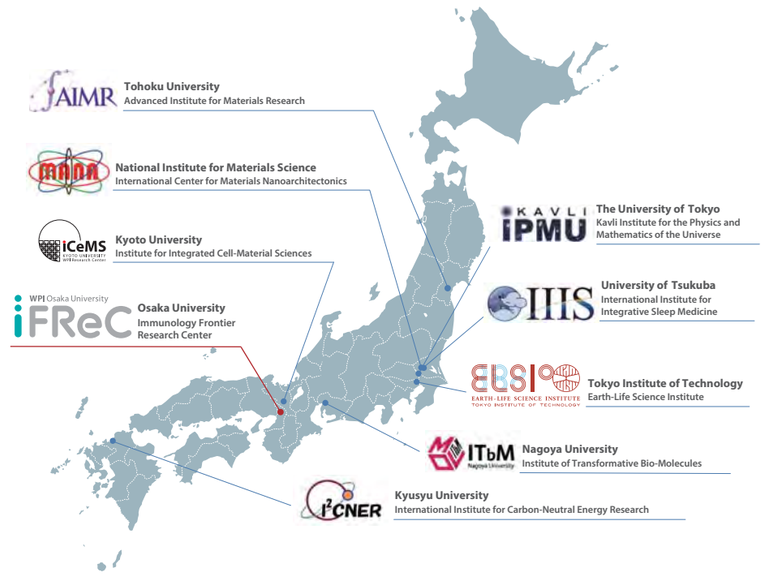
International Scientific Advisory Board

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

As of FY2015

Jeffrey Ravetch	The Rockefeller University, USA	**Immunology**
Christopher Goodnow	Garvan Institute of Medical Research, Australia Australian National University	**Immunology**
Richard Locksley	University of California, San Francisco	**Immunology**
Anne O'Garra	The Francis Crick Institute, UK	**Immunology**
Lewis L. Lanier	University of California, San Francisco , USA	**Immunology**
Yale E. Goldman	University of Pennsylvania, USA	**Imaging**
David Westhead	University of Leeds, UK	**Bioinformatics**
Vladimir Brusic	Boston University, USA	**Bioinformatics**
Mo Jamshidi	University of Texas, USA	**Bioinformatics**
Philip Chen	University of Macau, China	**Bioinformatics**

The Selected Nine World Premier International Research Centers



Administrative Office of IFReC

General Affairs Section

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

Accounting Section

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

Research Planning & Management Office

- Research promotion and support
  (Consultation for grants and patents, fusion research program, etc.)
- Establishing research environments
  (Facility and safety management, research agreement, etc.)
- Fostering young scientists
  (Winter School, 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	Kazuhiko Maeda
Assistant Professor	Takashi Satoh
	Kenta Maruyama
	Mikaël Martino
Postdoctoral Fellow	4
Research Assistant	7
Visiting Scientist	1
Support Staff	6

The immune system serves to defend against pathogens. Evolutionally conserved pattern-recognition receptors (PRRs) play a key role in the innate immune response to microbes ranging from bacteria to fungi, protozoa and viruses. After sensing microbial components, PRRs stimulate production of inflammatory factors such as cytokines/interferons by dendritic cells to induce an acquired immune response, which is 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 in our body. Therefore, both activation and inactivation of immune responses must be strictly controlled. To gain a deeper understanding of the immune system, we have examined the regulatory mechanisms of innate and acquired immune responses, and novel PRRs capable of sensing microbial components. We focused on the role of zinc-finger antiviral protein (ZAP) against viral infection.

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

ZAP acts as an RNA sensor and induces the degradation of murine leukemia retrovirus (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 (P-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.

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 antiviral effector molecules involved in this response have not been fully identified. Here, we show that zinc-finger antiviral 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 antiviral effector molecule that mediates the type-I-IFN-dependent host defense against SINV (Kozaki et al, Int Immunol 2015). Although we have clarified the importance of ZAP in anti-viral responses, we still do not fully understand how ZAP eliminates the viruses. Indeed, ZAP suppresses the replication of various viruses, such as SINV, Ebola virus, Marburg virus, MLV, HIV-1 and HBV, indicating that ZAP targets both positive-sense and negative-sense RNA viruses, retroviruses and DNA viruses. On the other hand, ZAP does not suppress the replication of VSV, a negative-sense RNA virus. ZAP inhibits the protein translation of viral components 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)

We have clarified the molecular mechanism of colchicine, a drug for gout attack. In response to Nigericin, monosodium urate, or silica particles, NLRP3 forms the 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 also 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).

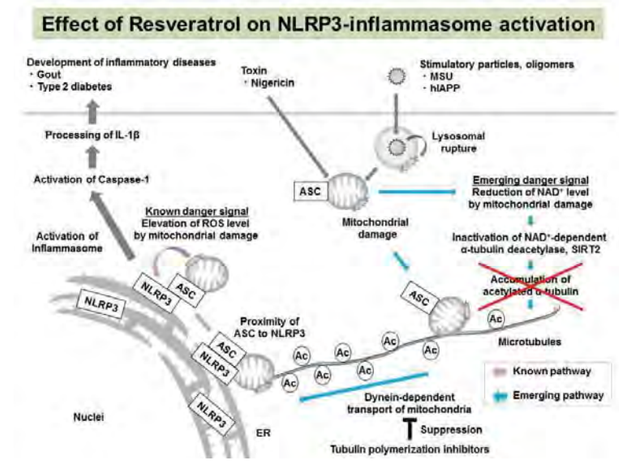


Figure 1.
Effect of resveratrol on NLRP3-inflammasome activation

Recent Publications

- Martino MM, Maruyama K, Kuhn GA, Satoh T, Takeuchi O, Muller R, Akira S. Inhibition of IL-1R1/MyD88 signalling promotes mesenchymal stem cell-driven tissue regeneration. Nat. Commun. 7, 11051 (2016).
- Yokogawa M, Tsushima T, Noda NN, Kumeta H, Enokizono Y, Yamashita K, Standley DM, Takeuchi O, Akira S, Inagaki F. Structural basis for the regulation of enzymatic activity of Regnase-1 by domain-domain interactions. Sci. Rep. 6, 22324 (2016).
- Misawa T, Saitoh T, Kozaki T, Park S, Takahama M, Akira S. Resveratrol inhibits the acetylated alpha-tubulin-mediated assembly of the NLRP3-inflammasome. Int. Immunol. 27, 425-434 (2015).
- Mino T, Murakawa Y, Fukao A, Vandenbon A, Wessels HH, Ori D, Uehata T, Tartey S, Akira S, Suzuki Y, Vinuesa CG, Ohler U, Standley DM, Landthaler M, Fujiwara T, Takeuchi O. Regnase-1 and Roquin regulate a common element in inflammatory mRNAs by spatiotemporally distinct mechanisms. Cell 161, 1058-1073 (2015).
- Kozaki T, Takahama M, Misawa T, Matsuura Y, Akira S, Saitoh T. Role of zinc-finger anti-viral protein in host defense against Sindbis virus. Int. Immunol. 27, 357-364 (2015).

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

Glycosylphosphatidylinositol (GPI) is a glycolipid that acts as a membrane anchor of 150 or more human cell surface proteins. Our laboratory aims to clarify the entire process of GPI-anchored protein (GPI-AP) biosynthesis, functional roles of lipid and glycan moieties of GPI-anchors, and molecular bases of diseases associated with GPI-anchor deficiencies. We reported several new findings in 2015.

1. Identification of PGAP4 gene involved in attachment of a side-branch to GPI

A number of mammalian GPI-APs, such as prion proteins, Thy-1 and renal dipeptidase, have N-acetylgalactosamine (GalNAc) side-branch attached to the first mannose (Figure 1). This side-branch can be elongated by galactose and sialic acid. Biological functions of the GalNAc side-branch had been unknown primarily because genes involved in generation of the side-branch had not been identified. Taking advantage of a monoclonal antibody that recognizes the GalNAc side-branch, we identified a gene necessary for transfer of GalNAc to GPI. The gene termed *PGAP4* (for *P*ost *G*PI *A*ttachment to *P*roteins *4*) encoded a Golgi-resident membrane protein that most likely is GalNAc transferase itself. In order to clarify the biological significance of the GalNAc side-branch, we are generating PGAP4 knockout mice.

2. Demonstration that endosome-to-trans Golgi network (TGN) retrograde transport system is critical for post-Golgi anterograde transport of GPI-APs

In the ER-to-plasma membrane anterograde transport of GPI-APs, ER-to Golgi transport is mediated by COPII coated transport vesicles whereas mechanisms of post-Golgi transport have been unclear. Aiming to clarify post-Golgi transport of GPI-APs, we developed a system for genome-wide screening of genes involved in ER-to-plasma membrane transport of GPI-APs and identified components of the Golgi-associated retrograde protein (GARP) complex, a tethering factor involved in endosome-to-TGN transport. In GARP-defective cells, post-Golgi anterograde transport of not only GPI-APs but also transmembrane proteins was impaired. We concluded that GARP-dependent endosome-to-TGN retrograde transport is required for recycling of factors critical to efficient post-Golgi anterograde transport of GPI-APs (Hirata T et al., Mol Biol Cell, 2015).

3. Characterization of new mutations that cause inherited GPI deficiencies (IGDs)

We reported the first cases of inherited GPI deficiency caused by mutations in *PIGY* and *PIGG* genes in collaborations with domestic and international research groups (Ilkovsky B et al., Hum Mol Genet, 2015; Makrythanasis P et al., Am J Hum Genet, 2016). Two affected individuals with homozygous hypomorphic *PIGY* mutations suffered from severe multi-system disease and early death. Two individuals with homozygous *PIGY* promoter mutations showed moderate developmental delay and microcephaly. Therefore, different mutations in the same *PIGY* gene caused very different symptoms and thus expanded the phenotype of IGDs. Five individuals from three families with biallelic loss-of-function *PIGG* mutations showed intellectual disability, early-onset seizures and hypotonia.

To date, pathogenic mutations that cause IGDs were identified in 14 of 27 genes involved in biosynthesis, transfer to proteins and maturation of GPI anchors.

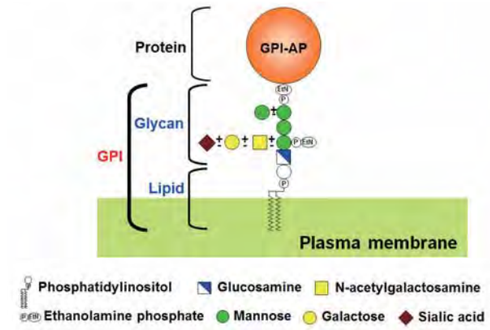


Figure 1.
The N-acetylgalactosamine (GalNAc)-containing side-branch of GPI anchors. Some GPI-anchored proteins, such as prion proteins, Thy-1 and renal dipeptidases, have a GalNAc side-branch linked to the first mannose. The GalNAc side-brunch can be elongated by galactose and sialic acid.

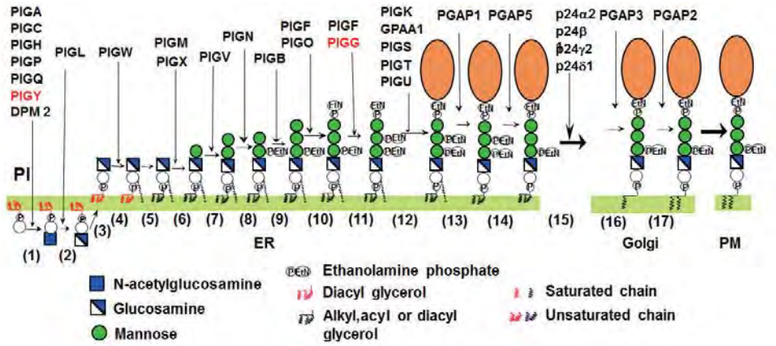


Figure 2.
Mutations in two genes *PIGY* and *PIGG* (shown in red) involved in GPI anchor biosynthesis were found to be causal to inherited GPI deficiency.

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- Hirata T, Fujita M, Nakamura S, Gotoh K, Motooka D, Murakami Y, Maeda Y, Kinoshita T. Post-Golgi anterograde transport requires GARP-dependent endosome-to-TGN retrograde transport. Mol. Biol. Cell 26, 3071-3084 (2015).
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Immunopathology

Atsushi Kumanogoh, MD/PhD

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

Our research team is involved in two approaches, that is, basic and clinical immunology. For the 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 and therapy of human immunological disorders, such as autoimmunity, allergy, immune deficiency, cancer/metastasis, and neurodegenerative diseases. We focus here on the involvement of Sema4A in functions of CD8-positie T cell responses.

Sema4A and CD8-positive T cells

Sema4A, a class IV transmembrane semaphorin, plays crucial roles in dendritic cells (DCs), macrophages, and T cells. Sema4A is highly expressed in polarized T helper type 1 (Th1) cells and is important for helper T-cell differentiation. In Sema4A-deficient mice, Th1 responses to heat-killed *Propionibacterium acne*—a Th1-inducing bacterium, are impaired in vivo. In contrast, T helper type 2 (Th2) responses against *Nippostrongylus brasiliensis*—a Th2-inducing intestinal nematode, are potentiated in these mice. TIM-2 appears to be the functional receptor for Sema4A, and the association between TIM-2 and Sema4A in Th1 cells negatively regulates Th2 cells. A recent study revealed a novel function of Sema4A associated with maintenance of regulatory T (Treg) cells. Sema4A directly interacts with the receptor neuropilin-1 (Nrp-1) expressed by Treg cells, and this interaction potentiates Treg-cell function and survival at inflammatory sites. In this pathway, Sema4A ligation of Nrp-1 restrains Akt phosphorylation in the cell body and at the immunologic synapse by recruiting phosphatase and tensin homologue (PTEN). This in turn promotes nuclear localization of the transcription factor Foxo3a, which plays an important role in the development and programming of Treg cells. This Sema4A/Nrp-1-dependent pathway is crucial for maintenance of immune homeostasis, and is involved in inflammatory colitis and Treg-cell stability in tumor tissues in vivo. These findings suggest that Sema4A is a potential target for therapies aimed at limiting Treg-cell–mediated tumor-induced tolerance without inducing autoimmunity. With respect to DC, Sema4A derived from both DCs and T cells is important for T cell–mediated immunity. Soluble Sema4A proteins and anti-Sema4A mAb bound to Sema4A on the surface of DCs enhance T-cell activation. Consistent with this, DCs derived from Sema4A-deficient mice stimulate allogeneic T cells poorly compared to wild-type DCs. By contrast, when CD4$^+$ T cells from Sema4A-deficient or wild-type mice are cultured with allogeneic DCs derived from wild-type mice, there are no differences in mixed lymphocyte reactions. These results suggest that DC-derived Sema4A is directly and critically involved in the activation of T cells reactive to alloantigens on DCs.

In this study, we show that a class IV semaphorin, Sema4A, regulates CD8$^+$ T cell activation and differentiation through activation of mTOR complex (mTORC) 1. Sema4A deficient CD8$^+$ T cells exhibited impairments in production of IFN-γ and TNF-α and induction of the effector molecules granzyme B, perforin, and FAS-L. Upon infection with OVA-expressing Listeria monocytogenes, pathogen-specific effector CD8$^+$ T cell responses were significantly impaired in Sema4A deficient mice. Furthermore, Sema4A deficient CD8$^+$ T cells exhibited reduced mTORC1 activity and elevated mTORC2 activity, suggesting that Sema4A is required for optimal activation of mTORC1 in CD8$^+$ T cells. IFN-γ production and mTORC1 activity in Sema4A deficient CD8$^+$ T cells were restored by administration of recombinant Sema4A protein. In addition, we show that plexin B2 is a functional receptor of Sema4A in CD8$^+$ T cells. Collectively, these results not only demonstrate the role of Sema4A in CD8$^+$ T cells, but also reveal a novel link between a semaphorin and mTOR signaling.

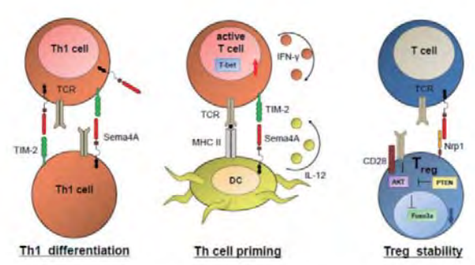

Figure 1.

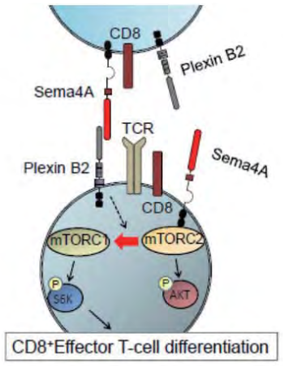

Figure 2.

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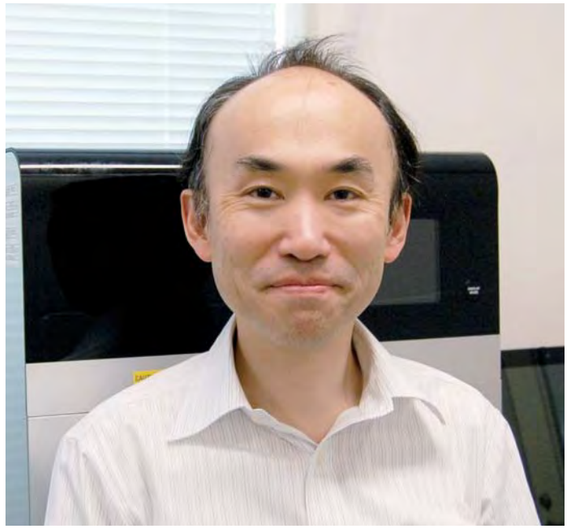
Immunochemistry



Hisashi Arase, MD/PhD

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

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

A) Interaction between immune receptors and herpesviruses

PILRα is one of the immune inhibitory receptors that are expressed on various immune cells. We previously found that both PILRα and PILRβ recognize CD99 as a host ligand (Shiratori et al. J. Exp. Med. 2004). In addition, we have identified PANP as a new ligand for PILR (Kogure et al. Biochem. Biophys. Res. Commun. 2011). PILRα is a unique receptor that that has binding sites for both sugar chain and protein structure (Wang et al. J. Immunol. 2008; Kuroki et al. Proc. Natl. Acad. Sci. USA. 2014). We found that PILRα associates with glycoprotein B (gB), an envelope protein of herpes simplex virus-1 (HSV-1), and the interaction between PILRα and gB is involved in membrane fusion during HSV-1 infection (Satoh et al. Cell 2008; Wang et al. J. Virol. 2009). We further analyzed host cell molecules that associate with HSV-1 gB and found that non-muscle myosin heavy chain (NMHC-IIA) associates with gB and is involved in HSV-1 infection (Arii et al. Nature 2010). We also found that Siglec-4 (MAG, myelin associated glycoprotein) associates with VZV gB and mediates VZV infection. Because Siglec-4 is specifically expressed in neural tissues, Siglec-4 seemed to be involved in neurotropic characteristic of VZV (Suenaga et al. Proc. Natl. Acad. Sci. USA. 2010; Suenaga et al. J. Biol. Chem. 2015).

B) PILRα plays an important role in regulation of inflammation

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

C) Identification of new sensing system for immune abnormalities

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

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 are targets for autoantibodies in autoimmune disease patients (Jin et al. Proc. Natl. Acad. Sci. USA. 2014; Tanimura et al. Blood. 2015). Autoantibody binding to misfolded proteins transported to the cell surface by MHC class II molecules was strongly correlated with susceptibility to autoimmune disease. This suggested that misfolded proteins, which normally would not be exposed to the immune system, can be targets for autoantibodies as 'neo self' antigens, which is involved in the pathogenicity of autoimmune diseases (Arase Adv. Immunol. 2016)(Figure 2).

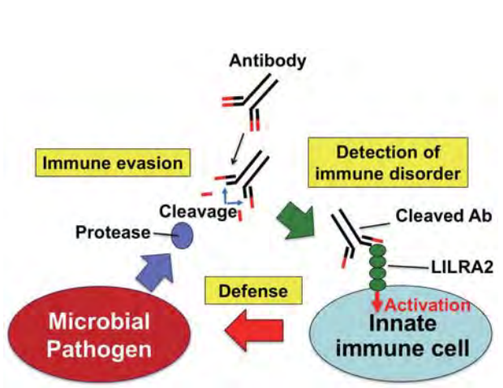


Figure 1. Microbially cleaved immunoglobulins are sensed by the innate immune receptor LILRA2
Microbial pathogens produce various proteases and degrade immunoglobulins to evade immune system. However, immune system has acquired a novel receptor, LILRA2, which specifically recognizes degraded immunoglobulins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

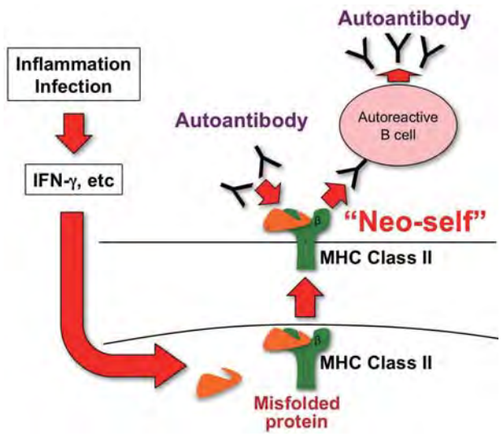


Figure 2. Misfolded proteins transported to the cell surface by MHC class II molecules are targets for autoantibodies
Cellular misfolded proteins are generally degraded in the cells and are not transported to outside the cells. Therefore, misfolded proteins transported to the cell surface by MHC class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

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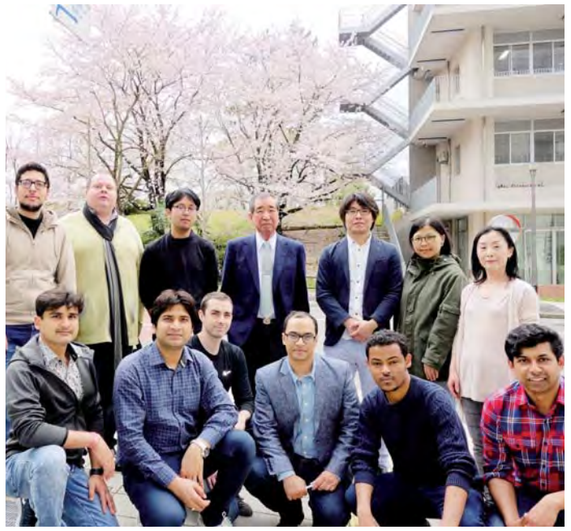
Immune Regulation



Tadamitsu Kishimoto, MD/PhD

Professor	Tadamitsu Kishimoto
Associate Professor	**Barry Ripley**
Assistant Professor	**Kazuya Masuda**
Postdoctoral Fellow	**5**
Research Assistant	**3**
Support Staff	**2**

A Summary of our Recent Research

1. Arid5a regulates naïve CD4$^+$ T cell-fate through selective stabilization of Stat3 mRNA (published)

We recently demonstrated that Arid5a in T cells regulates the half-life of Stat3 mRNA in an IL-6-dependent manner. Reduction of STAT3 level in Arid5a-deficient T cells led to the imbalance of STATs (STAT1, 3, 5) activation under Th17-polarizing conditions, which contributed to the alteration of the character of inflammatory T cells into that of anti-inflammatory T cells. Thus, Arid5a plays an important role in T cells through post-transcriptional control of inflammatory gene expression.

2. Immunomodulatory drugs inhibit TLR4-induced type-1 interferon production independently of Cereblon via suppression of the TRIF/IRF3 pathway (published)

Here we developed Cereblon-deficient mice using the CRISPR-Cas9 system. TLR-induced cytokine responses were unaffected by Cereblon deficiency in vivo. Moreover, IMiD treatment inhibited cytokine production even in the absence of Cereblon. The IMiD-induced suppression of cytokine production therefore occurs independently of Cereblon in mice. Further investigation revealed that IMiDs are potent inhibitors of TLR-induced type-1 interferon production via suppression of the TRIF/IRF3 pathway. These data suggest that IMiDs may prove effective in the treatment of disorders characterized by the ectopic production of type-1 interferon. Significantly, these properties are mediated separately from thalidomide's teratogenic receptor, Cereblon. Thus, certain therapeutic properties of Thalidomide can be separated from its harmful side effects.

3. Regulation of expression levels of Arid5a in macrophages through TLR4 signaling (ongoing study)

We recently found that Arid5a protein is rapidly increased and then quickly degraded in LPS-treated macrophages. Interestingly, Arid5a protein is highly phosphorylated and ubiquitinated when its protein levels reach peak maximum 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.

4. Arid5a deficiency protects against the development of bleomycin-induced pulmonary fibrosis (ongoing study)

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 the important role of Arid5a in lung tissue fibrosis.

5. Pathogenic role of Arid5a in endotoxin shock (ongoing study)

Interestingly, Arid5a KO mice are found to be completely resistant to lipopolysaccharide 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 recently found that Arid5a in T cells regulates production of IFN-γ through stabilization of *Tbx21* mRNA. Consistent with the data, IFN-γ protein level in serum was significantly reduced in LPS-treated Arid5a KO mice. We are also generating macrophage and T cell specific Arid5a KO mice to examine which cells are important for Arid5a to augment endotoxin shock.

6. The analysis of Arid5a function using Arid5a-transgenic mice (ongoing study).

We recently generated Arid5a-transgenic mice using DNA pronuclear injection. The phenotype of Arid5a-transgenic mice seemed to be normal; however, cytokine levels such as IL-6 and TNF-α in serum were significantly elevated in LPS-treated Arid5a transgenic mice compared to WT mice. Similarly, IL-6 level was increased in peritoneal macrophages following LPS stimulation. These results suggest that Arid5a promotes production of inflammatory cytokines in macrophages following LPS treatment. Currently we are investigating the mechanism of how Arid5a drives pro-inflammatory cytokine productions through posttranscriptional modulation.

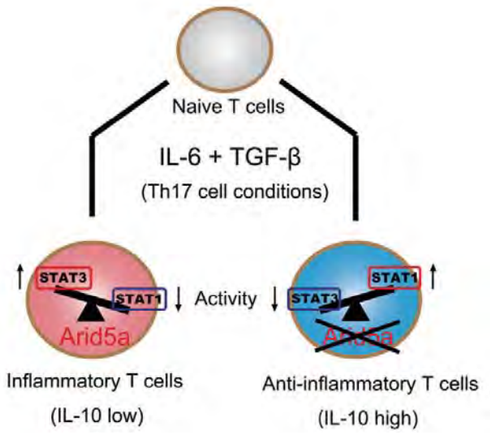


Figure 1.
T cell-fate decision by Stat3 stabilizing Arid5a.

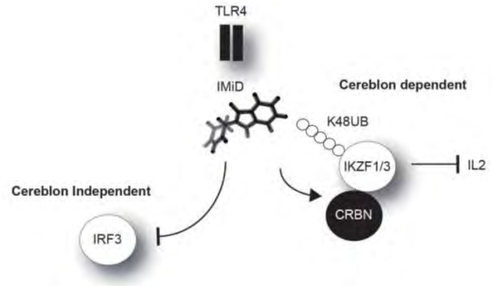


Figure 2.
The anti-inflammatory of immunomodulatory drugs (IMiDS) result from Cereblon dependent and independent pathways.

Recent Publications

- Chinen I, Nakahama T, Kimura A, Nguyen NT, Takemori H, Kumagai A, Kayama H, Takeda K, Lee S, Hanieh H, Ripley B, Millrine D, Dubey PK, Nyati KK, Fujii-Kuriyama Y, Chowdhury K, Kishimoto T. The aryl hydrocarbon receptor/microRNA-212/132 axis in T cells regulates IL-10 production to maintain intestinal homeostasis. Int. Immunol. 27(8), 405-415 (2015).
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Mucosal Immunology

Kiyoshi Takeda, MD/PhD

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Associate Professor	Eiji Umemoto
Assistant Professor	Hisako Kayama
	Shih-Han Tsai
Postdoctoral Fellow	2
Research Assistant	5
Support Staff	3

The intestine is a unique organ, inhabited by a large number of commensal microbes. The intestinal mucosa is protected from commensal microbes and pathogenic microorganisms by several types of barriers. One of these barriers is the mucus layers produced by intestinal secretory cells. Mucus is a viscous fluid enriched in mucin glycoproteins and the mucus layers play an indispensable role in the prevention of enteric pathogen infection and intestinal inflammation. Epithelial cells in the large intestine, where tremendous numbers of bacteria exist, are covered by thick mucus composed of two layers: the inner, firm mucus layer and outer, loose mucus layer. Many commensal bacteria are present in the outer mucus layer, whereas none are present in the inner mucus layer. The segregation of commensal bacteria and colonic epithelia by the inner mucus layer is critically involved in the pathogenesis of intestinal inflammation. Indeed, several mouse models of spontaneous colitis show the disappearance of the bacteria-free zone in the inner mucus layer. In the small intestine, a variety of cationic anti-microbial peptides including α, β-defensin that are produced by Paneth cell, and regenerating islet-derived 3 (Reg3) family proteins that are induced by pattern recognition receptor signaling, mediate the segregation of intestinal bacteria and epithelial cells. However, the large intestine contains no professional cells that produce anti-microbial peptides, although many bacteria are present. Thus, the mechanism by which mucus prevents bacterial access to the epithelial surface of the large intestine is unknown.

Ly6/Plaur domain containing 8 (Lypd8) segregates intestinal bacteria and colonic epithelia

Ly6/Plaur domain containing 8 (Lypd8) is a member of Ly6/Plaur family proteins characterized by the presence of Ly6/Plaur domain. We found that Lypd8 was a highly glycosylated GPI-anchored protein, highly and selectively expressed in epithelial cells of the colon and constitutively secreted into the intestinal lumen. We generated Lypd8-deficient mice to analyze the function of Lypd8 in vivo. In wild-type mice, there is a bacteria-free space just above the colonic epithelia, however this free space was not present, and many bacteria invaded the inner mucus layer and the colonic epithelia in Lypd8$^{-/-}$ mice. We next examined which kinds of bacteria invaded the inner mucus layer and the colonic epithelia by analyzing bacterial DNA in the colonic tissue of Lypd8$^{-/-}$ mice. Several bacterial genera were increased in Lypd8$^{-/-}$ colonic tissues, particularly *Proteus, Escherichia* and *Helicobacter* were tremendously increased. Thus, intestinal bacteria such as *Proteus*, *Helicobacter* and *Escherichia*, all of which are Gram-negative rod bacteria possessing multiple flagella, penetrated the inner mucus layer and were present adjacent to the epithelial cell layers of Lypd8$^{-/-}$ mice.

Lypd8-deficient mice are highly susceptible to intestinal inflammation

Proteus and *Helicobacter* are involved in the pathogenesis of inflammatory bowel diseases in both mice and humans. Therefore, we next analyzed the sensitivity to dextran sulfate sodium (DSS)-induced intestinal inflammation in Lypd8$^{-/-}$ mice. DSS administration induced severe intestinal inflammation with high mortality in Lypd8$^{-/-}$ mice. Oral administration of gentamicin, which is active for flagellated bacteria such as *Proteus*, *Escherichia* and *Helicobacter*, reduced the numbers of flagellated bacteria in the intestine and restored the bacteria-free zone just above the colonic epithelia in Lypd8$^{-/-}$ mice. Furthermore, gentamicin treatment ameliorated the intestinal inflammation in DSS-treated Lypd8$^{-/-}$ mice. Thus, invasion of the epithelial layer by flagellated bacteria such as *Proteus*, *Escherichia* and *Helicobacter* correlates with the increased sensitivity to intestinal inflammation in Lypd8$^{-/-}$ mice.

Lypd8 binds to bacterial flagella and thereby suppresses their motility

We next addressed the mechanisms by which Lypd8 inhibits bacterial invasion of the epithelial layer. Flow cytometric analysis revealed that Lypd8 binds to a subpopulation of the intestinal bacteria, and further showed that Lypd8 preferentially binds to flagellated bacteria such as *Proteus* and *Escherichia*. Scanning electron microscopic analysis of *Proteus mirabilis* incubated with recombinant Lypd8 showed that Lypd8 binds to flagella of *P. mirabilis*. We next investigated whether Lypd8 affects motility of flagellated bacteria. Lypd8 expressed on Caco-2 cells, human colonic epithelial cell line suppressed swarming motility of *P. mirabilis*. Furthermore, swimming motility of *P. mirabilis* and commensal *E. coli* was inhibited in semi-agar plate including recombinant Lypd8 protein. Thus, highly glycosylated Lypd8 associated and suppressed the motility of *P. mirabilis*.

Taken together, these findings suggest that Lypd8 mediates segregation of commensal flagellated bacteria from the intestinal epithelial layer in the large intestine, and thereby contributes to the maintenance of gut homeostasis.

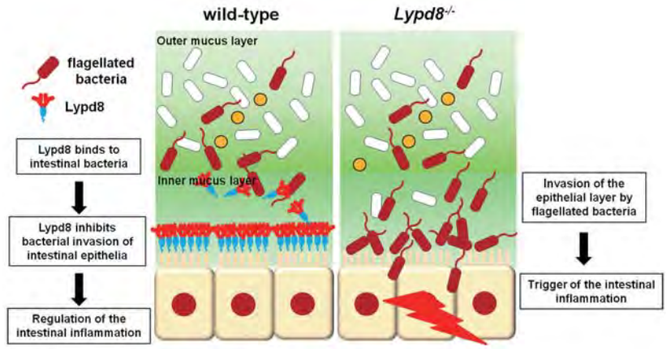

Figure. Lypd8 maintains gut homeostasis by segregating intestinal bacteria and colonic epithelia
Lypd8 is a highly glycosylated GPI-anchored protein, and highly and selectively expressed in epithelial cells on the uppermost layer of the intestinal gland and shed into the intestinal lumen. Lypd8 inhibits bacterial invasion of the colonic mucosa by binding to the intestinal bacteria, and thereby regulates the intestinal inflammation.

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- Masahata K, Umemoto E, Kayama H, Kotani M, Nakamura S, Kurakawa T, Kikuta J, Gotoh K, Motooka D, Sato S, Higuchi T, Baba Y, Kurosaki T, Kinoshita M, Shimada Y, Kimura T, Okumura R, Takeda A, Tajima M, Yoshie O, Fukuzawa M, Kiyono H, Fagarasan S, Iida T, Ishii M and Takeda K. Generation of colonic IgA-secreting cells in the cecal patch. Nat. Commun. 5, 3704 (2014).
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Molecular Immunology

Hitoshi Kikutani, MD/PhD

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Postdoctoral Fellow	1
Research Assistant	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 signals to neighboring wild type cells. Thus, LMP1 may contribute to EBV infection by suppressing host humoral responses.

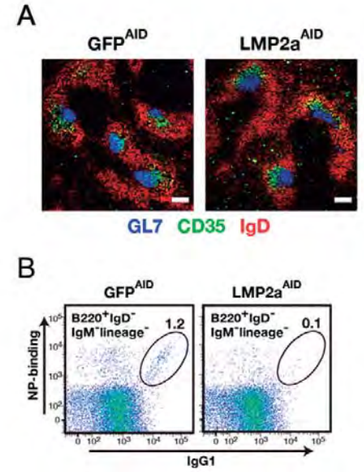

Figure 1. EBV LMP2A reduces the threshold for selection of high-affinity B cells. (A) The conditional expression of LMP2a by the AID (activation-induced cytidine deaminase) promoter-driven Cre did not alter GC size and structure in the spleen upon immunization. (B) Generation of antigen-binding B cells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

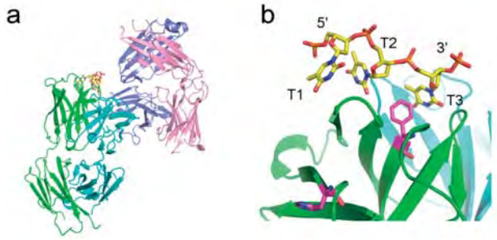

Figure 2. Crystal structure of the ligand-bound anti-DNA antibody, clone 71F12 derived from an acute SLE patient.
Fab of 71F12 and oligonucleotides (5'-TTTTT-3') were co-crystalized (a) and (b). In the crystal, electron density of 3 thymines were determined. 71F12 interacted with the third thymine base (T3) through base-stacking interaction, for which the conserved mutation Y33F we found in NGS analysis was required (b).

2) Phylogenetic and structural bases for anti-nuclear antibody generation in acute systemic lupus erythematosus

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

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

The crystal structure of the anti-DNA antibody 71F12 that exhibited high-affinity to both ds- and ssDNA, revealed that somatic mutation contributed to rigid antigen recognition largely through interaction with a nucleobase, but not with ribose or phosphodiester of DNA. Thus, we proposed that at least a portion of high-affinity anti-ssDNA antibodies could target nucleobases of unfolded dsDNA. The structure analysis also supports affinity maturation of anti-DNA lineage in SLE.

Recent Publications

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Experimental Immunology

Shimon Sakaguchi, MD/PhD

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Postdoctoral Fellow	3
Research Assistant	4
Support Staff	6

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

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 (Tregs). Yet how Treg cells effectively control potentially hazardous self-reactive T cells in humans remains an open question. In the previous year, we showed that Treg cells were able to 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, in particular, the expression of CD80 and CD86, via CTLA-4 expressed by Treg cells. This year, in collaboration with Kajsa Wing's group in Karolinska Institute, Sweden, we have addressed the role of CTLA-4 in immune regulation by conditionally deleting CTLA-4 expression in Treg cells for a limited period in adult mice (Klocke et al., PNAS, 2016). Such mice spontaneously developed fatal systemic autoimmune disease. Together with our previous findings with congenital Treg-specific CTLA-4 deficiency in mice and CTLA-4 gene mutations in humans (Wing et al., Science 2008; Yamaguchi et al., PNAS, 2013; Wing et al., Immunity, 2014; Schubert et al., Nature Med., 2014), the results indicate that CTLA-4-mediated control of self-reactive T cells by Treg cells is indispensable for maintaining self-tolerance, and can be a key target in controlling autoimmunity and tumor immunity.

In our study of FOXP3$^+$ CD25$^+$ CD4$^+$ T cells in humans, we have previously shown that they are heterogeneous in function and differentiation status, including suppressive or non-suppressive cells as well as resting or activated Treg cells (Miyara et al., Immunity, 2009). Our attempts to further differentiate suppressive FOXP3$^+$ T cells from non-suppressive FOXP3$^+$ T cells have revealed that CD15s (Sialyl Lewis x) is expressed by activated or effector FOXP3$^+$ Treg cells but not by non-suppressive FOXP3$^+$ T cells, enabling reliable distinction of the two populations (Miyara et al., PNAS, 2015). This year we have also analyzed tumor-infiltrating FOXP3$^+$ T cells, in particular, how FOXP3$^+$ T cell subpopulations contribute to tumor immunity (Saito et al., Nat. Med., 2016). In general, abundant Treg-cell infiltration into tumors is associated with poor clinical outcomes in various types of cancers. However, the role of Treg cells is controversial in colorectal cancers (CRCs), in which FOXP3$^+$ T-cell infiltration indicated better prognosis in some studies. We have shown that CRCs, which are commonly infiltrated by suppression-competent FOXP3^{hi} Treg cells, can be classified into two types by the degree of additional infiltration of FOXP3^{lo} non-suppressive T cells. The latter, distinguished from FOXP3$^+$ Treg cells by being CD45RA$^-$ and unstable in FOXP3 expression, secreted inflammatory cytokines. Indeed, CRCs with abundant infiltration of the FOXP3^{lo} T cells showed significantly better prognosis than those with predominant FOXP3^{hi} Treg-cell infiltration. Development of such inflammatory FOXP3^{lo} non-Treg cells appeared to depend on tissue secretion of IL-12 and TGF-β, and was correlated with tumor invasion of intestinal bacteria, especially *Fusobacterium nucleatum*. Our results indicate that functionally distinct subpopulations of tumor-infiltrating FOXP3$^+$ T cells contribute in opposing ways to determining CRC prognosis. Depletion of FOXP3^{hi} Treg cells from tumor tissues to augment tumor immunity can be an effective treatment of CRCs and other cancers, while strategies to locally increase FOXP3^{lo} non-Treg cells could be tumor-suppressive and -preventive.

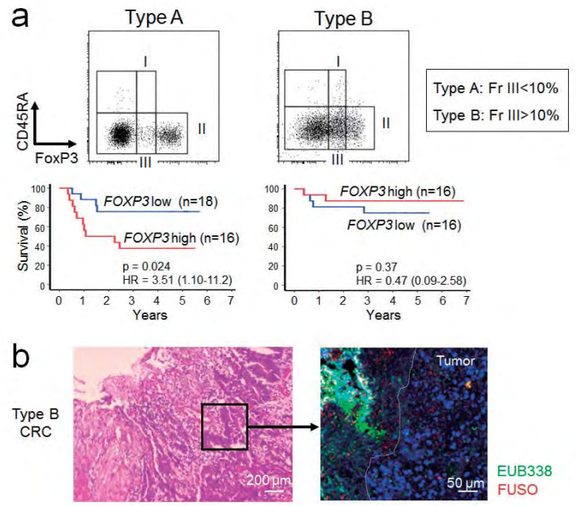

Figure 1. FOXP3$^+$ T-cell subsets in colorectal cancers (CRCs). (A) In half of CRCs, tumor-infiltrating FOXP3$^+$ T cells are predominantly effector or activated Treg cells, which are FOXP3^{high} CD45RA$^-$ (Fr. II); in the other half of CRCs, tumor-infiltrating FOXP3$^+$ T cells contain FOXP3^{low}CD45RA$^-$ non-Treg cells (Fr. III) in addition to Fr. II FOXP3^{high} effector Treg cells. The former type CRCs, designated as Type A, show poor prognosis when FOXP3 gene transcription by tumor tissues is high because the high expression is due to predominant presence of FOXP3^{high} Treg cells. In contrast, Type B CRCs show better prognosis when FOXP3 gene transcription by cancer tissues is high, because non-Treg cells, which produce inflammatory cytokines, contribute to the high transcription. (B) Predominant infiltration of FOXP3^{low} non-Treg cells in Type B CRCs can be attributed to tumor infiltration of intestinal bacteria, in particular, Fusobacteria.

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Cell Signaling

Takashi Saito, PhD

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 activation regulation in the relationship with other signals such as co-stimulation and innate signaling and cytoskeletal regulation.

[1] Regulation of activation signal at immune synapse

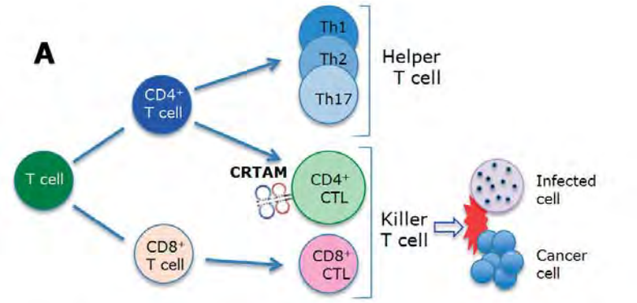
T cell activation is induced through TCR microclusters, which recruit TCR, kinase and adaptors and initiate activation signals. During analyses of dynamic movement of cytoplasmic molecules ZAP70 and SLP76, we found that SLP76 made clear clusters upon very weak stimulation while ZAP70 did not, and that SLP76 clusters were generated by outside-in signal through LFA1. Each TCR-MC was bordered by adhesion molecules; LFA-1, paxillin, Pyk2 and vinculin at initial phase. Because the centralized TCR-MC with surrounded adhesion ring resembles the immune synapse in micro-scale, we named the structure “microsynapse”. TCR-MCs, ZAP70, SLP76 and F-actin were detected at the center, and LFA1, Paxillin, Pyk2 are in the outside ring of the microsynapse. Down-modulation of microsynapse resulted in the reduction of the size of TCR-MC and clustering of F-actin and SLP76, consequently, decreasing pErk, IL-2 production and cell growth. TCR activation, particularly weak stimulation, was supported by the microsynapse. The microsynapse is essential for the generation of mature TCR-MCs and the following events of cell adhesion and T cell activation.

[2] Regulation of CD4 CTL development

We have cloned CRTAM as an early activation molecule predominantly expressed on CD8 T cells and NK cells upon activation. We demonstrated that CRTAM is an early activation marker and an adhesion receptor, and is bound to its ligand Necl-2. We found that a small proportion of $CD4^+$T cells also express CRTAM. $CRTAM^+CD4$ T cells possess mRNA expression profiles of both CD4+ and CD8+ T cells, that is, they express genes in cytotoxic T cells (CTL) such as perforin, granzyme B, Eomesdermin and IFNγ. $CRTAM^+CD4$ T cells but not $CRTAM^-CD4$ T cells exclusively secrete IFNγ, and exhibit cytotoxicity, indicating that $CRTAM^+$ CD4 T cells confer generation of $CD4^+$ CTLs. CRTAM induces the expression of Eomes and CTL-related genes through intracellular signaling of CRTAM, which induce CTL lineage T cells. Thus, CRTAM is also the first surface marker for CD4+CTL, and can be used for therapeutic targets for inflammatory diseases where CD4+ CTLs seems to induce the diseases. (Figure 1)

[3] T cell activation regulation by self-recognition

It has been suggested that T cells recognize self-antigen. T cells are semi-activated by self-recognition through continuous contact with APC presenting self-antigens, which subsequently enhance T cells responsiveness to foreign antigen, while T cells become anergic in the absence of the semi-activation. However, how the signaling events trigger T cells upon self-recognition has not been analyzed. We investigated the mechanism of semi-activation and its status of T cells by self-recognition. We show that self-recognition could trigger multiple signaling pathways downstream of TCR including enhanced phosphorylation of p38, Erk and S6, and most remarkably the calcium-calcineurin-NFAT pathway was activated leading to the accumulation of NFAT in the nuclei. Chip-Seq analysis shows that NFAT activated by self-recognition can bind to genes such as Egr that play a critical role in the activation and function of T cells. Indeed, transgenic Egr expression rescued IL-2 response in T cells after loss of self-recognition. This may partly represent the status of semi-activation of T cells upon self-recognition in vivo.



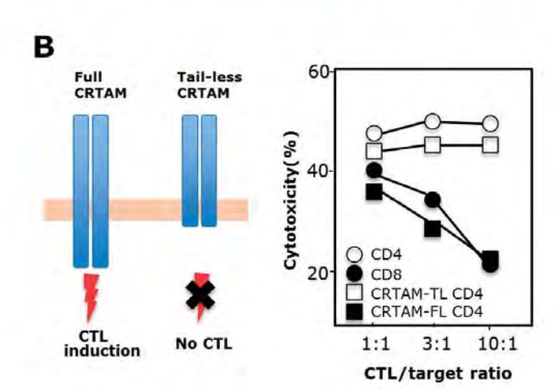


Figure 1. CRTAM determines $CD4^+$CTL lineage
[A] $CD4^+$CTLs are generated from CRTAM-expressing $CD4^+$T cells. CRTAM+ but not CRTAM- CD4+T cells develop CD4+T cells with CTL function, which may function in protecting infection and cancer, and in inducing inflammation depending on circumstances.
[B] CRTAM signals induce development of CD4+CTL. Full-length CRTAM (FL)- but tail-less CRTAM (TL)-expressing T cells induce $CD4^+$CTL exhibiting cytotoxicity.

Recent Publications

- Hashimoto-Tane A, et al. Micro adhesion rings surrounding TCR microclusters are essential for T cell activation. J. Exp. Med. (2016) in press.
- Takeuchi A, et al. CRTAM determines the CD4+ cytotoxic T lymphocyte lineage. J. Exp. Med. 213, 123-138, (2016).
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- Imanishi T, et al. Nucleic acid sensing by T cells initiates Th2 cell differentiation. Nat. Commun. 5, 3566 (2015).

Lymphocyte Differentiation

Tomohiro Kurosaki, MD/PhD

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Introduction

In the case of T cell-dependent humoral immune responses, after initial T-B cell interactions, B cells undergo brisk proliferation before entering the germinal center (GC) reaction. Once a GC is established in the B cell follicle, it develops a dark zone (DZ) and a light zone (LZ) and B cells migrate between these two zones. Proliferation and somatic hypermutation (SHM) occur in the DZ followed by B cell shuttling to the LZ, where they exit the cell cycle. Because antigen-presenting follicular dendritic cells (FDCs) and follicular helper T (T_{FH}) cells reside in the LZ, GC B cells expressing high-affinity BCRs in the LZ are selected in response to signals provided by cognate T_{FH} cells. Then, as cells transition from the LZ to DZ state, proliferation is induced. Such iterative cycles of diversification and selection are thought to be a driving force for subsequent affinity maturation. With successful selection, the LZ GC B cells, in addition to returning to the DZ, can enter into two terminal fates, memory B cells or plasma cells. Our laboratory has focused on understanding the cellular and molecular basis underlying fate decisions of LZ GC cells (back to the DZ GC, differentiation into memory B cells, or plasma cells).

Regulation of GC B cell proliferation by transcription factor Foxo1

Foxo1, forkhead box protein O1, is a transcriptional regulator that localizes to the nucleus and controls gene expression. Phosphorylation by Akt causes Foxo1 translocation to the cytoplasm where it can be degraded. Foxo1 is perhaps best known for its ability to promote cellular quiescence, thus phosphatidyl inositol 3'OH kinase (PI3K)-mediated activation of Akt and subsequent inactivation of Foxo1 is necessary for pre-B cell proliferation. However, the role of Foxo1 in mature B cells, particularly GC B cells, remained obscure. A strong hint that Foxo1 might play a critical and unconventional role in controlling gene expression within GC B cells has been provided by the discovery that GC-derived lymphomas frequently carry mutations in Foxo1 that prevent its inactivation by Akt.

To address Foxo1 function in GC biology, we used inducible gene knockout of Foxo1. We found that Foxo1 exerts a proliferative role, rather than promoting quiescence as previously thought. Mechanistically, upon sufficient provision of T cells help, $Foxo1^{-/-}$ LZ GC cells could not induce transcription factors, c-Myc and BATF, both of which are essential for GC B cell proliferation. Hence, Foxo1 is required for stimulating GC cell division, explaining the previous observations that GC-derived lymphoma frequently possesses a constitutively active form of Foxo1. Moreover, we found that the regulatory network through Foxo1 is highly context-specific (anti-proliferative in pre-B cells versus proliferative in GC B cells).

Selection mechanisms of GC cells into the memory compartment

One key unresolved question about the cell-fate decisions made in the GC is how memory B cells are selected and generated. Two models, stochastic and instructive, have been put forward to explain the memory-selection mechanism. Studies of a wide variety of genetic perturbations have shown that, overall, distortions in the affinity composition of GC B cells are indistinguishable from those in memory B cells, suggesting that memory B cells are selected stochastically from among GC B cells. The alternative "instructive" model postulates that selection into the memory B cells is regulated by a large number of means, such as extrinsic signals from T cells, including cytokines and cell-contact-dependent signals, and affinity-based signals through the BCR.

To address the selection and generation mechanisms of memory B cells, we established a mouse model for marking GC B cells and their progeny within a given time window. Using these mice, we found that LZ GC B cells with BCRs of lower affinity had higher expression of the transcriptional repressor Bach2 than those with BCRs of higher affinity and were predisposed to enter the memory B cell pool. Moreover, haploinsufficiency of Bach2 resulted in suppression of the generation of memory B cells and Bach2 expression in GC cells was inversely correlated with the strength of T cell help. Together, we propose a model in which weak help from T cells maintains relatively high expression of Bach2 in LZ GC B cells, which predisposes them to enter the memory B cell pool (Figure).

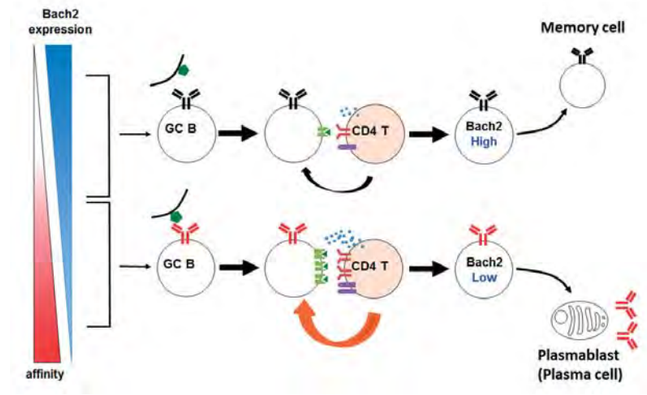

Figure . Mechanism of selection of memory B cells and plasma cells from germinal center B cells
The affinity of B cell receptor for antigen and the expression level of Bach2 are inversely correlated in germinal center B cells. Low affinity germinal center B cells, which receive weaker stimulation from antigen and antigen specific CD4 T cells, maintain a relatively high level of Bach2 expression and tend to be induced to form memory B cells in the germinal center. On the other hand, high affinity cells, which receive strong stimulation from antigen and antigen specific CD4 T cells, have difficulty maintaining Bach2 expression at high levels and tend to be induced to form plasmablasts (plasma cells).

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Malaria Immunology

Cevayir Coban, MD/PhD

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In general, our group is interested in host-pathogen interactions. Our infection model is malaria caused by *Plasmodium* parasites. We have several reasons to study this pathogen. First of all, malaria disease continues to be a global burden by killing millions every year due to its complications such as cerebral malaria. Yet there is no fully potent drug and/or vaccine against this disease (*WHO Report*, *2015*). Second, *Plasmodium* parasites are so diverse that each *Plasmodium spp*. causes different disease outcomes in humans as well as in mice and we still know very little about the reasons.

Death from malaria occurs due to organ-specific immunopathology caused by parasites; however, detailed understanding of this immunopathology is lacking. Therefore, our recent focus is to understand how immunopathology is caused by *Plasmodium* parasites at the tissue and cell 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 the host secreted against *Plasmodium* parasites.

One of the topics we investigate 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 target 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 the brain by ultra-high field MRI showed that the olfactory bulb is physically and functionally damaged by *Plasmodium* parasites. Live multiphoton imaging of the olfactory bulb confirmed that the complex trabecular small capillaries comprising the olfactory bulb show parasite accumulation and cell occlusion followed by microbleeding, events associated with high fever and cytokine storm. With these findings, we have provided evidence that olfactory functional impairment (loss of smell) could be a valuable early diagnosis marker for cerebral malaria (*Cell Host Microbe, 2014*).

The final goal of our research is the translation of our understanding of host-pathogen interactions into vaccines or drugs to treat disease. Given that there is still more to do for the development of potent vaccines against malaria, we focus on the adjuvants, because if rationally designed, adjuvants improve vaccine efficacy (*J Immunology, 2015; PNAS, 2014*). "Rational adjuvants" are the key for better and controllable immune responses. In recent years, we've developed a new adjuvant called synthetic hemozoin, a synthetic analog of *Plasmodium*-produced hemozoin, and completed its preliminary GLP non-clinical safety and toxicology studies in several animals and infection models (*Vaccine, 2016*). These studies performed according to GLP procedures in rats concluded that synthetic hemozoin is a safe adjuvant and displays very low autoimmune properties.

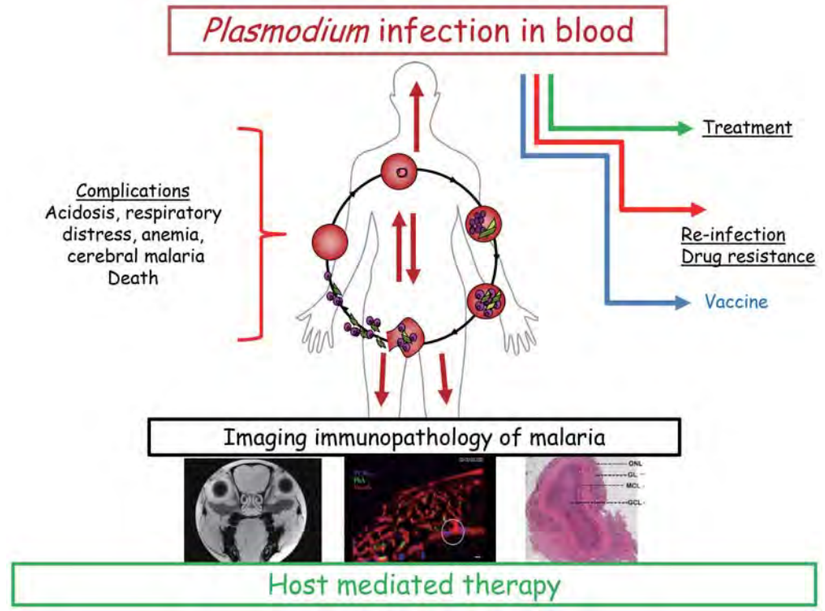

Figure.
Blood stage *Plasmodium* infection causes multiple and deadly complications, although easily treated if diagnosed early. However, high prevalence of drug resistance and the lack of successful vaccines are the drawbacks to efforts in the elimination of this disease. Therefore additional treatment modalities should be investigated to prevent and treat complications and prevent death caused by malaria. Our approach to this problem is to use cutting edge imaging technologies to understand immunopathology caused by *Plasmodium* parasites.

Recent Publications

- Lee MSJ. et al. Current status of synthetic hemozoin adjuvant: A preliminary safety evaluation. Vaccine 19, 34(18), 2055-2061 (2016).
- Onishi M. et al. Hydroxypropyl-β-Cyclodextrin spikes local inflammation that induces Th2 cell and T follicular helper cell responses to the coadministered antigen. Journal of Immunology 194, 2673-2682 (2015).
- Zhao H. et al. Olfactory plays a key role in spatiotemporal pathogenesis of cerebral malaria. Cell Host Microbe. 15, 551-563 (2014).
- Kobiyama K. et al. Nonagonistic Dectin-1 ligand transforms CpG into a multitask nanoparticulate TLR9 agonist. Proc. Natl. Acad. Sci. USA. 111, 3086-3091 (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, 705-716 (2012).

Vaccine Science

Ken J. Ishii, MD/PhD

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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.

Vaccine science with old, but newly evolving adjuvant researches

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).

Recently, we reported that nucleic acids such as dsDNA or RNA:DNA hybrid can be found in the cytosol of cancer cells such as B cell lymphoma (Koo CX et al J. Biol. Chem. 2015, Shen YJ et al, Cell Reports 2015), potentially activating innate and adaptive immunity to cancer cells.

In FY2015-2016, we continued to elucidate the mechanisms of, and to develop novel, vaccine adjuvants. We recently identified that two STING ligands, DMXAA and another natural cyclic dinucleotide such as cGAMP, were unexpectedly allergic. The ligands function as type-2 inducing adjuvants to co-administered antigens, and addition to other DNA-based adjuvants such as CpG DNA, abrogated STING-L-induced type-2 adjuvant effect, and synergized toward type-1 adjuvant effect (Tang CK et al PLoS One 2013, Temizoz B et al Eur. J. Immunol. 2015).

We coincidently found that hydroxypropyl-β-cyclodextrin (HP-β-CD), a common additive for many drugs, acts as a vaccine adjuvant. HP-β-CD induces host cell death derived dsDNA and local inflammation, and HP-β-CD also induces Th2 cells and T follicular helper cell responses to the co-administered antigen (Onishi M et al J. Immunol. 2015). HP-β-CD was also shown to be a potent mucosal adjuvant for intra-nasal Flu vaccination (Kusakabe T et al Vaccine 2016).

We also developed novel adjuvants such as hydroxyapatite, natural biomaterial of bone (Hayashi M et al Vaccine 2015, Fig.1), and non-aggregating Poly-A Tailed Immunostimulatory A/D Type CpG Oligodeoxynucleotides Applicable for Clinical Use (Aoshi T et al J. Immunol. Res. 2015).

Finally, we have conducted various preclinical and clinical studies to develop humanized K-type CpG ODN (code name: K3) and its 2nd generation CpG complexed with β-glucan (code name: K3-SPG), some of which were published (Kobiyama K et al Eur. J. Immunol. 2016)(Fig.2).

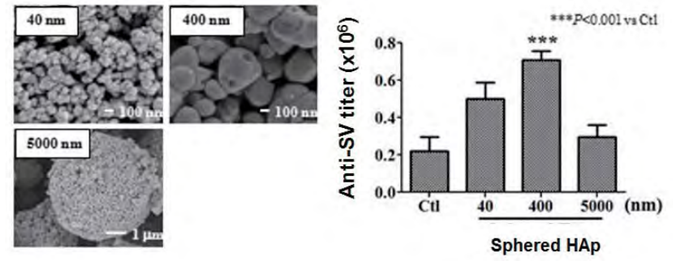


Fig. 1 Adjuvant activity of hydroxyapatite was dependent on its size irrespective to the shape. (Hayashi M. et al Vaccine 2015)

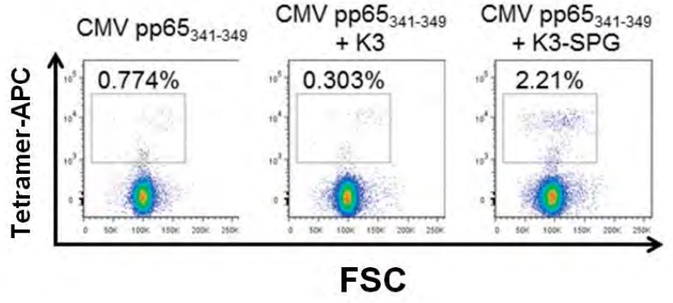
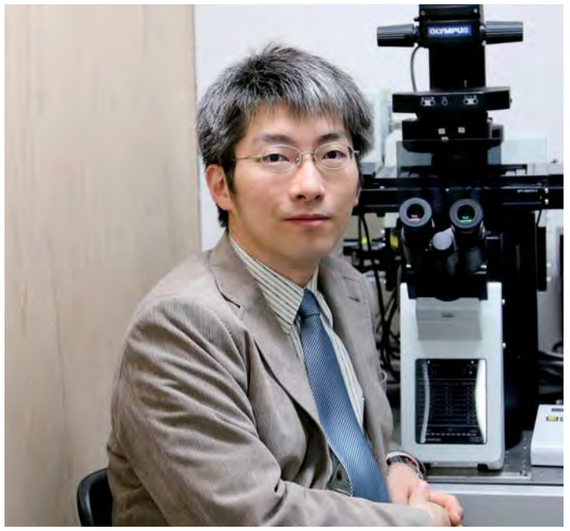


Fig. 2 Potent antigen-specific CD8+ T-cell proliferation by K3-SPG, a humanized CpG ODN complexed with β-glucan. (Kobiyama K et al Euro. J. Immunol. 2016)

Recent Publications

- Kobiyama K, et al. Species-dependent role of type I IFNs and IL-12 in the CTL response induced by humanized CpG complexed with β-glucan. Eur. J. Immunol. 46, 1142-1151 (2016).
- 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, 2673-2682 (2015).
- Temizoz B, et al. TLR9 and STING agonists synergistically induce innate and adaptive type-II IFN. Eur. J. Immunol. 45, 1159-1169 (2015).
- Kobiyama K, et al. Nonagonistic Dectin-1 ligand transforms CpG into a multitask nanoparticulate TLR9 agonist. Proc. Natl. Acad. Sci. USA. 111, 3086-3091 (2014).
- Desmet C and Ishii KJ. Nucleic acid sensing at the interface between innate and adaptive immunity in vaccination Nat. Rev. Immunol. 12, 479-491 (2012).

Immunoparasitology



Masahiro Yamamoto, PhD

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Interferon-γ (IFN-γ) is an important T-helper 1 (Th1) cytokine that inhibits the survival and growth of a wide range of intracellular pathogens such as viruses, bacteria, and parasites. Stimulation of innate immune cells such as macrophages by IFN-γ up-regulates almost 2000 effector genes encoding various IFN-γ-inducible proteins including immunity-related GTPases such as the MX proteins, p47 immunity-related GTPases (IRGs) and p65 guanylate-binding proteins (GBPs). MX proteins and GBPs have been shown to restrict replication of viruses. Moreover, IRGs and GBPs play roles in host defense against vacuole-forming bacteria including *Salmonella*, *Chlamydia*, *Mycobacteria* and *Listeria* by induction of antibacterial responses involving autophagic effectors, inflammasome and phagocytic oxidases.

Not only viruses and bacteria but also the vacuolar parasite *Toxoplasma gondii* is targeted by IFN-γ-inducible GTPases. IFN-γ is critically required for suppression of *T. gondii* replication inside PVs and cell-autonomous clearance. Nitric oxide that is produced by inducible nitric oxide synthase (iNOS) in the infected cells mainly inhibits the replication. On the other hand, *T. gondii* survival within infected cells is suppressed by cooperative action between IRGs and GBPs. Indeed, various types of cells such as macrophages, fibroblasts and astrocytes derived from mice lacking IRGs such as Irgm1 (also known as LRG-47), Irgm3 (IGTP), and Irga6 (IIGP1), or GBPs such as Gbp1, Gbp2, and a cluster of GBPs on murine chromosome 3 (GBP^{chr3}; Gbp1, Gbp2, Gbp3, Gbp5, and Gbp7) were defective for IFN-γ-mediated intracellular killing of *T. gondii*. After the formation of PVs, GBPs and a subfamily of IRG members called GKS-IRGs such as Irga6, Irgb6 (TGTP) and Irgb10 are shown to accumulate of PV membrane (PVM) and oligomelize dependently on GTP-binding to destroy PV membrane integrity and structure, resulting in cell-autonomous clearance by intracellular digestive pathways.

The regulatory mechanism of how IFN-γ-induced GTPases are recruited to PVs has gradually been elucidated. In the absence of essential autophagy-related proteins Atg3, Atg5, Atg7 and Atg16L1, and another subfamily of IRGs called GMS-IRGs such as Irgm1 and Irgm3, the recruitment of IFN-γ-inducible GTPases and the killing of *T. gondii* are severely impaired. Thus, Atg3/Atg5/Atg7/Atg16L1 and Irgm1/Irgm3 are required for proper targeting of GKS-IRGs and GBPs to *T. gondii* PVM and play positive roles in the cell-autonomous resistance to the pathogen. On the other hand, the inhibitory mechanism for the IFN-γ-inducible GTPase-dependent immunity remains unclear.

To explore the molecular mechanism to control the action of IFN-γ-inducible GTPases, we have attempted to identify binding partners of Gbp2, since a single deletion of *Gbp2* in mice has been shown to result in impaired in vitro and in vivo resistance to type II *T. gondii*. In the present study, we identify RabGDIα as a Gbp2-interacting protein. We have an interest in this protein for two reasons: One is because RabGDIα has been shown to participate in the regulation of Rab proteins, which like GBPs belong to another family of GTPases, and the other is because we demonstrate that overexpression of RabGDIα in cells impairs IFN-γ-induced reduction of *T. gondii* numbers. We have tested whether RabGDIα acts as a regulator of IFN-γ-inducible GTPases under physiological condition. Macrophages and fibroblasts from RabGDIα-deficient mice exhibit enhanced IFN-γ-dependent clearance of *T. gondii*. Moreover, the enhanced clearance by RabGDIα deficiency is accompanied by increased recruitment of Irga6 and Gbp2 to the parasite. Notably, Gbp2 is required for Irga6 recruitment, which is suppressed by direct and specific interactions of RabGDIα with Gbp2 through a lipid-binding pocket. Furthermore, a high dose of type II *T. gondii* infection in RabGDIα-deficient mice results in increased resistance, which is characterized by a decreased parasite burden in the brain. Taken together, our data have indicated that RabGDIα play a negative role in the Gbp2-Irga6 axis of IFN-γ-inducible GTPase-dependent cell-autonomous resistance to *T. gondii*.

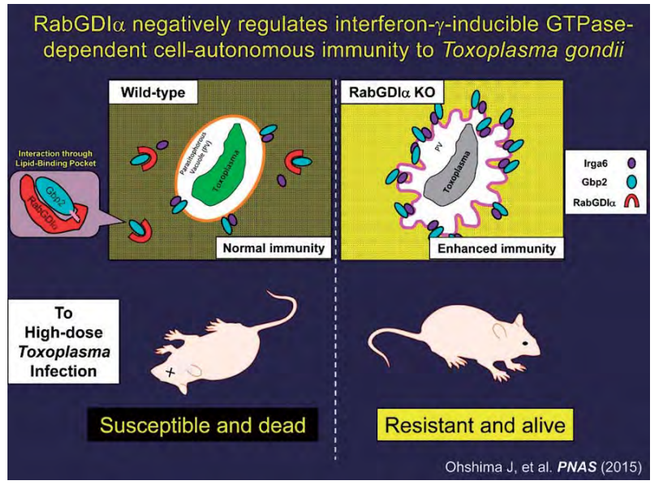

Figure. RabGDIα negatively regulates IFN-γ-inducible GTPase-dependent cell-autonomous immunity to *Toxoplasma gondii*

Recent Publications

- Lee Y, Sasai M, Ma J, Sakaguchi N, Ohshima J, Bando H, Saitoh T, Akira S, Yamamoto M. p62 plays a specific role in interferon-γ-induced presentation of a Toxoplasma vacuolar antigen. Cell Reports. 13, 223-233 (2015).
- Ohshima J, Sasai M, Liu J, Yamashita K, Ma JS, Lee Y, Bando H, Howard JC, Ebisu S, Hayashi M, Takeda K, Standley DM, Frickel EM, Yamamoto M. RabGDIα is a negative regulator of interferon-γ-inducible GTPase-dependent cell-autonomous immunity to Toxoplasma gondii. Proc. Natl. Acad. Sci. USA. 112, E4581-4590 (2015).

Biochemistry and Immunology

Shigekazu Nagata, PhD

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

We recently identified three P4-type ATPases (ATP8A2, ATP11A and ATP11C) and their subunit CDC50A as flippases that actively translocate PtdSer from outer to inner leaflets of the plasma membrane. Among these three flippases, ATP8A2 is specifically expressed in brains, while ATP11A and ATP11C are ubiquitously expressed in various cells including lymphocytes and hepatocytes. ATP11A and ATP11C contain two or three caspase-recognition sites in the middle of molecules, and their flippase activity is destroyed during apoptosis. A high concentration of Ca^{2+} inhibits their flippase activity, too.

There are two families of membrane proteins that support non-specific scrambling of phospholipids at plasma membranes. Five members (TMEM16C, 16D, 16F, 16G and 16J) of the TMEM16 family, that contain 10 transmembrane segments, function as Ca^{2+}-dependent scramblases by forming a dimer. Thus, when cells are activated, the high intracellular concentration of Ca^{2+} reversibly inactivates the flippases, and reversibly activates TMEM16F scramblase to expose PtdSer to the cell surface (Figure 1).

When the intracellular Ca^{2+} level returns to normal, the flippase re-establishes the asymmetrical distribution of phospholipids. Among these five members that function as scramblases, TMEM16F is ubiquitously expressed in various cells, while others are tissue-specific; they are specifically expressed in the brains or intestines. Human and mouse platelets express only TMEM16F as a Ca^{2+}-dependent scramblase. The TMEM16F$^{-/-}$ activated platelets cannot expose PtdSer, leading to the reduced ability to produce thrombin for blood clotting. In fact, human patients of Scott syndrome, a rare congenital bleeding disorder, were found to carry a loss of function mutation in TMEM16F.

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

The PtdSer, thus on the dead cell's surface, is recognized by macrophages for engulfment. Xkr4 and Xkr9 are tissue-specifically expressed in the brains and intestines, respectively. Whereas, Xkr8 is ubiquitously expressed in various tissues. When Xkr8$^{-/-}$ mouse embryonal fibroblasts (MEF) and fetal thymocytes undergo apoptosis, they do not expose PtdSer and can not be engulfed by macrophage. The unengulfed dead cells are believed to undergo secondary necrosis, and intracellular materials are released from dead cells. This may activate the immune system, leading to systemic lupus erythematosus-type autoimmune disease.

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

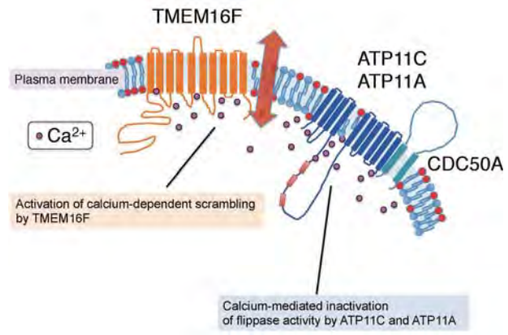

Figure 1. The molecular mechanism for PtdSer exposure in cells with high Ca^{2+}-concentration
The flippase comprised of P4-ATPase (ATP11A or ATP11C) and CDC50A, and a Ca^{2+}-dependent scramblase (TMEM16F) are schematically shown. In activated platelets, the intracellular Ca^{2+} concentration increases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

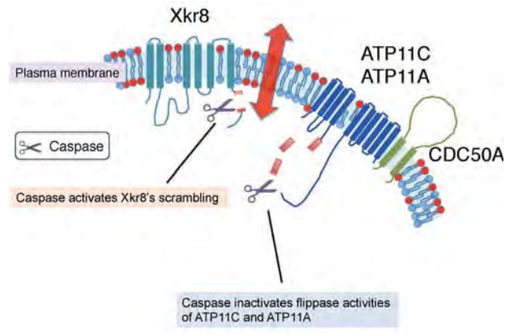

Figure 2. The PtdSer-exposure in apoptotic cells
A caspase-dependent phospholipid scramblase of Xkr8, and flippase (ATP11A/ATP11C associated with CDC50A) are schematically shown. When cells undergo apoptosis, caspase 3 or caspase 7 in the downstream of the caspase-cascade cleaves Xkr8 to activate its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

Recent Publications

- Gyobu S, et al. A role of TMEM16E carrying a scrambling domain in sperm motility. Mol. Cell Biol. 36, 645-659 (2016).
- Fujii T, Sakata A, Nishimura S, Eto K, Nagata S. TMEM16F is required for phosphatidylserine exposure and microparticle release in activated mouse platelets. Proc. Natl. Acad. Sci. USA 112, 12800-12805 (2015).
- Segawa K, Nagata S. An apoptotic 'eat me' signal: phosphatidylserine exposure. Trends Cell Biol. 25, 649-650 (2015).
- Segawa K, et al. Caspase-mediated cleavage of phospholipid flippase for apoptotic phosphatidylserine exposure. Science 344, 1164-1168 (2014).
- Suzuki J, Denning DP, Imanishi E, Horvitz HR, Nagata S. Xk-related protein 8 and CED-8 promote phosphatidylserine exposure in apoptotic cells. Science 341, 403-406 (2013).

Single Molecule Imaging

Toshio Yanagida, PhD

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Measurement of single T cell activation by APC

In the common model of T cell activation, T cells are expected to be activated by antigen-presenting cells (APC) on contact when an appropriate peptide is presented. However, by counting the number of activated T cells attached to APC by micro-pipette, we previously found that only 22% became activated even though the antigen was presented by APC. On the other hand, 11% of T-cells showed activation even when antigen peptide was absent, indicating that activation of T-cell is not deterministic. To understand how the immune system distinguishes between self and non-self in such non-deterministic manner of T cells, we further investigated the effect of regulatory T cells (Fig. 1), and found that in the presence of regulatory T cells, activation of T cells was suppressed in the absence of antigen peptide, whereas activation probability did not change in the presence of antigen peptide. This observation show that regulatory T cells act as a faucet for the leaky immune system.

Immune model predicting stochastic T cell activation

A numerical model to explain the adaptive immunity predicting stochastic T cell activation and suppression of self- response by regulatory T cell was made and evaluated. Theoretical modeling showed that T cell association to APC is crucial to maintain stable unresponsiveness in addition to inhibition of activation-signaling when ligands for stimulation are limited and competed among T cells. 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.

Raman observation of T cell activation

Using Raman spectroscopy, we succeeded in visualizing the activation status of living T cells without labeling. Raman spectra from activated T cells largely differed compared to naïve T cells, enabling the prediction of the activation status of T cells at the single cell level. Our analysis revealed that spectra of individual T cells gradually change from the pattern of naïve T cells to that of activated T cells during the first 24 h of activation. The presented results indicate that Raman spectrum enables the detection of dynamic change in individual cell state scattered in heterogeneous populations.

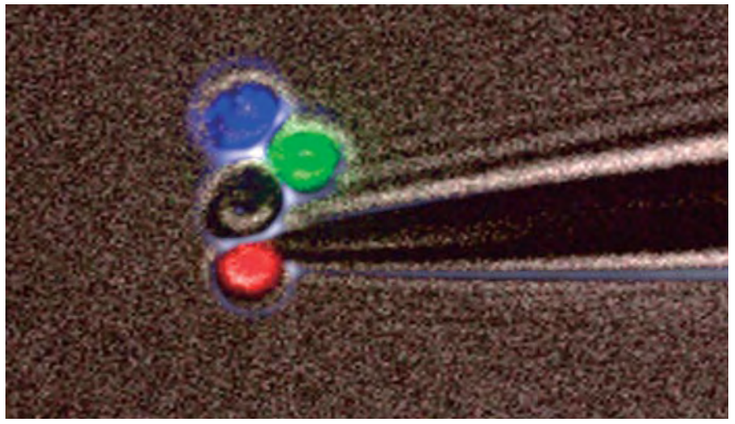

Fig. 1 Counting T-cell activation upon engagement with APC in the presence of regulatory T cell. Rhod2 stained T-cells (red) were held with glass micro-needle and placed on APC and the fluorescence was monitored. Regulatory T cell (blue) and conventional T cell (green) are also present on APC.

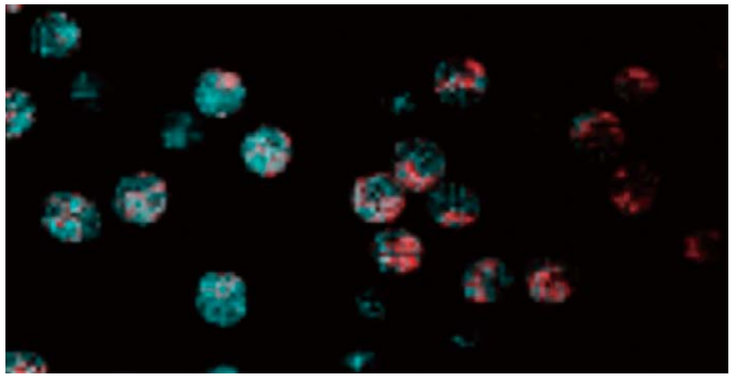

Fig. 2 Pseudo colored Raman image of T cells activated for 12 h. Images are pseudo colored so that naïve state is represented by blue whereas activated state is in red.

Recent Publications

- Morikawa TJ, Fujita H, Kitamura A, Horio T, Yamamoto J, Kinjo M, Sasaki A, Machiyama H, Yoshizawa K, Ichimura T, Imada K, Nagai T, Watanabe TM. Dependence of fluorescent protein brightness on protein concentration in solution and enhancement of it. Sci. Rep. 6, 22342 (2016).
- Iwaki M, Iwane AH, Ikezaki K, Yanagida T. Local heat activation of single myosins based on optical trapping of gold nanoparticles. Nano Letters, 15, 2456-2461 (2015).
- Fujita H, Esaki T, Masujima T, Hotta A, Kim SH, Noji H, Watanabe TM. Comprehensive chemical secretory measurement of single cells trapped in a micro-droplet array with mass spectrometry. RSC Adv. 5, 16968-16971 (2015).
- Ichimura T, Chiu LD, Fujita K, Kawata S, Watanabe TM, Yanagida T, Fujita H. Visualizing cell state transition using Raman spectroscopy. PLoS One. 9(1), e84478 (2014).
- Watanabe TM, Imada K, Yoshizawa K, Nishiyama M, Kato C, Abe F, Morikawa TJ, Kinoshita M, Fujita H, Yanagida T. Glycine insertion makes yellow fluorescent protein sensitive to hydrostatic pressure. PLoS One. 8(8), e73212 (2013).

Biofunctional Imaging

Yoshichika Yoshioka, PhD

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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 can be used to obtain images and spectra of the same mouse repeatedly over time, and the precise information, which is obscured by individual differences, can be obtained. Technical developments and refinements are important and necessary to obtain refined images and spectra (information).

Ultra high filed MRI and iron oxide nanoparticles

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 T2/T2* relaxation times. Combining high magnetic-field strength with high-sensitivity radio-frequency (RF) coils and optimal contrast agents will enable the visualization of immune cell dynamism and molecular events in vivo in both animals and humans.

SPIO has been used in preclinical and clinical diagnosis with MRI, particularly for liver, spleen, lymph nodes and bone marrow because nanoparticles such as SPIO are taken nonspecifically by these tissues and by macrophages. Many studies aimed at improving the unique physicochemical and biological properties of SPIO have been conducted to visualize a specific biological event including inflammation by modifying the particle structure, size, coating, and so on.

Our collaborator synthesized a new type of SPIO which shows a stealth property to macrophages. We thought that new immunological phenomena could be visualized by the new SPIO. Actually, the particle showed some characteristic contrasts in mice organs and tissues. One being kidney tissue. Figure 1 shows the unique contrast pattern obtained by the new SPIO. Figure 1 (a) MRI was obtained in vivo before and after the new SPIO administration. The new SPIO produced low signal intensity regions in the boundaries between cortex and outer medulla and between outer medulla and inner medulla. Although these low intensity regions are unique, the intensity in cortex regions were also low compared to the images obtained before administration. Using ex vivo high resolution MRI (Figure 1 (b)) we could confirm that the dark spots by the new SPIO were found mostly in the boundaries between cortex and outer medulla and between outer medulla and inner medulla. Several small dark spots were found in the cortex, which look like renal corpuscles. We examined the SPIO distribution in the cortex histologically after ex vivo MRI. The SPIO distributed in the lysosome of mesangial cells of glomerulus. These results indicate mesangial cells of control mice could recognize some substance which macrophages do not recognize. Since mesangial cells are considered to play some immunological roles, the new SPIO could be used as a tool to assess the immunological responses of the kidney and kidney disease regarding immune disorders.

Integration of MRI and MRS

MRS provides information non-invasively about metabolism of organs and tissues even at deep regions. We tried to obtain the spectra of bone marrow in mice femurs. Bone marrow spectra from 0.7 x 0.7 x 0.7 mm^3 regions could be obtained shown in Figure 2 (a, b). (a) and (b) are control and peroxisome proliferator-activated receptor γ (PPARγ) agonist (rosiglitazone) treated mice, respectively. Steatosis of bone marrow in the treated mice could be visualized. Figure 2 (c, d) are high resolution MRI of these mice femurs. Decreases of trabecular and compact bones in the treated mouse femur could be assessed quantitatively by our high resolution MRI (Shiomi Y, et al. JBC 2015). The important point is that the anatomical and metabolic information of mouse femurs could be obtained simultaneously by ultra-high field magnetic resonance.

Diverse information

MRI and MRS are non-invasive and provide diverse information such as in Figures 1 and 2. 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.

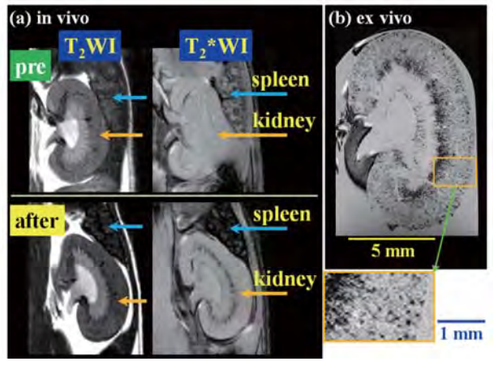

Figure 1.
(a), Abdomen MRI of the same live mouse before and after the new SPIO administration. Upper images were obtained before the SPIO administration. The lower after the administration. Kidney showed unique MRI contrast pattern. (b), ex vivo MRI of kidney after the new SPIO administration. Several small round dark spots could be seen in the cortex region. These are renal corpuscles. We found the new SPIO were taken by mesangial cells of glomeruli. Mesangial cells and glomeruli could be visualized by MRI.

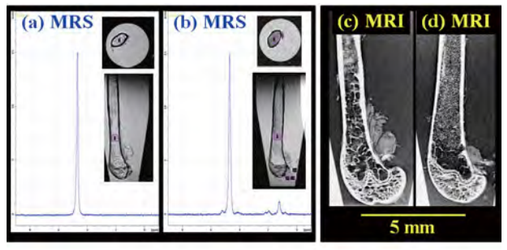

Figure 2.
MRS and MRI of mice femurs. (a,b), bone marrow spectra from 0.7 x 0.7 x 0.7 mm^3 regions in femurs. (a), control mouse. (b), peroxisome proliferator-activated receptor γ agonist treated mouse. Steatosis of bone marrow in the treated mice was found. (c, d), high resolution MRI of control and treated mice femurs. Assessment of trabecular and compact bones could be done quantitatively by high resolution MRI (Shiomi Y, et al. JBC 2015).

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- Arima Y, Kamimura D, Atsumi T, Harada M, Kawamoto T, Nishikawa N, Stofkova A, Ohki T, Higuchi K, Morimoto Y, Wieghofer P, Okada Y, Mori Y, Sakoda S, Saika S, Yoshioka Y, Komuro I, Yamashita T, Hirano T, Prinz M, Murakami M. A pain-mediated neural signal induces relapse in multiple sclerosis models. eLife 4, e08733 (2015).
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Immunology and Cell Biology

Masaru Ishii, MD/PhD

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

1. Intravital bone imaging revealing osteoclast and osteoblast dynamics in vivo

By using intravital multiphoton microscopy, we have elaborated a novel imaging system for visualizing inside bones. By improving the 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.

Furthermore, in collaboration with Dr. Kikuchi in the frame of IFReC, we have developed a new chemical probe for detecting proton secretion in bone resorption by osteoclasts (Nat Chem Biol, in press). By using this probe, we could grasp the real time-course of osteoclastic bone resorption which was finely regulated by cell-cell contact with bone-forming osteoblasts and other bone cell types.

2. DNA methylation regulates osteoclast differentiation

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 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).

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 yet 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).

4. Application of intravital imaging techniques for dissecting human immunology

Intravital imaging with multi-photon microscopy is an undoubtedly powerful tool for dissecting live cellular dynamics in intact tissues and organs and thus useful for studying immune system dynamics in vivo. However, the application is currently limited in animal models and may not be applicable for analyzing human samples. By collaborating with companies (supported by AMED) we are developing a new microscopy system for application to human tissues and organs in vivo. To date, we have succeeded in visualizing non-labelled human normal and cancer tissues, and we will be able to dissect human immunology in the future.

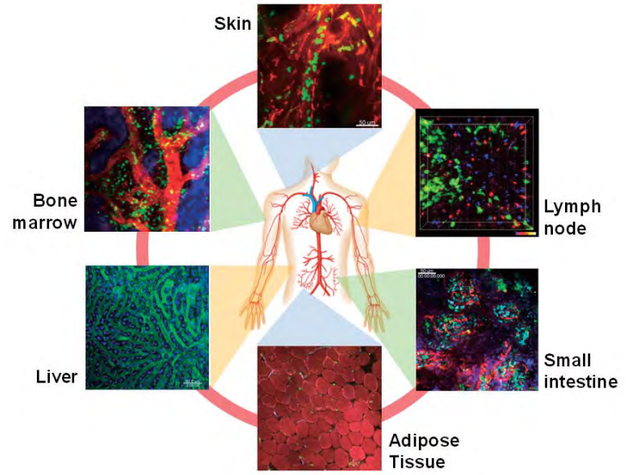

Figure . Intravital imaging for various immune systems
Immune cells are high dynamic and interconnecting various tissues and organs, by forming a 'soft-wired' network. We are elucidating the basic principle controlling the dynamic nature of immune cells by visualizing in vivo behaviors with advanced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

Recent Publications

- Maeda H, et al. Real-time intravital imaging of pH variation associated with cell osteoclast activity and motility using designed small molecular probe. Nat. Chem. Biol. (2016) in press.
- Sekimoto R, et al. Visualized macrophage dynamics and significance of S100A8 in obese fat. Proc. Natl. Acad. Sci. USA, 112, E2058-66 (2015).
- Nishikawa K, et al. Dnmt3a regulates osteoclast differentiation by coupling to an S-adenosyl methionine-producing metabolic pathway. Nat. Med. 21, 281-287 (2015).
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- Kikuta J, et al. Dynamic visualization of RANKL and Th17-mediated osteoclast function. J. Clin. Invest. 123, 866-873 (2013).

Nuclear Medicine

Jun Hatazawa, MD/PhD

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■ Research Assistant	**2**

In the clinical imaging of nuclear medicine, glucose analog tracer F-18 fluoro-deoxy-glucose (FDG) is widely used for the diagnosis of cancer. It is well known that cancer cells and inflammatory cells both utilize glucose in energy production. It is difficult to differentiate between cancer and inflammation by means of glucose analog radiotracer. We studied the usefulness of L-4-borono-2-18F-fluoro-phenylalanine (F-18 FBPA) as a tumor-specific probe, in comparison to F-18 FDG and C-11 methionine (Met), focusing on its transport selectivity for L-type amino acid transporter 1 (LAT1), an isoform highly up-regulated in cancers.

Cellular uptake and kinetic analyses were performed to evaluate the transport capacity and Km value. PET studies were performed in rat xenograft models of C6 glioma (n = 9) and in rat models of turpentine oil-induced subcutaneous inflammation (n = 9). The kinetic parameters and uptake values on static PET images were compared using the one-tissue compartment model (K1, k2) and maximum standardized uptake value (SUVmax).

The cellular analyses showed that the transport capacity by LAT1 was comparable among FBPA, Met and BPA, whereas that for a non–cancer-type isoform LAT2 was significantly decreased for FBPA as compared to Met. The Km value of F18-FBPA for LAT1 (196.8+/-11.4μM) was dramatically lower than that for LAT2 (2813.8 ± 574.5μM), suggesting the higher selectivity of F-18 FBPA for LAT1. K1 and k2 values were significantly smaller in F-18 FBPA PET (K1 = 0.04 ± 0.01 ml/ccm/min and k2 = 0.07 ± 0.01/min) as compared to C-11 Met PET (0.22 ± 0.09 and 0.52 ± 0.10, respectively) in inflammatory lesions. Static PET analysis based on the SUVmax showed significantly higher accumulation of F-18 FDG in the tumor and inflammatory lesions (7.2 ± 2.1 and 4.6 ± 0.63, respectively) as compared to both F18 FBPA (3.2 ± 0.40 and 1.9 ± 0.19) and C-11 Met (3.4 ± 0.43 and 1.6 ± 0.11). No significant difference was observed between F-18 FBPA and C-11 Met in the static PET images. The study showed the utility of F-18 FBPA as a tumor-specific probe of LAT1 with low accumulation in the inflammatory lesions.

Based on the experimental works, clinical studies were conducted after the approval of institutional review board to differentiate between brain tumor recurrence and radiation necrosis. F-18 FBPA PET accurately diagnosed patients with recurrent brain tumor and radiation necrosis with the threshold accumulation value of 2.0 of SUVmax.

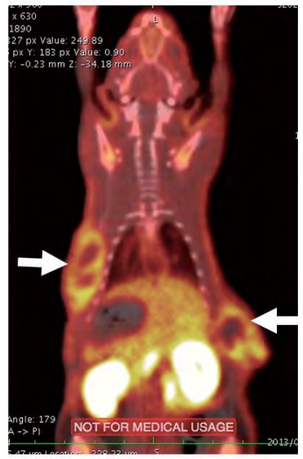

Figure 1. F-18 FBPA animal PET in rats transplanted with RCG-6 cancer cell (arrow)

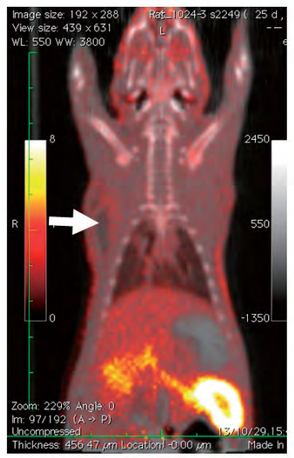

Figure 2. F-18 FBPA animal PET in rats with inflammation induced by subcutaneous oil injection (arrow)

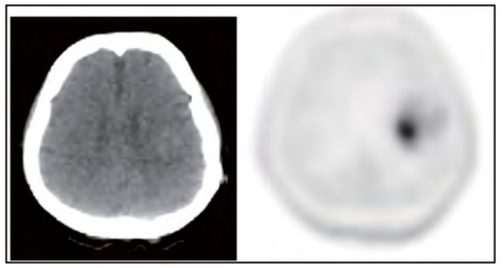

Figure 3. F-18FBPA PET in recurrent brain tumor (left: CT, right: F-18 FBPA PET)

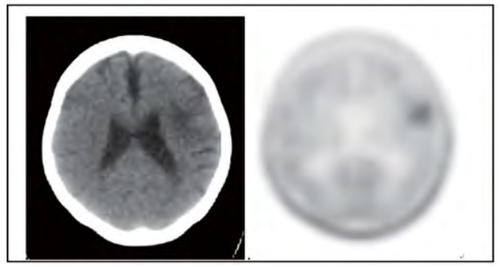

Figure 4. F-18FBPA PET in radiation necrosis (left: CT, right: F-18 FBPA PET)

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- Kainuma S, Miyagawa S, Fukushima S, Pearson J, Chen YC, Saito A, Harada A, Shiozaki M, Iseoka H, Watabe T, Watabe H, Horitsugi G, Ishibashi M, Ikeda H, Tsuchimochi H, Sonobe T, Fujii Y, Naito H, Umetani K, Shimizu T, Okano T, Kobayashi E, Daimon T, Ueno T, Kuratani, T, Toda K, Takakura N, Hatazawa J, Shirai M, Sawa Y. Cell-sheet Therapy with Omentopexy Promotes Arteriogenesis and Improves Coronary Circulation Physiology in Failing Heart. Mol Ther. 23, 374-386 (2015).

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Chemical Imaging Techniques

Kazuya Kikuchi, PhD

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	Masafumi Minoshima
Support Staff	1

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

Intravital imaging by two-photon excitation microscopy (TPEM) has been widely utilized to visualize cell functions. However, small molecular probes (SMPs) commonly used for cell imaging cannot be simply applied to intravital imaging because of the challenge of delivering them into target tissues, as well as their undesirable physicochemical properties for TPEM imaging. Here, we designed and developed a functional SMP with an active-targeting moiety, higher photostability, and fluorescence switch, and imaged osteoclast activity by injecting the SMP into living mice.

We designed a new pH-activatable probe "pHocas" (pH-activatable fluorescent probe for osteoclast activity sensing), consisting of an amide-substituted BODIPY fluorophore with an electron-deficient character, which prevents its degradation during laser irradiation of TPEM. pHocas possesses an *N,N*-dialkyl-*o*-toluidine moiety that serves as pH-dependent fluorescence via photo-induced electron transfer (PeT), as well as two bisphosphonate groups for bone-specific delivery (Fig. 1). The fluorescence emission of pHocas increased at lower pH and maintained under continuous light irradation, indicating that it can be used to visualize pH variation in the acidic compartment created by active osteoclasts.

Next, we explored the utility of pHocas for intravital imaging of osteoclast activity in living mice over a long period of time. After subcutaneous injection of pHocas into transgenic mice expressing the red fluorescent protein tdTomato in osteoclasts. Time-lapse imaging revealed the local appearance and disappearance of fluorescence signals under the basal membrane of osteoclasts accompanied by membrane fluctuations. Interestingly, we found that the motility of bone-resorbing osteoclasts, which is believed to be static, fluctuates in a time-dependent manner. We suggest that these bone-resorbing osteoclasts have a different function, in contrast to the static osteoclasts, which may be required to explore the signals that activate bone resorption by slightly moving the bone surface.

Design of a protein tag and fluorogenic probe for live-cell imaging of intracellular proteins

Specific protein labeling by synthetic fluorescent probes and complementary protein tags is an emerging technology that enables conditional labeling at specific time points. We previously developed protein-labeling systems using the Photoactive Yellow Protein (PYP) as a protein tag in combination with a fluorogenic probe, FCANB. FCANB has a triblock modular structure: hydroxy cinnamic acid acts as the PYP ligand, fluorescein the fluorophore, and nitrobenzene the quencher moiety. Although FCANB enhances its fluorescence upon reaction with the PYP-tag, it is cell-impermeable and the labeling rate ($t_{1/2}$~1 h) is not sufficiently fast.

We re-designed the fluorogenic probe AcFCANB, as a membrane-permeable probe by incorporating two acetyl groups into the fluorophore. Once incorporated inside the cells, AcFCANB is rapidly digested by cellular innate esterases to recover the anionic FCANB. Next, to accelerate the labeling rate, we focused on the surface charges of the PYP-tag. Based on the structure of PYP, three aspartic acids (D53, D71, D97) and one glutamic acid (E74) exist on the same face as a reactive cysteine residue (C69). We speculated that charge reversal at these residues would reduce repulsive forces, and facilitate interaction between FCANB and PYP-tags. To this end, a series of cationic PYP mutants were designed and created by point mutation of the acidic amino acids: D53R, D71R, E74R, D97R (Fig. 2).

Time course fluorescence measurement of FCANB in the presence of PYP mutants revealed that all the mutants showed improved binding rates compared with that of the wild-type PYP. The highly efficient molecular dynamics calculations used here were sufficient to recapitulate the binding rates of large charged ligands on charged proteins. The resulting AcFCANB/PYP 4R pair enabled no-wash imaging of intracellular proteins in a desirable time frame ($t_{1/2}$ < 30 min).

Fig. 1
(left) Chemical structure of pHocas for imaging of osteoclast activity (right) Two-photon time lapse imaging of bone tissue after the injection of pHocas. Excited at 940 nm. Scale bars: 20 μm.

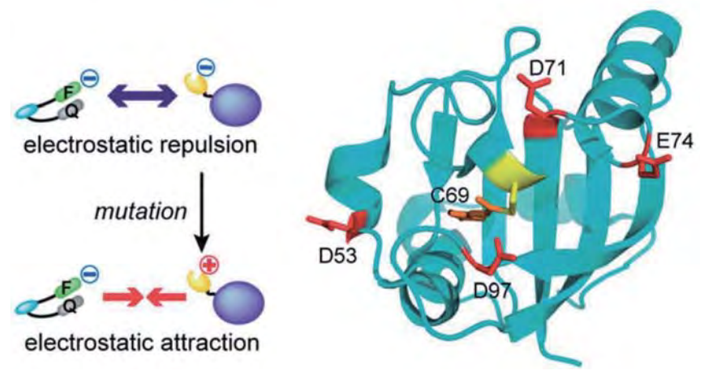

Fig. 2
(left) Schematic illustration for the mechanism of labeling acceleration. (right) Structure of PYP showing the reactive C69 surrounded by the four acidic residues targeted for mutation.

Recent Publications

- Maeda H, Kowada T, Kikuta J, Furuya M, Shirazaki M, Mizukami S, Ishii M and Kikuchi K. Real-time Intravital Imaging of pH Variation Associated with Osteoclast Activity, Nat. Chem. Biol. 12, (2016), in press. DOI: 10.1038/nchembio. 2096
- Kamikawa Y, Hori Y, Yamashita K, Jin L, Hirayama S, Standley DM and Kikuchi K. Design of a Protein tag and Fluorogenic Probe with Modular Structure for Live-Cell Imaging of Intracellular Proteins. Chem. Sci. 7, 308–314 (2016).
- Hori Y, Hirayama S, Sato M and Kikuchi K. Redesign of Fluorogenic Labeling System to Improve Surface Charges, Brightness, and Binding Kinetics for Imaging Functional Localization of Bromodomains, Angew. Chem. Int. Ed. 54, 14368–14371 (2015).

Biophotonics

Nicholas Isaac Smith, PhD

Associate Professor	**Nicholas Isaac Smith**
■ Assistant Professor	**Alison Hobro**
	Nicolas Pavillon

The biophotonics lab uses and develops different optical techniques to study biological and immunological processes. Primarily label-free, we exploit the different physical interactions of light with the sample to image or measure cellular information. Raman spectroscopic imaging is one of our main tools, and we develop new imaging modes to go along with the Raman measurements. In practice, this means illuminating the sample with a high intensity laser beam and measuring the wavelength shifts of the scattered light. By resonance with the endogenous molecules in a living cell or tissue sample, we can observe the spatial and chemical distributions in the sample. In combination with this, we are investigating methods of using other wavelengths of light to simultaneously measure different features of cells or tissue. Our targets are typically lymphocytes, macrophages and their response to stimulation.

We combined quantitative phase imaging with Raman imaging, which are both label-free imaging modalities that provide complimentary information on the cell response, where protein distribution in the cell is imaged mainly by the phase mode, while lipid and other components are more dominant in the Raman imaging mode (Pavillon et al 2013, Pavillon et al 2014). For the purposes of discriminating cell lines, we found that it is possible to image the cells using the phase mode, allowing the Raman signals to be collected in an optimized mode instead of relying on Raman for imaging. The outcome of this is that it is possible to use an optimized label-free approach to discriminate cells. Targets of this type of discrimination have been lymphocytes (Hobro et al 2016), but can be of any cell type. We termed the new mode hybrid scanning and found that it behaves very well for applications such as label-free cell screening, with some high-throughput characteristics (Pavillon et al 2015).

We also extended our studies in using gold nanoparticles in a cell to enhance the Raman signal. Using photoreduction, we showed that it is possible to generate gold nanoparticles within a cell (Smith et al 2014), which can then be used to enhance the Raman signal. Most of our work is label-free and uses only a laser or other light source, the nanoparticle enhanced projects offer different avenues to study a cell response.

In collaboration with the Coban lab, which is focused on malaria immunology, we have been studying the application of Raman scattering to diagnosis of malaria and then more recently to the observation of uptake processes in macrophages. Upon exposure to the malaria byproduct hemozoin, macrophages engulf and transport the nano-sized crystals. Using Raman imaging we were able to observe this process and identify some cellular changes occurring during uptake. In particular we found a transient lipid response that occurs during or soon after the initial uptake (Hobro, et al 2015). The Raman measurement of lipids formed or concentrated near the particles is consistent with the appearance of vesicles during uptake.

Through collaboration with our colleagues in the department of Applied Physics, we also worked on a number of projects aimed at extending the applications and capabilities of imaging techniques. We have hosted a number of students from physics, and believe that sharing students, projects, and ideas between our labs enables innovative research.

In addition to main lab activities, Dr. Hobro is running a Kakenhi project "Detection and identification of virions in host bodily fluids and immune cells" on the measurement by both Raman and absorption spectroscopy aimed at identifying molecules of interest in a complex biomolecular background such as those that occur in plasma or inside a cell. Raman techniques can readily identify single types of molecules when measured in isolation, but a major challenge is to find limits of detection among competing signals from other molecules. Dr. Pavillon is also running a Kakenhi project, "Full-field holographic optical coherence tomography combined with Raman spectroscopy for in vivo microscopic imaging of biological tissue", which is also a project where we collaborate with the Photonics Advanced Research Center at Osaka University. This project is based on simultaneous Raman and phase measurements geared towards tissue imaging, using a longer wavelength broadband source to allow penetration of turbid samples such as tissue. These additional projects are ongoing.

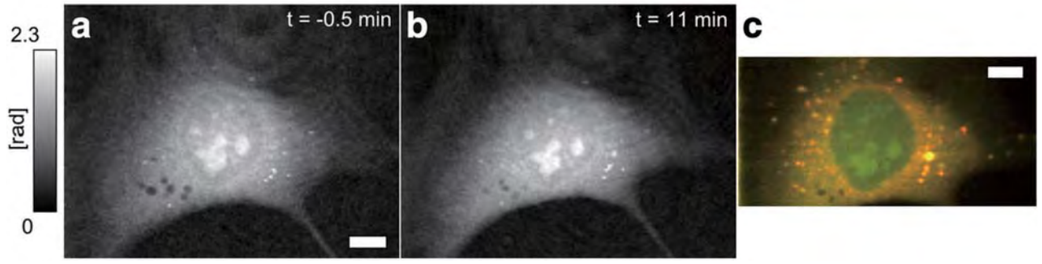

Figure 1. Multimodal label-free imaging of MEF (fibroblast) cells, from Pavillon et al 2015.
The black and white mode gives quantitative phase information while the colored image is constructed from Raman scattering bands at 2853cm^{-1} (red, lipids) and 2973cm^{-1} (green, C-H stretching). The phase map is dominated by proteins.

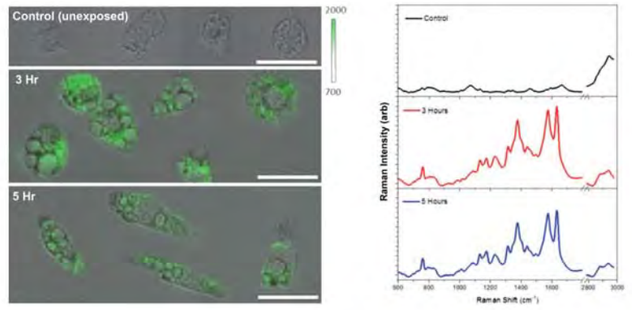

Figure 2. Raman imaging of hemozoin uptake by macrophage cells, from Hobro et al 2015.
The black and white image is brightfield, while the green channel overlay represents the distribution of hemozoin in the cells. The spectra on the right-hand side show the sensitivity of the measurement to the presence of hemozoin, represented by the strong peaks.

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- Hobro AJ, Kumagai Y, Akira S and Smith NI. Raman spectroscopy as a tool for label-free lymphocyte cell line discrimination. Analyst 2016 (in press).
- Hobro AJ, Pavillon N, Fujita K, Ozkan M, Coban C and Smith NI. Label-free Raman imaging of the macrophage response to the malaria pigment hemozoin. Analyst 140, 2350-2359 (2015).
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Immune Response Dynamics

Kazuhiro Suzuki, MD/PhD

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

During the last century, extensive studies were performed on individual organ systems and yielded huge progress in biomedical science. However, because the organ systems in our body mutually communicate to cross-regulate their functions, it is nearly impossible to precisely predict responses of one organ system without knowing the states of other organ systems. Thus, the importance of studies that clarify interconnections among multiple organ systems is being increasingly recognized. In this regard, recent studies that have revealed the cellular and molecular basis for connections between the nervous and immune systems represent the new trend. Studies during the last decade have demonstrated that peripheral nerves of the autonomic and somatosensory nervous systems regulate both the development and functions of immune cells through multiple modes of action (Ordovas-Montanes, et al. Trends immunol. 36: 578, 2015).

Adrenergic nerves constitute efferent pathways of the sympathetic nervous system and coordinate organ functions through secretion of a neurotransmitter, noradrenaline. In the 1980s, comprehensive morphological studies mapped adrenergic nerve fibers in both primary and secondary lymphoid organs (Felten, et al. J. Immunol. 135: 755s, 1985). In secondary lymphoid organs, including spleen and lymph nodes, the adrenergic nerve fibers exhibit a uniform pattern of distribution, where they mainly supply T-cell zones but are absent from B-cell follicles. These findings established an anatomical basis for connections of the sympathetic nervous system with the immune system. Pharmacological studies of the same period demonstrated that noradrenaline bound to the surface of lymphocytes, which was almost exclusively mediated by β_2-adrenergic receptors (Brodde, et al. Life Sci. 29: 2189, 1981), indicating that inputs from the sympathetic nervous system can directly act on immune cells. However, it was unclear how lymphocyte behaviors are influenced by adrenergic nerves that project to lymphoid organs.

After spending several hours in a lymph node, lymphocytes exit from the lymph node into lymph, return to blood flow through the thoracic duct, and travel to other lymphoid organs to continue antigen surveillance in the whole body. We recently found that inputs from adrenergic nerves control lymphocyte egress from lymph nodes through β_2-adrenergic receptors (Nakai, et al. J. Exp. Med. 211: 2583, 2014). Activation of lymphocyte β_2-adrenergic receptors augments signals through CCR7 and CXCR4, chemokine receptors that promote lymph node retention of lymphocytes, and consequently inhibit their lymph node egress. Additionally, in murine models of inflammatory diseases, including multiple sclerosis and allergic dermatitis, we showed that administration of a β_2-adrenergic receptor agonist ameliorates the diseases, which is accompanied by reduced lymph node egress of pathogenic T lymphocytes and their recruitment to target organs (Suzuki and Nakai, Clin. Exp. Neuroimmunol. 7: 10-17, 2016).

Our findings have established a novel mechanism by which adrenergic nerves control the immune system and implied its therapeutic potential for inflammatory diseases (Figure). However, some questions remain unsolved. First, the molecular mechanisms of the crosstalk between β_2-adrenergic receptors and chemokine receptors are unclear. Second, it remains to be visualized how adrenergic nerves interact with lymphocytes and influence their migratory behaviors in lymph nodes. Finally, the physiological significance of this neural control mechanism for lymphocyte recirculation through lymph nodes remains to be addressed. We are currently working on these questions to reveal the whole picture of adrenergic control of lymphocyte trafficking. Our research contributes to comprehensive understanding of immune regulation by the nervous system.

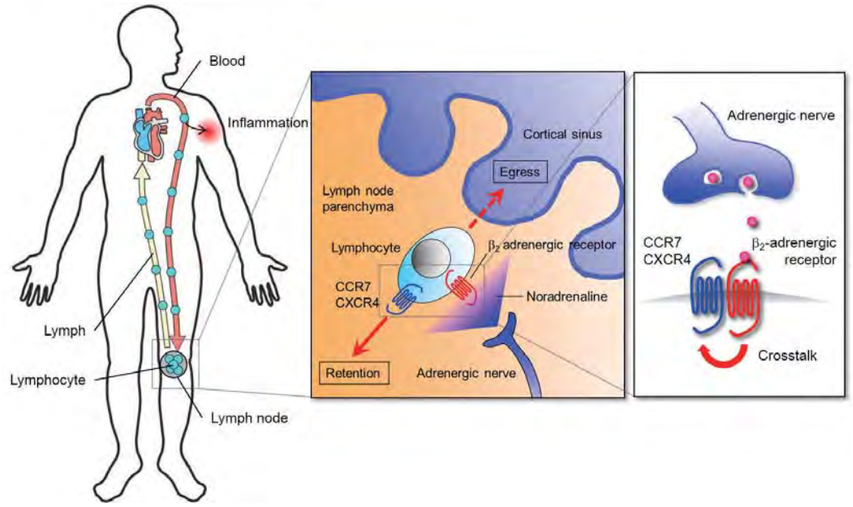

Figure. Control of lymphocyte trafficking by adrenergic nerves
Activation of β_2-adrenergic receptors expressed on lymphocytes inhibits their egress from lymph nodes by enhancing retention-promoting signals mediated by chemokine receptors, CCR7 and CXCR4.

Recent Publications

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- Gray EE, Friend S, Suzuki K, Phan TG and Cyster JG. Subcapsular sinus macrophage fragmentation and CD169$^+$ bleb acquisition by closely associated IL-17-committed innate-like lymphocytes. PLoS One. 7, e38258 (2012).
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Brain-Immune Interaction

Ben Seymour, MD/PhD

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

Homeostasis and defense after injury

A core question we aim to answer is how does the brain and immune system cause changes to behavior after injury? Although reduced motivation and pain may be adaptive, for instance allowing us to prioritize recuperation and recovery, they may also underlie pathological states such as excessive fatigue and depression. We have been studying brain activity and behavior in a human model of thermal injury (i.e. burns), to see how our ability to control the injury (by cooling it) influences both behavior and autonomic nervous system activity. We have found that the extent to which humans perceive their ability to find relief of the pain is tightly linked with efferent autonomic output, providing a potentially important mechanism by which the brain can modulate peripheral loci involved in the inflammatory response. We've also identified the pathway in the brain that underlies this – from the anterior cingulate cortex to the brainstem periaqueductal grey (submitted), identified as a possible key link in the brain-immune pathway.

Using insights from engineering and information science, we've begun to use these findings to construct control theory models of homeostasis after injury, which aim to unite behavior and immune system function within a single model. Following a large translational award from Arthritis Research UK, we now aim to use this to identify immune and brain markers of fatigue in inflammatory arthritis (rheumatoid arthritis patients), and probe their modulation by biologic therapies.

Neurogenic inflammation

Following tissue injury, the local inflammatory response is coordinated by a population of small umyelinated nerve fibers called c-fibers. Understanding the role of these fibers is critical to understand disorders such as psoriasis, which is dependent on neurogenic control. We have been studying a human model of neurogenic inflammation involving topical application of the TRP channel ligand capsaicin, which causes is vasoactive oedema, hyper-sensitivity and pain. In particular, we have been using integrated brain imaging techniques (MEG and fMRI) to try and identify if the brain has an internal representation of peripheral inflammation. Our provisional results suggests that this is indeed the case. If this is confirmed, this means that the brain has the critical substrate from which it can make 'intelligent' responses to control the inflammatory response.

We have also been developing objective behavioural and physiological tests on the local inflammatory response as a tool for identifying the cognitive influences on immune processes, with collaborators within IFReC and industry. This is being done as a precursor to identify the potential psychological causes of vaccine-induced pain. This is a major problem for the HPV vaccination program in Japan, with a large consequent mortality.

Rodent injury and neuroimaging

A further component of our lab aims to develop animal models of injury that can be used to test more mechanistic questions about the role of the immune system in peripheral injury and pain. We have developed a surgical plantar flap model of injury, which causes immediate and prolonged behavioural responses associated with pain. We have subsequently probed this in various murine knock-out models, including MyD88 and TRIF, and shown that they reduce pain-related responses. This identifies possible target pathways for understanding how peripheral immune mediators might modulate the development of chronic pain.

With collaborators within IFReC, we have also initiated a neuroimaging program to identify regions of the brain that are involved in injury and pain, using manganese-enhanced MRI and functional MRI. We aim to apply these methods to identify the immune control of brain activity associated with inflammation and pain in mice (in the plantar flap model) and the rat (in the CFA inflammatory arthritis model).

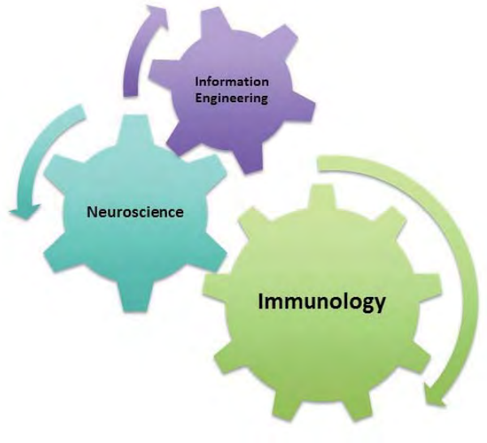

Figure 1.

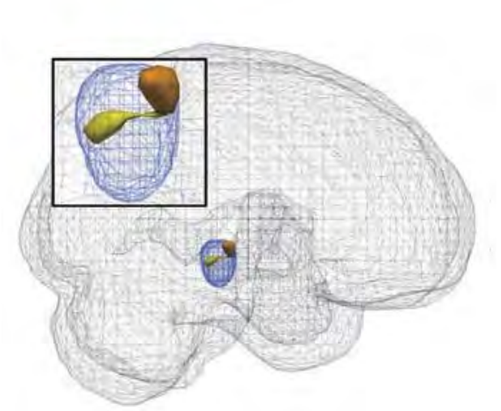

Figure 2.
Potential brain region underlying efferent control during injury.

Recent Publications

- Chassang S, Snowberg E, Seymour B, Bowles C. Accounting for behaviour in treatment effects - New applications for blind trials. PLoS ONE 10(6), w0127227 66, (2015).
- Pauli W, Larsen T, Collette S, Tyszka J, Seymour B, O'Doherty J. Distinct contributions of subregions of the human substantia nigra to appetitive and aversive learning. Journal of Neuroscience. 35, 14220-33 68 (2015).
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- Seymour B, Maruyama M, Martino BD. "When is a loss a loss? Excitatory and inhibitory processes in loss-related decision-making." Current Opinion in Behavioral Sciences 5, 122-127 (2015).
- Maruyama M. Neural substrates for understanding math expressions. Clinical Neuroscience 33, 936-938 (2015).

Information Systems

Yutaka Hata, PhD

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

(1) We first propose an automated single macrophage tracking method in 11.7 T magnetic resonance (MR) images. The method is based on motion artifacts and spatial/temporal noise reduction algorithms for preprocessing, a background subtraction method for detection, and the Hungarian method for tracking. The method has been applied to a brain-stroke mouse model, and has provided trajectory of macrophages and numerical results of macrophages including numbers, densities, velocities, etc. Imaging and quantification of macrophage kinematics will strongly assist the progress of immunology research. Figure 1 shows the tracking imaging result.

(2) A new simulation method for swarming motile cells says the chemotactic bacteria is developed on the basis of the kinetic chemotaxis model. The Monte Carlo algorithm is employed to reproduce the run-and-tumble motions of cells interacting with the chemical cues in the local environment. The reaction-diffusion equations to describe the spatial and temporal variations of chemical cues are also numerically solved coupling with the Monte Carlo simulation. We apply the method to the traveling population wave of bacteria generated by the chemotaxis and division of bacteria. We also carry out the analytical calculation of the continuum model for this problem and compare the analytical results with the numerical results. It is found that the traveling wave solutions exist for the specific division rates. The numerical results also indicate the possibility of an interesting instability problem which will be analyzed theoretically.

(3) We proposed a 4D macrophage simulator based on evolving cellular automata. The simulator can imitate and visualize macrophages' movement in 3D space with time progress. First, macrophages' data were extracted from some images observed by a two-photon microscope. The extracted data were used as training data for learning and evolving cellular automata. The cellular automata used in this research are stochastic cellular automata (SCA) in 3D space and can evolve by using genetic algorithms. The results from the proposed simulator are similar to movements of real macrophages. Figure 2 shows the 4D simulator result.

(4) We studied several basic researches on computer engineering on medical science.

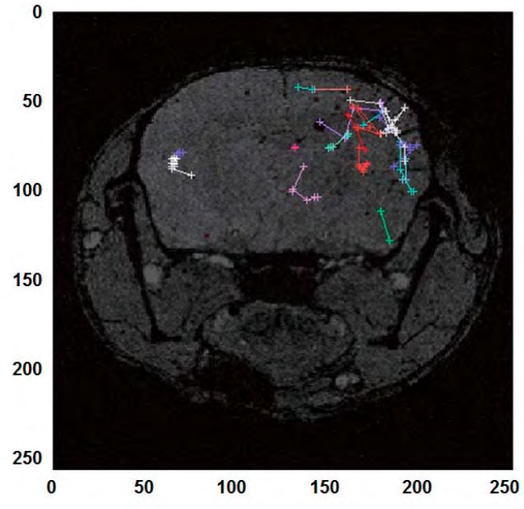

Figure 1. Macrophage tracking result
Each color shows individual macrophage trajectory.

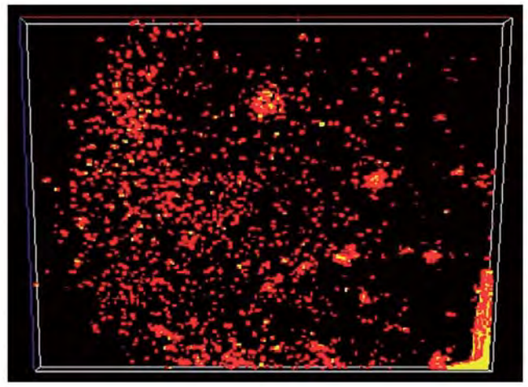

Figure 2. Result of the 4D simulator
Red objects show macrophages and yellow objects show T-cells.

Recent Publications

- Tashita A, Kobashi S, Mori Y, Morimoto M, Aikawa S, Yoshioka Y, Hata Y. Macrophage tracking using the Hungarian algorithm in time lapse MR images. Proceeding of 7th International Conference on Emerging Trends in Engineering & Technology, 169-173 (2015).
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- Sato K, Shima S. Various oscillation patterns in phase models with locally attractive and globally repulsive couplings. Phys. Rev. E 92, 042922 (2015).

Systems Immunology

Daron M Standley, PhD

Professor	Daron M Standley
Associate Professor	Kazutaka Katoh
Assistant Professor	Kazuo Yamashita
Postdoctoral Fellow	2
Support Staff	4

Our laboratory mainly focuses on two areas of research: Post transcriptional regulation of immune responses and analysis of high-throughput antibody and TCR sequence data. Below we summarize our progress in these two areas.

Post-transcriptional regulation of immune responses

Post-transcriptional regulation can be mediated by RNA-binding proteins (RBPs) or by micro RNAs (miRNAs), both of which are capable of either stabilizing or destabilizing messenger RNAs (mRNAs). Our lab has been investigating both types of regulators in collaboration with experimental groups. In parallel, we have developed novel in silico methods for such purposes.

Our interest in RBP-mediated post transcriptional regulation began with our collaboration with the Akira Lab in the biochemical characterization of Regnase-1, an endoribonuclease that targets a number of mRNAs related to inflammatory responses (Matsushita et al., 2009). One of the open questions in this project is how Regnase-1 recognizes its target mRNAs. It has now been established that Regnase-1 recognizes a conserved stem-loop structure and that its targets overlap with those of another post-transcriptional regulator, Roquin (Mino et al., 2015); however Regnase-1 and Roquin appear to utilize different motifs for target RNA recognition. In vitro binding assays indicated that Regnase-1 RNase activity requires not only the RNase-containing PIN domain, but also the presence of an N-terminal domain (NTD) (Yokogawa et al., 2016). Using molecular docking, we predicted that the NDT and PIN domains interact physically; resulting in stabilization of the RNase active site residues. The docked model was subsequently confirmed by NMR spectroscopy (Yokogawa et al., 2016) (Figure 1). As an extension of this work, we have developed an all-purpose tool, aaRNA, for the prediction of RNA binding residues (Li et al., 2014), and have applied it to a number of RNA-binding proteins: Arid5a-Stat3 3'UTR interaction (Masuda et al., 2016), docking of ssDNA and anti-DNA antibody in SLE (with the Kikutani Lab; in preparation) and interaction between Apobec3g and HIV RNA (with the Koyanagi Lab, Kyoto Univ; in preparation).

In order to comprehensively understand the functional role of miRNAs in the regulation of immune responses, we analyzed the intersection between miRNA targets and genes preferentially expressed in immune cells. We developed a consensus prediction method using a random forest framework and applied it to both human and mouse miRNAs. We observed modest, yet statistically significant, differences in the targeting profiles of immune-related mRNAs as compared to other mRNAs (Figure 2A-C) and qualitative differences in the binding target profiles of circulating miRNAs compared to non-circulating miRNAs (Figure 2D-F) (submitted).

High-throughput antibody and TCR sequence data

One of the major goals of our lab is to discover immune-based biomarkers and therapeutics from human antibody and TCR repertoires. This goal requires that we are able to identify the targets of antibodies and TCRs from sequence data. Although this is a very ambitious goal, we have already made substantial progress in one aspect of the work: rapid and accurate 3D modeling of antibody structures from sequence (Yamashita et al., 2014). We have also recently found that different donors immunized with the same vaccine can produce widely different antibodies at the sequence level, which are highly similar at the structural level, and that the structurally-related antibody groups are more likely to be functionally related (in preparation). Based on these findings, we subsequently showed that we could group anti-HIV antibody sequences according to their target epitope with high accuracy. First we developed a mathematical model that could separate anti-HIV antibodies from other antibodies with > 95% accuracy (Figure 3A), and showed that such separation would be nearly impossible using only sequence information (Figure 3B). We also found that we could predict epitope-MHC interactions with high accuracy. For example, we could predict the correct MHC type for 41 known cytomegalovirus epitopes with an accuracy of > 97%. Work is ongoing now to extend these predictions to identify the correct TCR sequence from among thousands of possibilities. Both of these preliminary results are encouraging with respect to our goal of leveraging high-throughput sequence data to identify B and T cell populations associated with disease.

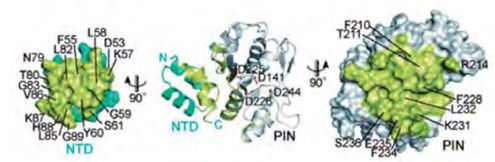

Figure 1. Docked model of the Regnase-1 NTD and PIN domains.
The figure shows the agreement between the docked model (center) and chemical shift perturbations on the NTD (left) and PIN (right) domains.

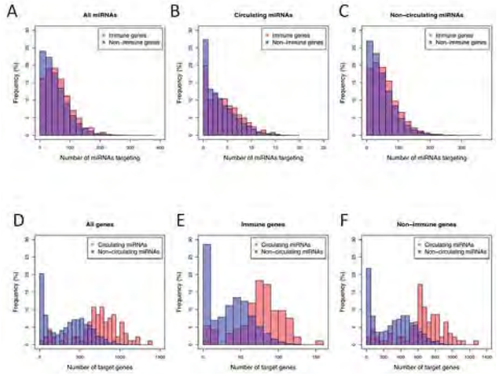

Figure 2. Circulating miRNAs preferentially regulate immune-related mRNAs.
The upper panel (A-D) shows the distributions of human immune-related and non-immune related mRNAs in terms of the number of targeting miRNAs. Immune-related mRNAs had more miRNA interactions in terms of all miRNAs (A), circulating miRNAs (B) and non-circulating miRNAs (C). Panel D-F shows the distributions of human miRNAs in terms of predicted targets. Circulating miRNAs targeted qualitatively more mRNAs regardless of whether all mRNAs (D), immune-related mRNAs (E) or non-immune related mRNAs were considered (F).

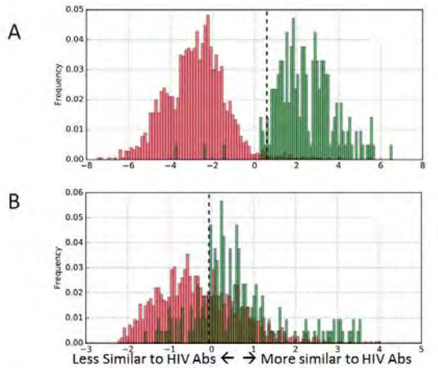

Figure 3. Separation of anti-HIV (green) and other antibodies (red) using structural information.
Figure 3A shows the successful separation of anti-HIV antibodies from other antibodies using modeled structural information. Figure 3B shows that separation using only sequence information is not possible.

Recent Publications

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- Mino T. et al. Regnase-1 and Roquin regulate a common element in inflammatory mRNAs by spatiotemporally distinct mechanisms. Cell 161, 1058-1073 (2015).
- Yokogawa M. et al. Structural basis for the regulation of enzymatic activity of Regnase-1 by domain-domain interactions. Sci. Rep. 6, 22324 (2016).
- Li S, Yamashita K, Amada KM & Standley DM. Quantifying sequence and structural features of protein-RNA interactions. Nucleic. Acids Res. 42, 10086-10098 (2014).
- Masuda K. et al. Arid5a regulates naive CD4+ T cell fate through selective stabilization of Stat3 mRNA. J Exp Med 213:605-619 (2016).
- Yamashita K. et al. Kotai Antibody Builder: automated high-resolution structural modeling of antibodies. Bioinformatics 30, 3279-3280 (2014).

Quantitative Immunology

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

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

Quantitative Approaches

Accurate quantification of biological responses is critical for understanding the dynamics of complex systems. Previously, we developed a fluorescent protein reporter system for the quantitative monitoring of IFN-a6 (Kumagai et al., 2007). Now we are trying to increase the "dimensions" 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. We are also developing automated computational algorithms to extract important quantities to understand interferon regulation from such time lapse imaging data.

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

Data Integration

The accumulation of high-throughput ("omics") datasets has brought biology into the big data era and the need for approaches that integrate, summarize and extract relevant information that reveals the relation between biological components. We are developing methods that integrate measurements of transcription factor binding with transcriptome data from different experimental conditions, and with protein-protein interaction data from public databases, to obtain insight into signaling and gene regulatory immune networks.

We apply these methods to study the mechanisms behind respiratory diseases, such as chronic obstructive pulmonary disease (COPD) and silicosis (Diez et al., 2015). A common feature of these diseases is that inflammation and disease progression are irreversible even after removing exposure to the harmful components (tobacco for COPD and silica for silicosis). Combining ATAC-seq and RNA-seq measurements of transcriptional activity in a mouse model of silicosis, we study the regulatory pathways associated with irreversible inflammation.

Mathematical Modeling

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

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

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

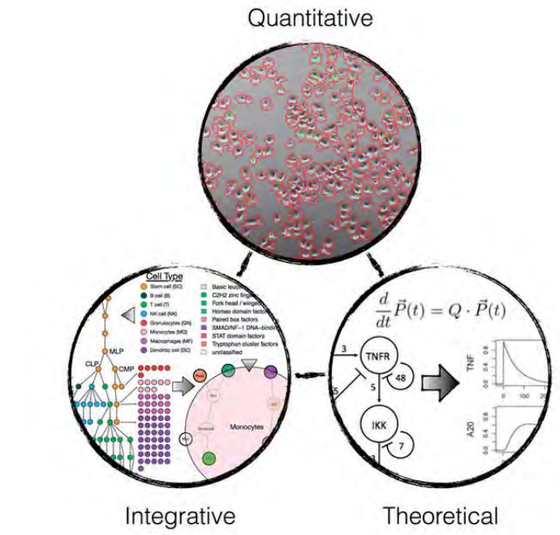

Figure. The Quantitative Immunology Research Unit combines quantitative, integrative and theoretical approaches.

Recent Publications

- Vandenbon A, Dinh V H, Mikami N, Kitagawa Y, Teraguchi S, Ohkura N, & Sakaguchi S. Immuno-Navigator, a batch-corrected coexpression database, reveals cell type-specific gene networks in the immune system. Proc. Nati. Acad. Sci. USA. 1604351113 (2016).
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- 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 9, e1003323 (2013).
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Next Generation Optical Immune Imaging

- Associate Professor **Noriko Takegahara**
- Visiting Academic staff **Kazuaki Tokunaga**

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 mechanism of generation of polyploidy. Myeloid cells such as macrophages and osteoclasts have a pronounced potential for cell fusion during development. Especially for osteoclasts, polyploidization is necessary to acquire sufficient bone-resorbing activity. However, precise mechanism of cell fusion is not fully understood. Our aim is to try to make advances in understanding the cellular and molecular mechanisms underlying polyploidization and cell fusion.

Identification of a novel mechanism for osteoclast polyploidization

During developmental programs, some mammalian somatic cells obtain additional sets of chromosomes by various mechanisms, including endocycles, endomitosis, incomplete cytokinesis, and cell fusion. Endocycles, endomitosis, and incomplete cytokinesis are directly associated with the proliferative state of the cell. By contrast, cell fusion is entirely independent of cell proliferation. Osteoclasts are specialized polyploid cells that resorb bone. Polyploidy is a hallmark of mature osteoclasts. Upon stimulation with receptor activator of nuclear factor kappa-B ligand (RANKL), myeloid precursors commit to becoming polyploid osteoclasts, largely via cell fusion. Although generation of polyploid osteoclasts is thought to occur due to cell fusion, independently of cell proliferation, some reports have pointed out a relationship between cell proliferation and osteoclast differentiation. These reports prompted us to investigate whether cell-cycle progression has an impact on polyploidization during osteoclastogenesis, and if so, how, and to what extent, the cell cycle regulates the polyploidization of osteoclasts. In 2015, we demonstrated that in addition to cell fusion, incomplete cytokinesis also plays a role in osteoclast polyploidization. RANKL stimulation transiently increased basal proliferation and induced polyploidy by incomplete cytokinesis as well as cell fusion. Polyploid cells generated by incomplete cytokinesis had the potential to subsequently undergo cell fusion. In addition, nuclear polyploidy (i.e., they contained nuclei with more than the diploid complement of chromosomes [> 2N]) was also observed in osteoclasts in vivo, suggesting the involvement of incomplete cytokinesis in physiological polyploidization. Furthermore, RANKL-induced incomplete cytokinesis was reduced by inhibition of Akt, resulting in impaired multinucleated osteoclast formation. Our findings reveal an unexpected pattern of cell division and fusion during the generation of polyploid osteoclasts.

Although our findings reveal an importance of incomplete cytokinesis in the formation of polyploid osteoclasts, a normal physiological process, the physiological significance of the phenomenon still remains unclear. This phenomenon may be just one aspect of the phenotype of terminally differentiated cells, or a consequence of stress response that preserves cell function. Alternatively, polyploid nuclei may create genetic diversity, which could promote better adaptation to chronic injury or stress. A full understanding of the mechanisms of polyploidization is necessary in order to reveal the physiological significance of osteoclast polyploidization via cell fusion and incomplete cytokinesis.

Exploration of fusion master molecule(s)

Tetraploid cells generated by cell fusion or incomplete cytokinesis had the potential to subsequently undergo cell fusion, indicating that tetraploids should express molecules required for cell fusion. By isolating the tetraploid cells, we performed gene screening and identified transmembrane molecules of which expression was detected in fusion competent cells but not in cells that do not obtain permission of cell fusion. We continued to investigate the biological function of these molecules using molecular and cellular biological methods, genetic methods, and optical methods such as flow cytometry and microscopy, and found that overexpression of these molecules enhanced multinucleation while knocked down transmembrane molecules inhibited multinucleation of osteoclasts. To clarify mechanisms underlying cell fusion, further analysis of transmembrane molecules is currently in progress.

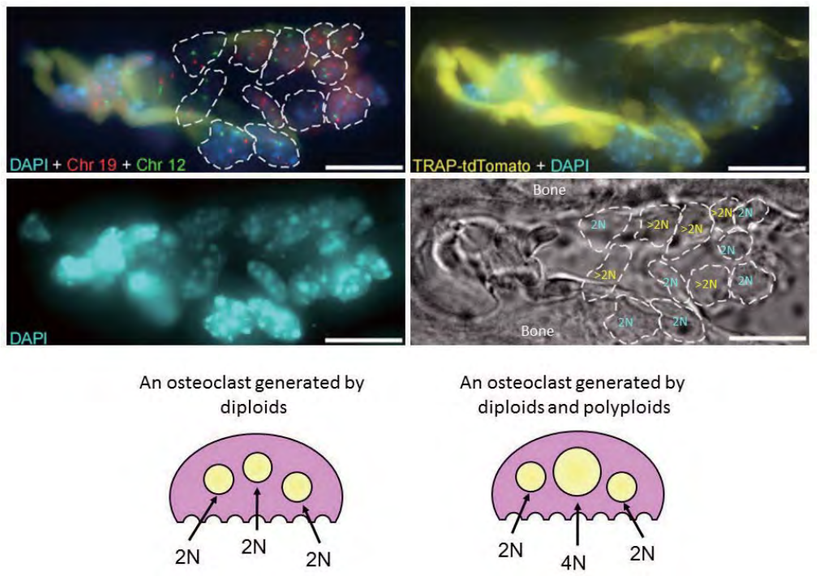

Figure.
Diploid and polyploid nuclei are observed in osteoclasts in vivo. Representative micrographs of fluorescence *in situ* hybridization (FISH).

Recent Publications

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Immuno-Genomics

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

The ultimate goal of immunology is health care: The 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.

Development of Immuno-Navigator, a database for gene coexpression in the immune system

The huge amounts of data in public databases contain enormous potential for unraveling complex dynamics in cellular biology. However, traditional biology lacks the means to analyze such big data. Moreover, it is hard to compare data of different laboratories. Thus, the extraction of meaningful information from public data is very difficult in practice.

During the last year, we continued our work on Immuno-Navigator (http://sysimm.ifrec.osaka-u.ac.jp/immuno-navigator/), a platform which allows researchers to use thousands of gene expression datasets for addressing key questions in immunology (Vandenbon *et al*., *PNAS*, 2016). The database at present contains both human and mouse gene expression data, and functions for exploratory analysis of this data. We added new functions allowing integrative study of coexpression data and experimental data, such as ChIP-seq data. Using our platform, researchers can explore information hidden in the data without concern for technical obstacles, and generate hypotheses regarding gene functions. Fig. 1 shows a conceptual overview of the usage of this database.

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. In previous studies, we investigated the role of Foxp3, Treg-specific gene expression profiles, and characteristic DNA hypomethylation in Tregs (Morikawa *et al*., *PNAS*, 2014).

In one of the applications of our Immuno-Navigator database, we predicted candidate regulators involved in the control of genes with enhanced expression in Tregs (Vandenbon *et al*., *PNAS*, 2016). Experimental validation showed that expression of one candidate regulator, integrin β8 (Itgb8), in Tregs was indeed associated with Treg-specific epigenetic features (see also Fig. 1). Further applications of our analysis platform on genes with repressed expression in Tregs suggested several candidate regulators involved in this repression. We are currently conducting experiments to elucidate the roles of these regulators in the establishment of functionally stable Treg cells.

Epigenetic changes in dendritic cells upon stimulation by lipopolysaccharide

Several of our previous studies have revealed links between transcription factors 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 understand the role of Akirin2 in recruiting the SWI/SNF complex to NF-κB target promoters (Tartey *et al*., *EMBO J*., 2014). However, details about the control of stimulus-induced epigenetic changes remain unknown.

In a collaborative study with Dr. Yutaro Kumagai of the Quantitative Immunology Unit, we are analyzing the temporal changes in histone modifications in dendritic cells upon LPS stimulation, using ChIP-seq experiments. Focusing on promoters and enhancers of LPS-inducible genes, we observed that changes in histone modifications follow a well-defined pattern, where some modifications appear early after stimulation, and others at later time points. Integrative analysis of our data with transcription factor binding data revealed that the binding of some transcription factors at promoters and enhancers coincides with induction times of some histone modifications at these regions. We believe this analysis can help reveal interactions between transcription factors and histone modifiers and their role in controlling the response to immune stimuli.

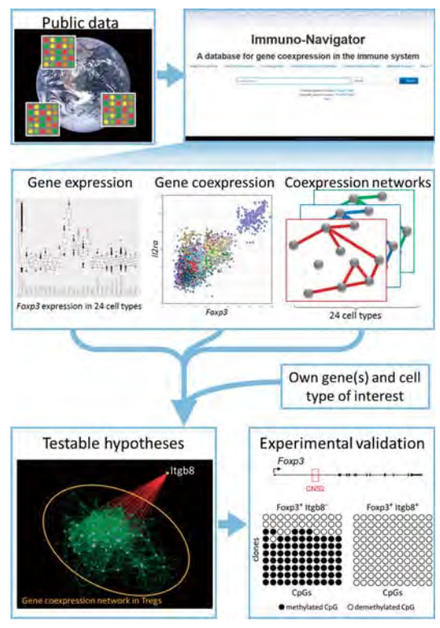


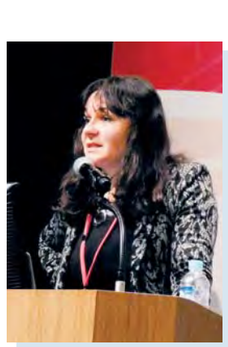
Fig. 1 Conceptual overview of the usage of the Immuno-Navigator database.
We collected a large amount of publicly available gene expression data from human and mouse immune cells into our database. Using our database, researchers can easily inspect gene expression and correlation of expression in different cell types. Using lists of genes of interest as input, researchers can generate promising hypotheses, and perform validation experiments.

Recent Publications

- Vandenbon A, et al. Immuno-Navigator, a batch-corrected coexpression database, reveals cell type-specific gene networks in the immune system. Proc. Natl. Acad. Sci. USA 113, 2393-2402 (2016).
- Mino T, et al. Regnase-1 and Roquin regulate a common element in inflammatory mRNAs by spatiotemporally distinct mechanisms. Cell 161, 1058-1073 (2015).
- 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, 2673-2682 (2015).
- Yang X, et al. VEGF-B promotes cancer metastasis through a VEGF-A-independent mechanism and serves as a marker of poor prognosis for cancer patients. Proc. Natl. Acad. Sci. USA 112, 2900-2909 (2015).
- 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, 5289–5294 (2014).

Symposia & Seminars

The 7th International Symposium of IFReC : Immunology at the Forefront



Date : January 21-22, 2016

Venue : KNOWLEDGE THEATER, Grand Front Osaka

This symposium provided a forum for the newest developments in wide-ranging areas of immunology. The symposium was effectively organized in combination with the 5th NIF Winter School by IFReC and Singapore Immunology Network, and an opportunity for the young researchers to form a global network.

Jan. 21

Speaker	Title
Chyi-Song Hsieh Washington University in St. Louis, USA	The development of the regulatory T cell receptor repertoire
Leonie Taams King's College London, UK	Cellular and molecular mechanisms of immune regulation in rheumatoid arthritis
Yenan Bryceson Karolinska Institutet, Sweden	Natural killer cells remember
Masahiro Yamamoto Osaka University, Japan	IFN-γ-inducible cell-autonomous immunity to intracellular pathogens
Rikinari Hanayama Kanazawa University, Japan	Mechanisms of hemophagocytosis and heterolysis by phagocytes

Jan. 22

Speaker	Title
Christoph Scheiermann Ludwig Maximilians University Munich, Germany	Mechanisms mediating circadian leukocyte trafficking
Kazuhiro Suzuki Osaka University, Japan	Adrenergic control of lymphocyte recirculation through lymph nodes
Naoto Kawakami Ludwig Maximilians University Munich, Germany	In vivo imaging of autoimmunity in the Central Nervous System
Masakatsu Yamashita Ehime University, Japan	Determination of T-cell fate by glutamine metabolism
Koji Hase Keio University, Japan	Interkingdom interaction shapes the mucosal immune system
Keishi Fujio University of Tokyo, Japan	Suppression of B cell functions by regulatory cytokines
Yoshihiro Baba Osaka University, Japan	Regulatory function of B cells in autoimmune inflammation
Tri Giang Phan Garvan Institute, Australia	Where and how memory cells are reactivated in the secondary antibody response in the lymph node

IFReC Seminars

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

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

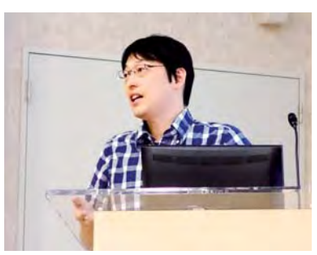
Date	Speaker	Affiliation	Title
2015			
May 19	**Masashi Watanabe**	National Cancer Institute, NIH, USA	The role of tumor suppressor p53 in CD4 T cell function and homeostasis
Jun. 22	**Bali Pulendran**	Emory University School of Medicine, USA	Systems based approach to vaccine design
Jul. 9	**Serkan Göktuna**	Bilkent University, Turkey	IKKe promotes intestinal tumorigenesis via establishing a pro-inflammatory microenvironment
	Tieu Lan Chau	University of Liege, Belgium	A role for APPL1 in TLR3/4-dependent TBK1 and IKKe activation in macrophages
Sep. 24	**Scott I. Simon**	University of California Davis, USA	Engineering the innate immune response to cutaneous Staph-aureus infection
Sep. 30	**Yihai Cao**	Karolinska Institutet, Sweden	Angiogenesis in diseases
Oct. 9	**Chen Dong**	Tsinghua University, China	Molecular mechanisms in T cell differentiation
Oct. 30	**Eddy F. Y. Liew**	University of Glasgow, UK	The role of IL-33 in infectious and inflammatory disease
Nov. 6	**Andreas Diefenbach**	University of Mainz Medical Centre, Germany	Transcriptional control of cell fate decision in the innate immune system
Nov. 16	**Takeshi Egawa**	Washington University, USA	A c-MYC-initiated transcriptional cascade in the regulation of lymphocyte clonal expansion in protective immunity
2016			
Jan. 26	**Jean Pieters**	University of Basel, Switzerland	Regulation of pathogen survival and immune responses through coronin 1-mediated activation of cell surface signaling
Feb. 5	**André Veillette**	University of Montréal/ McGill University, Canada	Receptors controlling NK cell functions
Feb. 19	**Fijs W.B. Van Leeuwen**	Leiden University Medical Center, The Netherlands	Hybrid fluorescence navigation surgery : from molecule to man

IFReC Colloquia

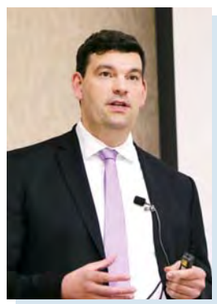
IFReC colloquia are a series of discussion meetings for IFReC members held once every other month. At each colloquium, three speakers from IFReC laboratories give talks about their latest research progress followed by intensive discussion. After the colloquium, a small social gathering is held to further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

Date : **20th Colloquium : April 15, 2015**
21st Colloquium : June 10, 2015
22nd Colloquium : August 5, 2015
23rd Colloquium : December 9, 2015
24th Colloquium : February 10, 2016

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



	Speaker	Title
20th	**Etsushi Kuroda** (Vaccine Science)	Fine particle kills alveolar macrophage to release IL-1 alpha that induces bronchus-associated lymphoid tissue in the lung
	Hideaki Fujita (Single Molecule Imaging)	Predicting activation status of T-cell
21st	**Yuji Yoshida** (Immunopathology)	Semaphorin 4D contributes to rheumatoid arthritis by inducing inflammatory cytokine production: Pathogenic and therapeutic implications
	Mikael Martino (Host Defense)	Inhibition of IL-1R1/MyD88 signaling promotes mesenchymal stem cell-driven tissue regeneration
	Kazuo Yamashita (Systems Immunology)	Modeling B cell repertoires at atomic resolution
22nd	**Mohamed El Sherif Badr** (Cell Signaling)	Mechanism and status of T cell activation by self-recognition
	Ryo Shinnakasu (Lymphocyte Differentiation)	Selection of germinal center B cells into the memory compartment
	Katsumori Segawa (Biochemistry and Immunology)	Caspase-mediated cleavage of phospholipid flippase for phosphatidylserine exposure during apoptosis
23rd	**Naganari Ohkura** (Experimental Immunology)	The development of regulatory T cells
	Yuichiro Hori (Chemical Imaging Techniques)	Fluorogenic probes for uncovering physiological function of N-glycan
	Szandor Simmons (Immunology and Cell Biology)	Implications of Spns2-deficiency on S1P-driven lymphocytes egress, HEV-integrity and immunity
24th	**Yuki Mori** (Biofunctional Imaging)	4D single cell tracking with MRI in living mouse brain
	Yutaro Kumagai (Quantitative Immunology Unit)	Understanding type I interferon production by quantifying epigenomics and single cell gene expression
	Fumiji Saito (Immunochemistry)	Immune evasion by Plasmodium falciparum via an inhibitory receptor

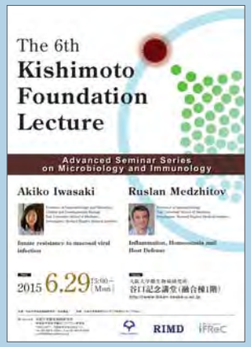


The 6th Kishimoto Foundation Lecture

Date : June 29, 2015

Venue : Taniguchi Memorial Hall, Osaka University

Host : Research Institute for Microbial Diseases & IFReC



Akiko Iwasaki

Professor, Yale University School of Medicine
Investigator, Howard Hughes Medical Institute

"Innate resistance to mucosal viral infection"

Ruslan Medzhitov

Professor, Yale University School of Medicine
Investigator, Howard Hughes Medical Institute

"Inflammation, Homeostasis and Host Defense"

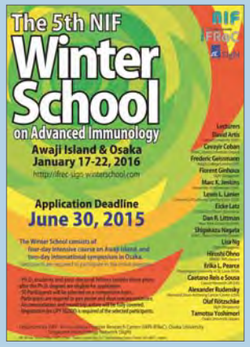
Events

The 5th NIF Winter School on Advanced Immunology

The 5th NIF Winter School on Advanced Immunology was held with Singapore Immunology Network (SIgN) in Japan on 17-22 January 2016. The program comprised a 4-day lecture course on Awaji Island and a 2-day international symposium in Osaka. Forty-nine young researchers from around the world, selected from 191 applicants, learned cutting-edge immunology from 16 world-renowned senior researchers at the course and 13 up-and-coming researchers at the symposium. The Winter School was highly evaluated by the participants and the lecturers as an excellent opportunity for networking with young peers through active discussions and communications as well as its high scientific level.

Date： **January 17- 22, 2016**

Venue： **Awaji Yumebutai International Conference Center, Hyogo /**
KNOWLEDGE THEATER, Grand Front Osaka
（The 7th International Symposium of IFReC）



Lecturer	Title
Caetano Reis e Sousa The Francis Click Institute, UK	Dendritic cell development and function
Frederic Geissmann Memorial Sloan-Kettering Cancer Center, USA	Resident and passengers: The architecture and functions of a layered myeloid system
Florent Ginhoux Singapore Immunology Network, Singapore	Dendritic cell and macrophage ontogeny
Eicke Latz University of Bonn, Germany / UMass Medical School, USA	Activation and regulation of inflammasomes in inflammatory diseases
Marc K. Jenkins University of Minnesota, USA	Tackling the helper T cell repertoire
Dan R. Littman New York University School of Medicine, USA	Mechanism of Th17 cell programming by the intestinal microbiota
Alexander Y. Rudensky Memorial Sloan Kettering Cancer Center, USA	Differentiation and function of regulatory T cells
Olaf Rötzschke Singapore Immunology Network, Singapore	Dissection of allergic reactions by systems biology in an allergen-saturated tropical-urban environment
David Artis Weill Cornell Medical College of Cornell University, USA	Immune regulation at barrier surfaces
Lewis L. Lanier University of California San Francisco, USA	Innate lymphoid cells and natural killer cells
Shigekazu Nagata IFReC, Osaka University, Japan	Apoptosis and exposure of phosphatidylserine
Tamotsu Yoshimori Osaka University, Japan	Autophagy: The intracellular self-degradation system fighting against diseases
Erika L. Pearce Max Planck Institute for Immunobiology and Epigenetics, Germany	Metabolic interactions in the tumor microenvironment
Hiroshi Ohno RIKEN Center for Integrative Medical Science, Japan	Gut microbiota, host defense and immunity
Cevayir Coban IFReC, Osaka University, Japan	Tissue-specific immunopathology during malaria
Lisa F.P. Ng Singapore Immunology Network, Singapore	Cellular and molecular mechanism of alphavirus pathogenesis: Implications for disease interventions

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 up to the cutting-edge of their research in an easy-to-understand manner. The lectures are open to all Osaka University members and held in the evening so that the participants can attend after work. During the lecture, participants can interact with the speaker, asking questions in an informal setting. Initiated in December 2013, the series was held ten times until the end of FY2015 and the questionnaire results from the participants show a high level of satisfaction throughout the series. The average number of participants is 45. 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.

This event was proposed for "Osaka University's Improvement Idea Awards Contest" as an activity model. In this proposal, we introduced IFReC "Immunology Lecture Series" to provide an opportunity for young researchers to explain their research in plain words for support staff (secretaries, technical staff and administrative staff) of each department in Osaka University in order to increase the understanding and motivation of them as well as to improve the presentation skills of young researchers. This proposal was admirably selected for the award.

Venue : Biken Hall, Osaka University
Date : July 22, 2015
Speaker : Masahiro Yamamoto (Immunoparasitology)
Title : Immune mechanism against parasites
— How Toxoplasma hijacks a host body?

Date : March 17, 2016
Speaker : Fuminori Sugihara (Biofunctional Imaging)
Title : Life phenomena observed
— MRI as a research tool

Emergency Drill

IFReC held a fire drill to raise all staff's awareness on their safety and security in their work environment. The staff was reminded of the procedures for evacuation and how to use fire extinguishers under the instruction of a staff member from the Osaka University Department of Safety and Hygiene.

Date : November 11, 2015
Participants : All IFReC Staff

Explanation Session for Preventing the Misuse of Funds

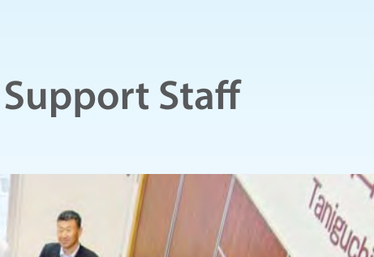
IFReC conducted a seminar to communicate the necessity for compliance by ensuring the awareness of staff of the usage rules for competitive research funding and staff obligations when using the staff purchasing system.

The bilingual session was designed to ensure that all IFReC members are fully informed of procedures and responsibilities.

Date : February 10, 2016
Venue : Taniguchi Memorial Hall, Osaka University

The 1st Workshop for WPI Research Support Staff

The Research Planning and Management Office at IFReC took initiative to organize the first workshop for support staff of WPI institutes including administrative and technical staff, and others such as university research administrators. Forty-one participants from all nine WPI institutes shared the successful achievements of each institute and discussed support for globalization, promotion of interdisciplinary research, and system reformation in order to realize world-visible research institutes.



Date : May 18 - 19, 2015

Venue : Taniguchi Memorial Hall, Osaka University /
IFReC Research Building (for IFReC introduction tour)

Participants : Research support staff at nine WPI centers;
Kavli-IPMU, iCeMS, AIMR, MANA, I²CNER, IIIS, ELSI, ITbM, and IFReC

DAY 1（May 18）	
Opening remarks	**Nobuo Sakaguchi** (Administrative Director, IFReC)
Explanation of proceedings	**Akihiko Takagi** (Research Planning and Management Office, IFReC)
KAVLI IPMU	**Kavli-IPMU, The University of Tokyo** Research support at Kavli IPMU
WPI Osaka University iFReC	**IFReC, Osaka University** Towards a new style of research support - IFReC Research Planning and Management Office -
iCeMS	**iCeMS, Kyoto University** An outline of iCeMS
AIMR	**AIMR, Tohoku University** Research support at AIMR
mana	**MANA, National Institute for Materials Science** Omotenashi is our catchphrase -MANA Administration Division-
Panel Discussion The role, expectations and reality of research support in WPI	**Moderator** Akihiko Takagi (Research Planning and Management Office, IFReC) **Panelists** Tomiyoshi Haruyama (Administrative Director, Kavli-IPMU) Shinichi Sato (Deputy Administrative Director, AIMR) Tomoko Narisawa (Technical Support Staff, ELSI) Ayato Sato (Head of Research Promotion, ITbM)

DAY 2（May 19）	
I²CNER	**I²CNER, Kyushu University** Approaches by I²CNER Administration Division
IIIS	**IIIS, University of Tsukuba** Solving the problem of sleep -Research support at IIIS
ELSI EARTH-LIFE SCIENCE INSTITUTE TOKYO INSTITUTE OF TECHNOLOGY	**ELSI, Tokyo Institute of Technology** Outline of research support at ELSI
ITbM Nagoya University	**ITbM, Nagoya University** Approaches by ITbM and future challenges
Closing remarks	Nobuo Sakaguchi (Administrative Director, IFReC)
Tour of IFReC	① Prof. Akira's Laboratory, Integrated Life Science Bldg. 10F ② Core Instrumentation Facility and Server room, IFReC Bldg. 4F ③ MRI, IFReC Bldg. 3F ④ Administrative Office, IFReC Bldg. 2F

Japanese Class

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 FY2015, international staff and Japanese volunteers enjoyed both traditional and trendy aspects of Japanese culture, with traditional “Tanabata”, and cooking trendy “Deco-Onigiri” (decorated rice ball). 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.

June 30, 2015 Tanabata& Soumen

November 26, 2015 Onigiri

Outreach Activities

Science Cafe

The series of science cafes is a long- lasting IFReC outreach activity to promote communication among researchers and the general public. It also enhances people's understanding of immunology researches and the researchers involved in them. In FY2015, IFReC science cafes were held at Osaka University Suita campus and at CAFE Lab. in the center of Osaka City. CAFE Lab. at Grand Front Osaka attracts a lot of office workers, who can feel free to stop by the venue after their work time. About 170 people in total participated in our science cafes.

Science Cafe on the Edge at 2015 Icho Festival
[Latest knowledge on gut immunity]

Date : May 2, 2015
Venue : Techno Alliance Building, Osaka University
Guest : Kiyoshi Takeda (Graduate School of Medicine, Osaka University / Mucosal Immunology, IFReC)

Science Cafe on the Edge 16
[Immunology research revealed by bioimaging techniques]

Date : January 22, 2016
Venue : CAFE Lab, Grand Front Osaka
Guest : Naoto Kawakami (Ludwig-Maximilians University Munich, Germany),
Masaru Ishii (Graduate School of Medicine, Osaka University / Immunology and Cell Biology, IFReC)

Super Science High School Student Fair 2015

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

The SSH Student Fair 2015 was held in Osaka and more than 200 schools, including several schools from overseas, held booths with posters to present their researches. WPI institutes held a collaborative booth and introduced the research activities of each institute using posters, booklets and demonstrations. Several WPI researchers including Akiko Nakai (Immune Response Dynamics, IFReC) gave short talks at "Researchers' mini-live talk".

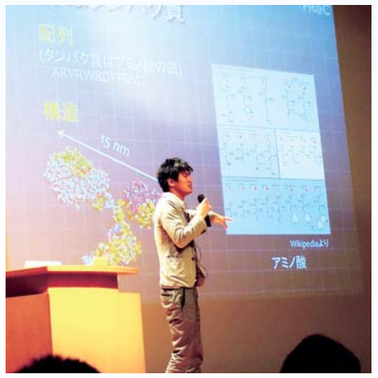
Date： August 5-6, 2015
Venue： INTEX Osaka
Host： MEXT, JST
Support： Boards of Education (Osaka Prefecture and Osaka City)

The 5th Annual WPI Joint Symposium

The 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 students' interest in science.

The 5th WPI joint symposium entitled "Science to sense and feel" was held in Kyoto and gathered about 400 participants. The program included talks by WPI scientists, a special talk by the president of Kyoto University, panel discussion, and booth exhibition.

Kazuo Yamashita (Systems Immunology, IFReC) gave a talk titled "Computer fighting against Viruses", and joined the panel discussion on laboratory notebooks. IFReC also opened a booth to introduce its research activities and held a demonstration experiment to show stained immune cells in a mouse lung with a laser microscope, which attracted a lot of visitors.



Date： December 26, 2015
Venue： Clock Tower Centennial Hall, Kyoto University (Kyoto)
Host： Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University
Cohost： WPI Institutes
Support： MEXT, JSPS, Boards of Education (Kyoto Prefecture and Kyoto City)

Speakers
- Koh Nagata (Assistant Professor, iCeMS, Kyoto University)
- Kazuo Yamashita (Assistant Professor, IFReC, Osaka University)
- Ken Takai (Principal Investigator, ELSI, Tokyo Institute of Technology)
- Jyuichi Yamagiwa (President, Kyoto University)

Navigator
Hejcik Pavel (Science Communicator, Miraikan - The National Museum of Emerging Science and Innovation)

Guest for panel discussion
Aya Ota (Freelance writer)

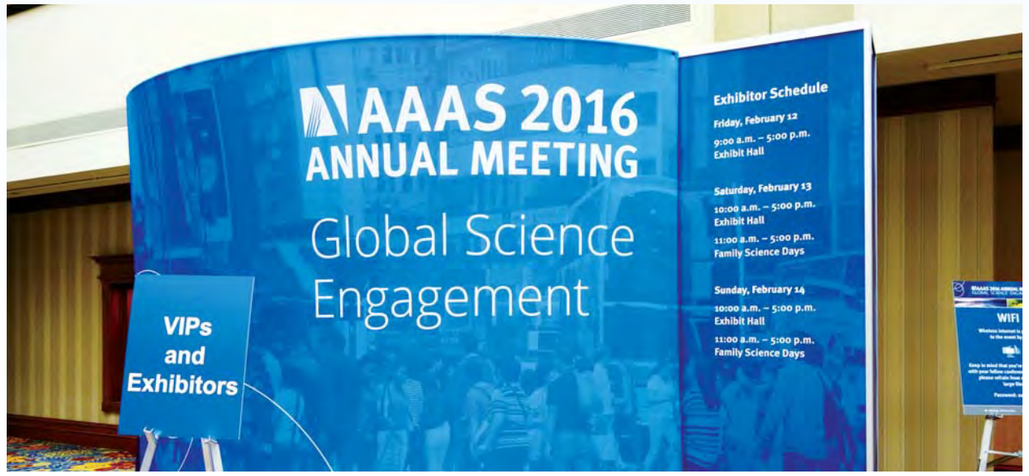
AAAS 2016 Annual Meeting

The American Association for the Advancement of Science (AAAS) is the biggest international scientific society in the world, and its mission is to “advance science and serve society”. The AAAS 2016 Annual Meeting was held in the US capital and it offered a broad range of activities including lectures, symposia, seminars and exhibits, with the theme of "Global Science Engagement".

WPI institutes held a collaborative booth to introduce the WPI program and the institutes’ activities using posters, booklets and a demonstration experiment. More than 300 participants visited the booth and gained interest in the WPI program and world leading researches in Japan.

Date： **February 11-15, 2016**

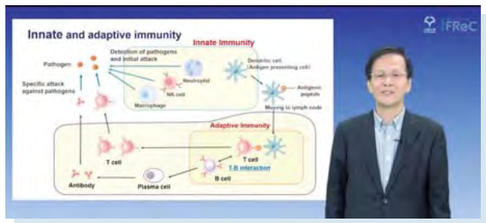
Venue： **Washington Marriott Wardman Park (Washington D.C., USA)**



IFReC on edX

Osaka University joined edX, one of the major Massive Open Online Course (MOOC) platforms, founded by Massachusetts Institute of Technology (MIT) and Harvard University. As the first course of OsakaUx (edX courses by Osaka University), eight IFReC researchers including Director Akira provided lectures from July to August 2015, with the support of the Teaching and Learning Support Center of Osaka University. The course provided fundamental knowledge in immunology as well as some advanced topics including cutting-edge research results. About 12,000 people enrolled in the course.

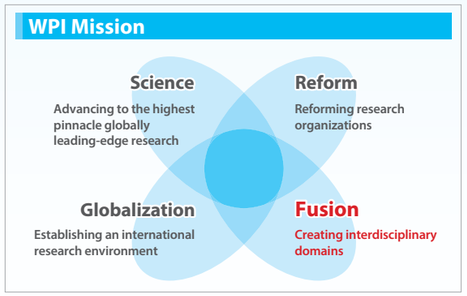
The second period of the program is scheduled to be distributed in FY2016. This time, IFReC researchers from bioinformatics and imaging fields will join the program.



Research Projects

Support Programs for Fusion Researches

One of the goals of the 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, the members of which are researchers from different groups / backgrounds. This program effectively encourages interaction and fusion among different groups. So far, 25 projects have received financial support from IFReC and some of the studies have been successfully published.

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. This program was introduced as a platform to foster young pioneers in the fusion field and to further promote interdisciplinary research at IFReC.

Five Combined Research Program projects and one Dual Mentor Program project ended in September 2015 and 5 projects are still in progress.

Evaluation Workshop for Research Support Program

All the project leaders presented their research results 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 leaders. The projects which ended in 2015 were given awards according to the score.

Date : October 7, 2015

Venue : Taniguchi Memorial Hall, Osaka University







Project Leader	Collaborators	Project Title
2012		
Masahiro Yamamoto	D. Standley E-M. Frickel	Trilateral analysis of interferon-γ-mediated cellular innate immunity against toxoplasma gondii
Masako Kohyama	C. Coban K. Suzuki F. Sugihara T. Aoshi	Role of tissue macrophage in malaria infection, and their developmental control by parasite metabolite
Barry Ripley	D. Standley G. Kurisu (IPR)	Role of arid5A in the selective control of IL-6 mRNA stability and development of TH17 cells
Fuminori Sugihara	R. Hanayama K. Kikuchi M. Kohyama S. Satoh S. Akira	In vivo imaging of germinal center development in mouse spleen using MRI
Tomoyuki Yamaguchi	H. Fujita S. Sakaguchi T. Watanabe H. Machiyama C. Furusawa S. Esaki	Imaging analysis of immune activation and regulation
Dual Mentor Program Takeshi Yoshida	R. Hanayama K. Suzuki D. Diez	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 N. Trost	Visualizing information processing of immune cells via combination of fluorescent reporter and single molecule imaging
Naganari Ohkura	A. Vandenbon S. Nakamura S. Yamazaki S. Kato M. Hashimoto	Development of an epigenome-based computational classification system for the treatment of autoimmune diseases
Kazuhiro Suzuki	Y. Baba	Visualizing activation of germinal center B cells using genetically encoded calcium indicators
Alexis Vandenbon	S. Sakaguchi H. Morikawa N. Ohkura	Identification of key factors for inducing functionally stable regulatory T cells

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

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 IFReC young researchers who wish to participate in research activities at overseas institutions. The program aims to develop the practical skills and abilities of young researchers in international collaborative research and to develop their network with researchers overseas. Four researchers used this support program in FY2015.

Young Scientist Support Program for Research Abroad

Name	Country	Conferences attended
Chen Ting	Canada	ISMRM (International Society for Magnetic Resonance Medicine)
Alison Jane Hobro	Germany / UK	1. PhysCell 2015 2. EMBO Meeting 2015 3. Visit to Chemistry department at Lancaster University
Szandor Simmons	Australia	4th European Congress of Immunology
Alison Jane Hobro	USA	SciX 2015
Mayuri Takana	USA	Keystonesyposia; Immunity in Skin Development, Homeostasis and Disease (B5)

Support provided for 43 overseas visits by young researchers in the past five years

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

Data

Common Facilities

IFReC and its parent institution, the Research Institute for Microbial Diseases (RIMD) are located on the same site, constituting a large research complex. It contains the Core Instrumentation Facility, the Animal Resource Center and the Network Administration Office, all of which are jointly operated by IFReC and RIMD. The Core Instrumentation Facility is equipped with various highly advanced instruments and skilled technicians provide in-house services to IFReC and RIMD researchers. The Animal Resource Center consists of three buildings for specific pathogen-free (SPF) animals and the live immuno-imaging facility. With a large 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. **Main Building, Research Institute for Microbial Diseases**
4. **South Building, Research Institute for Microbial Diseases**
5. **Cutting-edge Research Building for Infectious Diseases**
6. **Animal Resource Center for Infectious Diseases**

Animal Resource Center for Infectious Diseases

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

Live Immuno-Imaging Facility

- SPF animal 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.

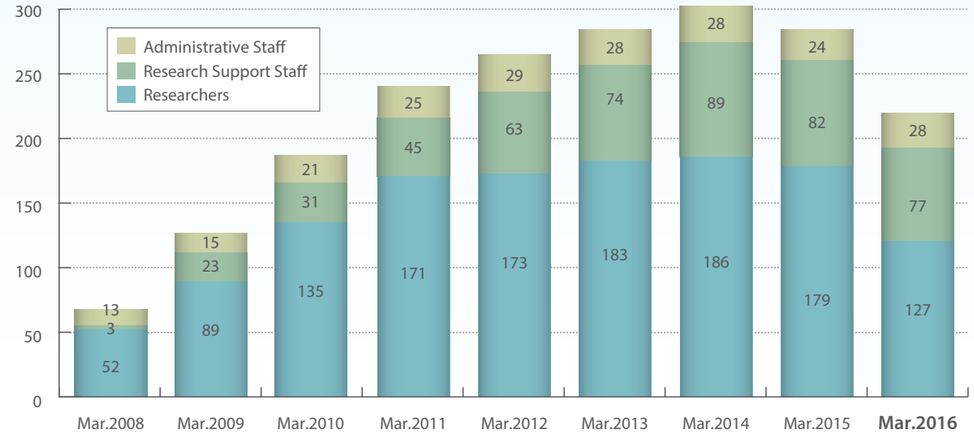
The Kishimoto Foundation was established in 2008 in honor of Tadamitsu Kishimoto, who, during the 1980s and 90s, elucidated the function of interleukin-6 (IL-6), a key molecule for stimulating immune responses. He later developed the anti-IL6 receptor-based therapy, tocilizumab, to treat immune disorders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

FY2015 Kishimoto Fellowship Recipients

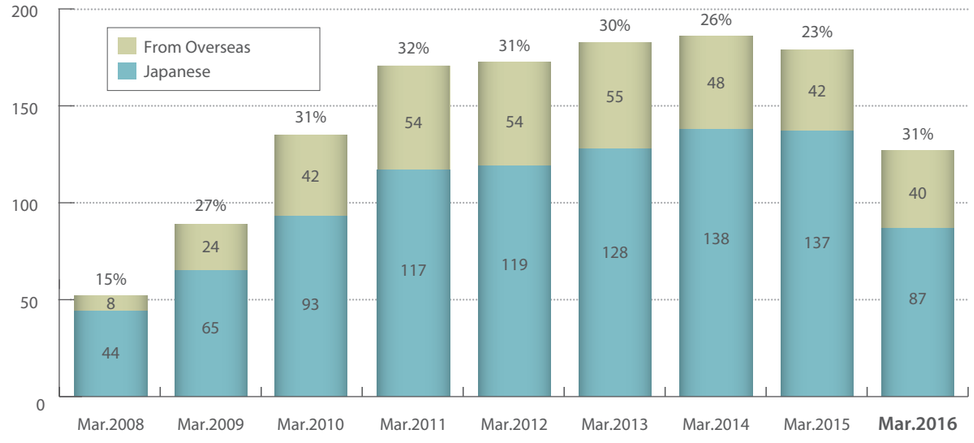
Position of recipient	Name (initials)	Nationality	Host researcher	Period
Specially Appointed Researcher	P. D.	India	Kishimoto	Nov. 1, 2012 - Oct. 31, 2015
Specially Appointed Researcher	D. M.	UK	Kishimoto	Dec. 1, 2013 - Mar. 31, 2017
Specially Appointed Researcher	Y. R.	China	K. Ishii	Apr. 1, 2014 - Mar. 31, 2017
Specially Appointed Researcher	J. H.	China	Arase	May 1, 2014 - Apr. 30, 2015
Specially Appointed Researcher	K. R.	Slovenia	Akira	Nov. 16, 2014 - Nov. 15, 2015
Specially Appointed Researcher	L. H.	Taiwan	Coban	Dec. 16, 2014 - Jul. 31, 2015
Specially Appointed Researcher	M. B.	Egypt	Saito	Apr. 1, 2015 - Dec. 31, 2015
Specially Appointed Researcher	T. N.	Germany	Seymour	Apr. 1, 2015 - Mar. 31, 2017
Research Fellow	H. H.	Jordan	Kishimoto	Jun. 8, 2015 - Aug. 6, 2015
Research Fellow	Y. C.	China	Yamamoto	Jul.1, 2015 - Dec. 28, 2015
Specially Appointed Researcher	P. L.	Australia	Coban	Oct. 1, 2015 - Sep. 30, 2016
Specially Appointed Researcher	D. H.	Australia	Sakaguchi	Oct. 16, 2015 - Oct. 15, 2016
Research Fellow	N. N.	Vietnam	Kishimoto	Nov. 2, 2015 - Jan. 28, 2016
Research Fellow	T. T.	Vietnam	Kishimoto	Nov. 2, 2015 - Jan. 28, 2016

Composition

Number of IFReC Staff



Number of Researchers



Major Awards

Shimon Sakaguchi — Thomson Reuters Citation Laureate

Sakaguchi was elected as a Thomson Reuters Citation Laureate 2015. The Laureates are cited so frequently in the previous two or more decades that these scientists typically rank in the top 0.1% in their research fields. The laureates are recognized to have written multiple high-impact articles over many years.

Sakaguchi at the Thomson Reuters Citation Laureate Award Ceremony (June, 2016)

Six IFReC Researchers — The World's Most Influential Scientific Minds 2015

Highly Cited Researchers are researchers with papers that have a large number of citations from all over the world as selected by Thomson Reuters. In 2015, some 3,000 researchers from 21 fields were selected as being some of the most influential researchers in the world.

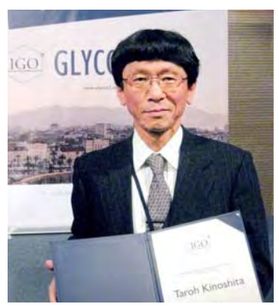
Seven selections were from IFReC for a total of six researchers (Director Akira was selected for two categories).

Biology & Biochemistry
- Shizuo Akira, Host Defense, IFReC

Immunology
- Shizuo Akira, Host Defense, IFReC
- Shimon Sakaguchi, Experimental Immunology, IFReC
- Ken Ishii, Vaccine Science, IFReC
- Kiyoshi Takeda, Mucosal Immunology, IFReC
- Masahiro Yamamoto, Immunoparasitology, IFReC
- Hiroaki Hemmi, Immune Regulation, IFReC (~2015)

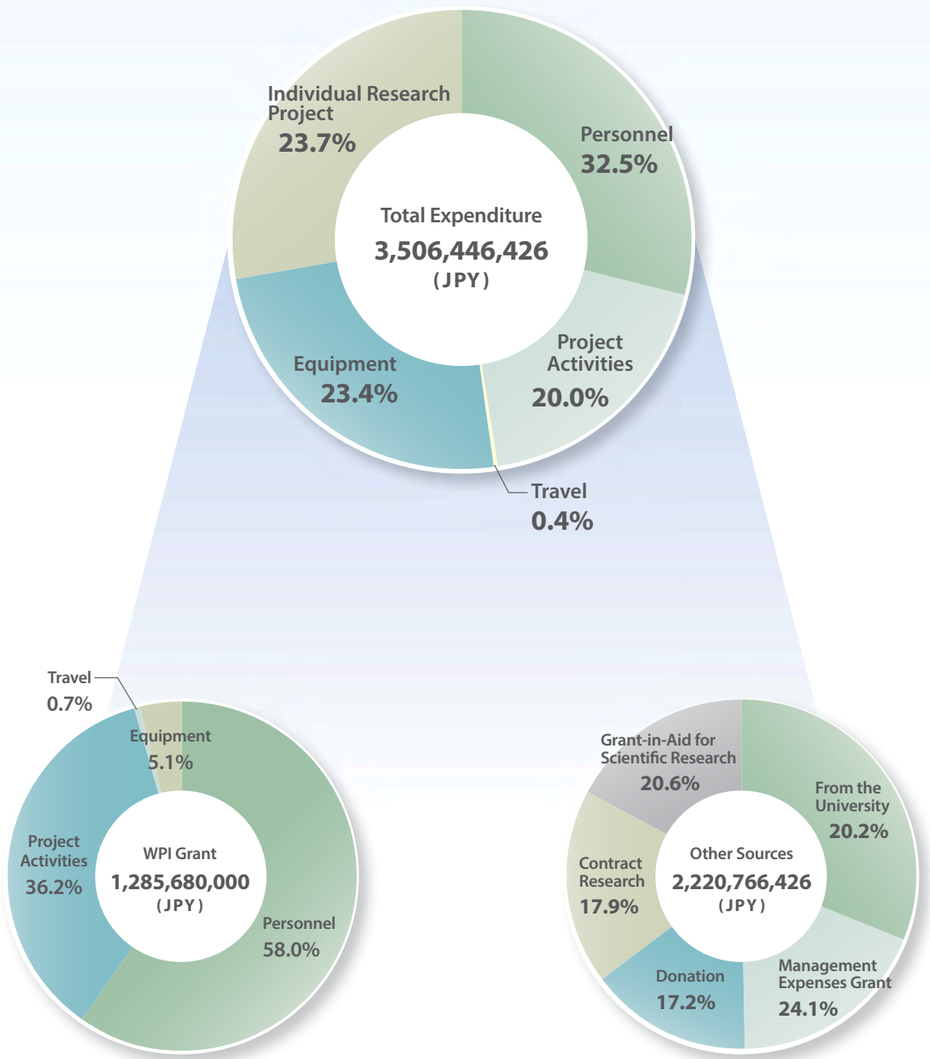
Taroh Kinoshita — International Glycoconjugate Organization Award

Kinoshita was awarded the International Glycoconjugate Organization (IGO) Award 2015 and presented an award lecture at XXIII International Symposium on Glycoconjugates held in Split, Croatia on September 15, 2015. The title for the award lecture was "Glycosylphosphatidylinositol-anchored proteins: biosynthesis, transport, shedding and deficiencies".



Finance

Breakdown of total expenditure at IFReC in FY2015



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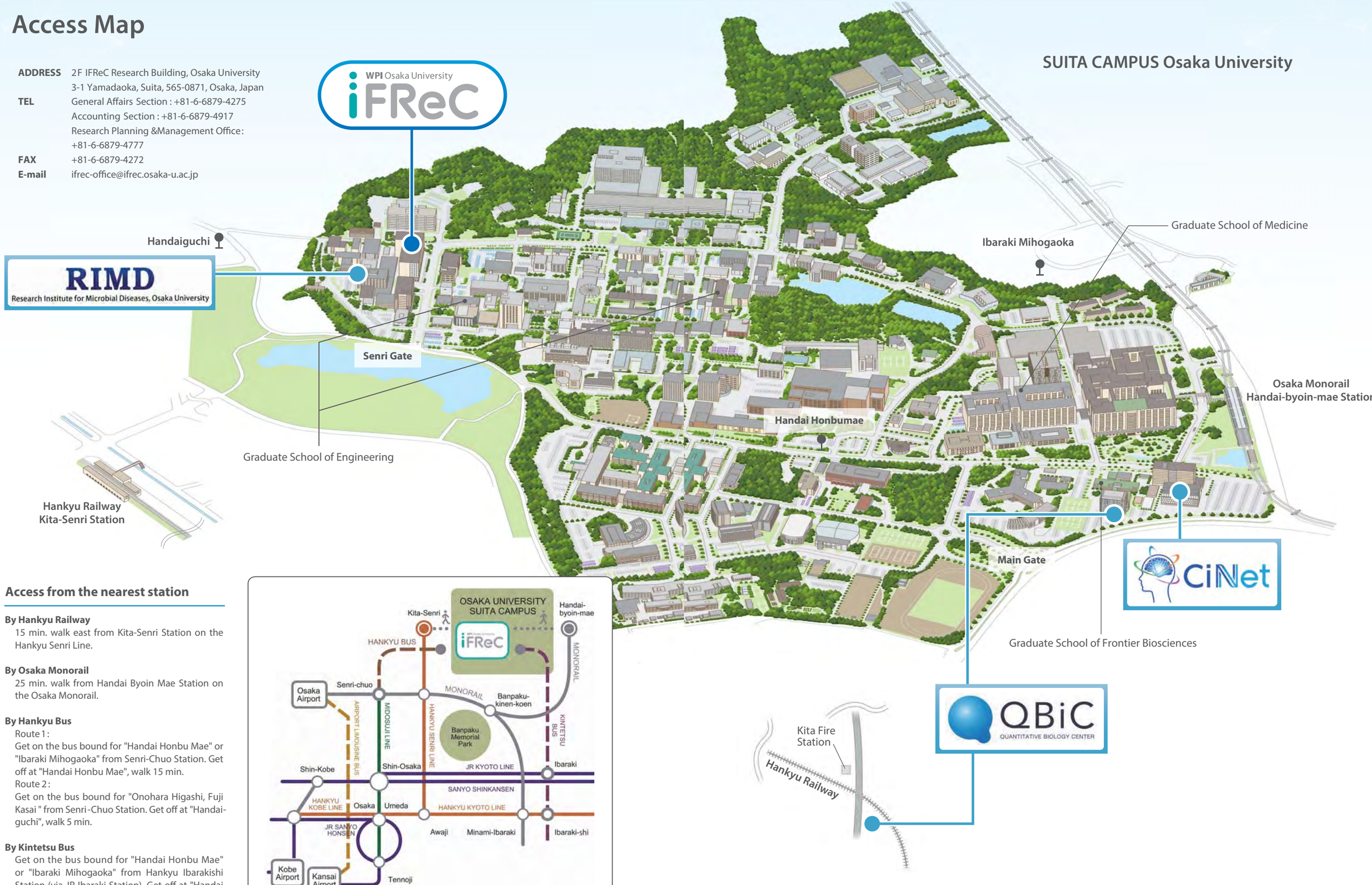

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