World Premier International
Research Center

Osaka University

# Immunology Frontier Research Center

## Annual Report of IFReC FY 2018

Osaka University

WPI Immunology Frontier Research Center FY2018



Edit : Research Planning & Management Office, IFReC
Published in June, 2019

wpi
World Premier International
Research Center Initiative

WPI Osaka University
iFReC

# CONTENTS


Message from the Director ..... 1

**Organization**

Organization Chart ..... 4
Committee and Advisory Board for IFReC ..... 6
Administrative Office of IFReC ..... 8

**Laboratories**

Immunology Group ..... 10
Imaging Group ..... 50
Informatics Group ..... 62
Units for Combined Research Fields ..... 66

**Events & Outreach Activities**

Cluster Science Days 2018 and the 10th International Symposium of IFReC ..... 70
Osaka-Heidelberg / Mannheim Sympoium on Immune Plasticity ..... 71
Next Gen Immunology in Health and Disease ..... 72
The 8th NIF Winter School on Advanced Immunology ..... 74
Visitors to IFReC ..... 76
IFReC Seminars ..... 78
IFReC Colloquia ..... 80
Science Café ..... 82
Students Visit / Science Agora ..... 83
Osaka University Co-Creation Festival / Workshop Festival ..... 84
Super Science High School Student Fair ..... 85
"Kagaku Zanmai" in Aichi / The 7th WPI Science Symposium ..... 86
AAAS Annual Meeting 2019 in Washington DC ..... 87
Japanese Language Classes ..... 88

**Research Projects**

Advanced Postdoc System ..... 90
Young Scientist Support Program for Research Abroad ..... 91

**Data**

Common Facilities ..... 94
Kishimoto Foundation Fellowship ..... 95
Major Awards ..... 96
Composition ..... 98
Finance ..... 99
Selected Articles ..... 100
Publications ..... 104

**Access Map** ..... 110


# Message from the Director

As the Director of the Immunology Frontier Research Center (IFReC) at Osaka University, I am very pleased to present the IFReC annual report for fiscal 2018.

From 2017, IFReC has been one of the members of the "WPI Academy". Furthermore, IFReC created a new mark in its history with a novel academic-industry partnership agreement. This governance system is without precedent and has attracted the attention of universities and enterprises as a way to show the new direction of research universities.

IFReC hopes to expand as a center that can provide a wide field for collaborative research. As part of this strategy, IFReC newly formed academic collaborative partnerships with the University of Bonn and Heidelberg University, Germany in 2018. We also hope IFReC will be a place along the career paths that focuses the capabilities of a wide variety of talented international researchers.

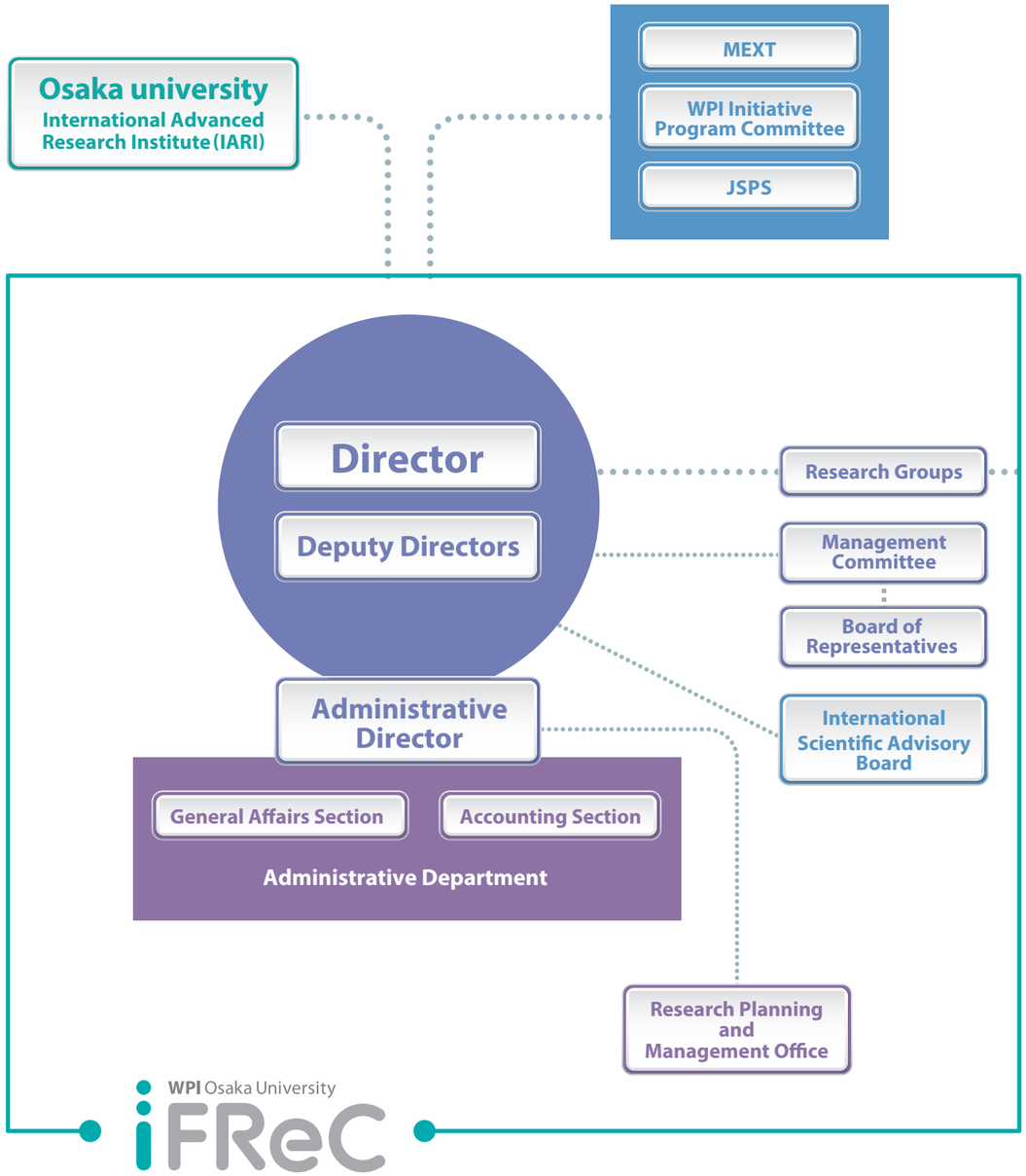
From July 2019, Professor Kiyoshi Takeda will succeed me as the director of IFReC. In the next decade, IFReC will aspire to further development under the leadership of Prof. Takeda, who is a world top class immunologist in the fields of innate immunity and gut immunity.

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

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

# Organization

# Organization Chart




As of March, 2019

## Immunology Group

| | |
|---|---|
| Host Defense | Shizuo Akira |
| Immunoglycobiology | Taroh Kinoshita |
| Immunopathology | Atsushi Kumanogoh |
| Immunochemistry | Hisashi Arase |
| Immune Regulation | Tadamitsu Kishimoto |
| Mucosal Immunology | Kiyoshi Takeda |
| Immune Regulation | Hitoshi Kikutani |
| Experimental Immunology | Shimon Sakaguchi |
| Cell Signaling | Takashi Saito |
| Lymphocyte Differentiation | Tomohiro Kurosaki |
| Lymphocyte Development | Fritz Melchers |
| Malaria Immunology | Cevayir Coban |
| Vaccine Science | Ken J. Ishii |
| Immunoparasitology | Masahiro Yamamoto |
| Biochemistry and Immunology | Shigekazu Nagata |
| Molecular Neuroscience | Toshihide Yamashita |
| Molecular Immunology | Sho Yamasaki |
| Stem Cell Biology and Developmental Immunology | Takashi Nagasawa |
| Aging Biology | Eiji Hara |
| Oncogene Research | Masato Okada |
| Signal Transduction | Nobuyuki Takakura |

## Imaging Group

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

## Informatics Group

| | |
|---|---|
| Systems Immunology | Daron M. Standley |
| Statistical Immunology | Yukinori Okada |

## Units for Combined Research Fields

| | |
|---|---|
| Quantitative Immunology | Diego Diez |

## Joint Research Chair of Innovative Drug Discovery in Immunology

| | |
|---|---|
| Chugai-pharm | Kunihiro Hattori<br>Ryusuke Omiya<br>Junichi Hata |
| Otsuka-pharm | |

## Common Facilities

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

## Cooperative Institutions

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

**Overseas**
- Indian Institute of Science Education and Research, India

# Committee and Advisory Board for IFReC

## World Premier International Research Center Initiative (WPI)

### Program Director

As of Mar. 2019

| | |
|---|---|
| **Akira Ukawa** | Director, Center for World Premier International Research Center Initiative, JSPS, Japan |

### Deputy Program Director

| | |
|---|---|
| **Minoru Yoshida** | Group Director, Chemical Genomics Research Group, RIKEN, Center for Sustainable Resource Science, Japan |

### Program Committee Members

| | |
|---|---|
| **Rita Colwell** | Distinguished Professor, University of Maryland, USA |
| **Richard Dasher** | Consulting Professor, Stanford University, USA |
| **Victor Joseph Dzau** | President, National Academy of Medicine, USA |
| **Michinari Hamaguchi** | President, Japan Science and Technology Agency (JST), Japan |
| **Toshiaki Ikoma** | Professor Emeritus,The University of Tokyo, Japan |
| **Maki Kawai** | Director General, Institute for Molecular Science, National Institutes of Natural Sciences, Japan |
| **Klaus von Klitzing** | Director, Max Planck Institute for Solid State Research, Germany<br>Nobel Laureate in Physics (1985) |
| **Makoto Kobayashi** | Honorary Professor Emeritus, High Energy Accelerator Research Organization, Japan<br>Nobel Laureate in Physics (2008) |
| **Kiyoshi Kurokawa** | Professor Emeritus, National Graduate Institute for Policy Studies, Japan |
| **Chuan Poh Lim** | Chairman, Agency for Science, Technology and Research, Singapore |
| **Hiroshi Matsumoto** | President, RIKEN, Japan |
| **Ryozo Nagai** | President, Jichi Medical University, Japan |
| **Michiharu Nakamura** | Counselor to the President, JST, Japan |
| **〈Chairperson〉<br>Ryoji Noyori** | Director-General, Center for Research and Development Strategy, JST, Japan<br>Nobel Laureate in Chemistry (2001) |
| **Norihiko Suzuki** | Chair of the Board/President, Akita International University |
| **Harriet Wallberg** | Former President, Karolinska Institutet, Sweden |
| **Jean Zinn-Justin** | Scientific Adviser, IRFU/CEA, France |

## WPI Academy

In FY 2017, MEXT established the WPI Academy to be the vanguard in internationalizing and further renovating Japan's research environment. The WPI Academy is a much-anticipated upgrade of WPI institutes, and is expected to position Japan as a hub at the pinnacle of international researcher circulation.

The five WPI centers including IFReC are regarded to have achieved "world-premier status", and thus became the initial members of the WPI Academy.

In the decade ahead, the research institutes of WPI and WPI Academy will work together to hold public relations and outreach activities.

### Academy Director

As of Mar. 2019

| | |
|---|---|
| **Toshio Kuroki** | Special Advisor, Research Center for Science Systems, JSPS, Japan |

### Academy Officer for IFReC

| | |
|---|---|
| **Takehiko Sasazuki** | University Professor, Institute for Advanced Study, Kyushu University, Japan |

## International Scientific Advisory Board from abroad

As of Mar. 2019

| | | |
|---|---|---|
| **Jeffrey Ravetch** | The Rockefeller University, USA | **Immunology** |
| **Christopher Goodnow** | The Australian National University, Australia | **Immunology** |
| **Richard Locksley** | University of California, San Francisco, USA | **Immunology** |
| **Lewis L. Lanier** | University of California, San Francisco, USA | **Immunology** |
| **Anne O'Garra** | The Francis Crick Institute, UK | **Immunology** |
| **Yale Goldman** | University of Pennsylvania, USA | **Imaging** |

# Administrative Office of IFReC

## General Affairs Section

- Employment and acceptance procedures for researchers and staff
- Management of work hours
- Social insurance (for part-time staff), employment insurance
- IFReC Kishimoto Foundation Fellowship Program
- Procedures related to research support (MTA, patents, modified animals, pathogens, human genome, animal experiments)
- Procedures related to safety and hygiene
- University's dormitory
- Procedures related to international students
- Issuance of various certificates
- 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 and Management Office

- Research promotion and support
  (Consultation for grants and patents, etc.)
- Establishing research environments
  (Facility and Safety management, Research agreement, etc.)
- Fostering young scientists
  (Winter School, Advanced postdoc program, Orientation, etc.)
- Organizing scientific events
  (Symposia, Colloquia, Seminars, etc.)
- Public relations
  (Publishing, Website, Outreach to citizens, etc.)


As of April, 2019



# Laboratories

# Host Defense

## Shizuo Akira, MD/PhD

| Professor | Shizuo Akira |
|---|---|
| Associate Professor | **Kazuhiko Maeda** |
| | **Takashi Satoh** |
| Assistant Professor | **Hiroki Tanaka** |
| | **Kenta Maruyama** |
| Postdoctoral Fellow | **3** |
| Research Assistant | **5** |
| Visiting Scientist | **6** |
| Support Staff | **11** |

We are focused on the innate immune system, which is an evolutionally conserved host defense mechanism against various pathogens. Innate immune responses are initiated by pattern recognition receptors (PRRs), which recognize specific structures of microorganisms. Toll-like receptors (TLRs) are capable of sensing organisms ranging from bacteria to fungi, protozoa, and viruses, and play a major role in innate immunity. Individual TLRs recognize different microbial components, and give rise to different patterns in gene expression.

### Identification of an atypical monocyte and committed progenitor involved in fibrosis, SatM (Figure 1)

Macrophages consist of at least two subgroups. M1 macrophages are pro-inflammatory and have a central role in host defense. On the contrary, M2 macrophages are associated with responses to anti-inflammatory reactions, and tissue remodeling. Monocytes and macrophages comprise a variety of subsets with diverse functions. It is thought that these cells play a crucial role in homeostasis of peripheral organs, key immunological processes, and development of various diseases. Among these diseases, fibrosis is a life-threatening disease of unknown aetiology. Its pathogenesis is poorly understood, and there are few effective therapies. The development of fibrosis is associated with activation of monocytes and macrophages. Recently, we have identified a new macrophage subset that is Ceacam1+Msr1+ Ly6C-F4/80-Mac1+ monocytes, and named it SatM (segregated-nucleus-containing atypical monocytes). SatM is regulated by CCAAT/enhancer binding protein beta (C/EBPβ), and is critical for fibrosis. We are further investigating the physiological role of SatM and its related subsets.

### Molecular mechanism of endoribonuclease Regnase-1 in inflammation

Regnase-1 is a member of CCCH-type zinc finger proteins. Regnase-1-deficient mice develop spontaneous autoimmune diseases accompanied by splenomegaly and lymphadenopathy. Regnase-1 has endonuclease activity and destabilizes a set of mRNAs through cleavage of their 3' UTRs such as IL-6 and IL-12 p40 in macrophages and c-Rel, Ox40, and IL-2 in CD4+ T cells. Regnase-1 itself is cleaved by Malt1 protease after T cell receptor stimulation, resulting in the enhancement of T cell activation. Dynamic control of Regnase-1 expression is critical for modulation of T cell activation. Based on these findings, we promote understanding of the precious roles of Regnase-1 in immune and non-immune cells by using tissue-specific Regnase-1 deficient mice and mutant mice.

Regnase-1 is inactivated in response to external stimuli through posttranslational modifications including phosphorylation, yet the precise role of phosphorylation largely remains unknown. As the molecular mechanism of Regnase-1, we showed that IL-17 induces the phosphorylation of Regnase-1 in an Act1-TBK1-IKKi-dependent manner, especially in non-hematopoietic cells. Phosphorylated Regnase-1 is released from the endoplasmic reticulum into the cytosol, thereby losing its mRNA degradation function, which leads to expression of IL-17 target genes (Figure 2). IL-17-induced Regnase-1 phosphorylation is completely blocked in two Regnase-1-mutant (Regnase-1$^{AA/AA}$ and Regnase-1$^{\Delta CTD/\Delta CTD}$) mice. Thus, Regnase-1 plays a critical role in the development of IL-17-mediated inflammatory diseases via the Act1-TBK1-IKKi axis. Blockade of Regnase-1 phosphorylation sites may be promising for treatment of T helper 17-associated diseases.

Damage to intestinal epithelial cell (IEC) layers during intestinal inflammation is associated with inflammatory bowel disease. As a new function of Regnase-1 in non-immune cells, we showed that Regnase-1 controls colon epithelial regeneration by regulating protein kinase mTOR (the mechanistic target of rapamycin kinase) and purine metabolism. During dextran sulfate sodium-induced intestinal epithelial injury and acute colitis, IEC-specific Regnase-1-deficient (Regnase-1$^{\Delta IEC}$) mice were resistant to body weight loss, maintained an intact intestinal barrier, and showed increased cell proliferation and decreased epithelial apoptosis. Chronic colitis and tumor progression were also attenuated in Regnase-1$^{\Delta IEC}$ mice. Regnase-1 predominantly regulates mTORC1 signaling. Metabolic analysis revealed that Regnase-1 participates in purine metabolism and energy metabolism during inflammation. Increased expression of ectonucleotidases contributed to the resolution of acute inflammation in Regnase-1$^{\Delta IEC}$ mice. Regnase-1 deficiency in IECs has beneficial effects on the prevention and/or blocking of intestinal inflammatory disorders.

We are further studying to achieve the goal of a comprehensive understanding of the innate immune system and to develop an effective treatment for immune-related inflammatory diseases.

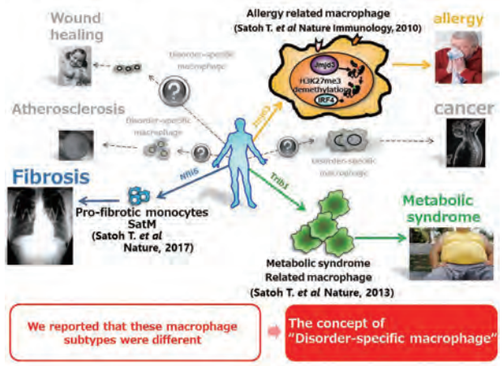



**Figure 1.**
**Functional diversity of disorder-specific macrophages.**

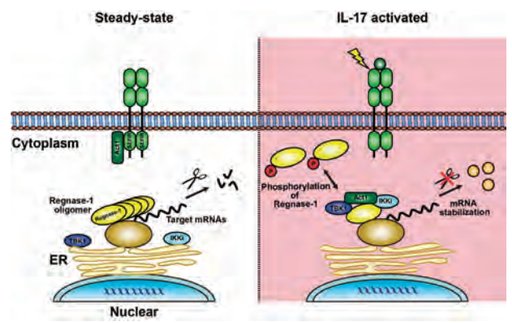



**Figure 2.**
**IL-17 induces Regnase-1 phosphorylation in an Act-1/TBK1/IKKi – dependent manner. Phosphorylation of Regnase-1 is released from the endoplasmic reticulum into the cytosols, resulting in loss of the mRNA degradation function.**

## Recent Publications


- Tanaka H., et al. Phosphorylation-dependent Regnase-1 release from endoplasmic reticulum is critical in IL-17 response. J. Exp. Med. (2019) in press.
- Maeda K., et al. Innate immunity in allergy. Allergy (2019) in press.
- Nagahama H., et al. Regnase-1 controls colon epithelial regeneration via regulation of mTOR and purine metabolism. Proc. Natl. Acad. Sci. USA. 115, 11036-11041 (2018).
- Kanemaru H., et al. Antitumor effect of Batf2 through IL-12 p40 up-regulation in tumor-associated macrophages. Proc. Natl. Acad. Sci. USA. 114, E7331 (2017).
- Satoh T., et al. Identification of an atypical monocyte and committed progenitor involved in fibrosis. Nature 541, 96-101 (2017)

# Immunoglycobiology

Taroh Kinoshita, PhD
Yoshiko Murakami (Co-PI), MD/PhD

| Professor | Taroh Kinoshita<br>Yoshiko Murakami |
|---|---|
| Research Assistant | 1 |
| Visiting Scientist | 2 |
| Support Staff | 4 |

We are interested in biosynthesis, functions and deficiencies of glycosylphosphatidylinositol (GPI) and GPI-anchored proteins (GPI-APs). In 2018, we made the following advances in the studies on GPI biosynthesis and GPI deficiency.

### Studies on GPI-AP biosynthesis

Mammalian glycosylphosphatidylinositols (GPIs) have been well characterized as membrane anchors of many cell-surface proteins. However, free, non-protein-linked GPIs on the mammalian cell surface remain largely unexplored. To investigate free GPIs in cultured cell lines and mouse tissues we used a T5-4E10 monoclonal antibody (T5 mAb), which recognizes non-protein-linked GPIs that have an *N*-acetylgalactosamine (GalNAc)-side-chain linked to the first mannose. The GalNAc must be at the non-reducing terminus to be recognized by T5 mAb. We show that free GPIs bearing the GalNAc-side-chain are present on the surface of Neuro2a and CHO but not HEK293, K562 and C2C12 cells. Further, the free GPIs are present in mouse pons, medulla oblongata, spinal cord, testis, epididymis and kidney. Taking advantage of a panel of double mutant CHO cells defective in GPI-transamidase and the GPI remodeling pathway, we demonstrate that free GPIs take the same structural remodeling pathway on the way from the ER to the plasma membrane as protein-linked GPI-anchors. Our results indicate that free GPIs are normal components of the plasma membrane in some tissues (Wang, Y. et al., *J. Biol. Chem*., 2019).

The GalNAc side chain of GPI is further modified by galactose in some GPI-APs. The enzyme GPI-GalT that mediates this galactosylation has not been identified. We identified a gene encoding GPI-GalT (Wang, Y. et al., manuscript in preparation).

### Studies on inherited GPI deficiencies (IGDs)

In collaborations with Dr. Campeau's group in Canada that identified the first cases of IGD having mutations in PIGS gene, we characterized functional abnormalities caused by those mutations. PIGS gene encodes a subunit of GPI transamidase that transfers GPI to precursor proteins to generate GPI-APs. We found the patients' mutations cause partial loss of function of PIGS (Nguyen, T.T.M. et al., *Am. J. Hum. Genet*., 2018). Also, in collaboration with clinicians and medical geneticists working with IGD, we reported the first cases of IGD with mutations in PIGB gene encoding the enzyme that transfers the third mannose to GPI. We found similarity between PIGB-IGD and DOORS syndrome. Some of the symptoms of DOORS syndrome might be caused by GPI-AP deficiency (Murakami, Y. et al., manuscript under review). To date, pathogenic mutations that cause IGDs were found in 20 of 27 genes for biosynthesis and maturation of GPI. Knowledge gained from these studies contributes to our deeper understanding of IGDs, progress in development of better diagnostics of IGDs, and effective measures to ameliorate symptoms of IGDs.

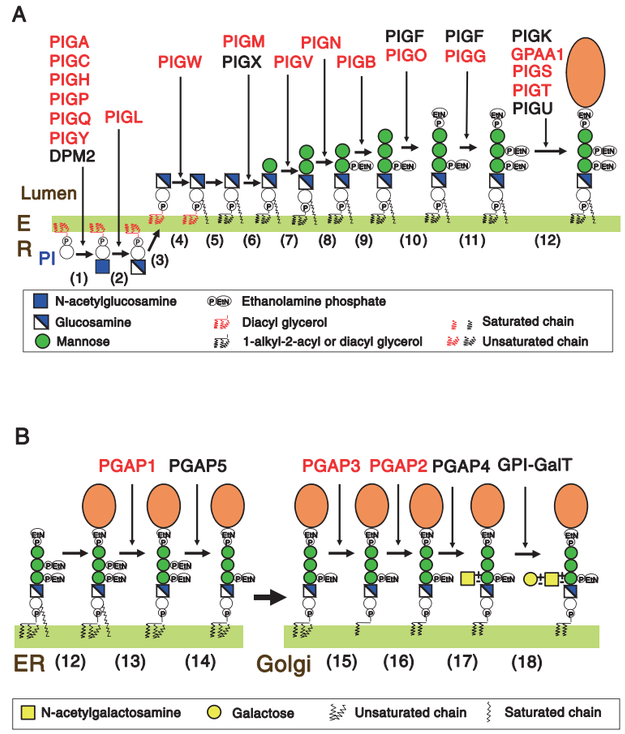



**Figure.**
**A. GPI biosynthesis and transfer to proteins in the ER. B. Maturation of GPI in the ER and the Golgi apparatus.**
**Genes in red, genes for which pathogenic mutations that cause inherited GPI deficiencies are known.**

## Recent Publications


- Wang Y, Hirata T, Maeda Y, Murakami Y, Fujita M and Kinoshita T. Free, unlinked glycosylphosphatidylinositols on mammalian cell surfaces revisited. J. Biol. Chem. 294, 5038-5049 (2019).
- Hirata T, Mishra SK, Nakamura S, Saito K, Motooka D, Takada Y, Kanzawa N, Murakami Y, Maeda Y, Fujita M, Yamaguchi Y & Kinoshita T. Identification of a Golgi GPI-N-acetylgalactosamine transferase with tandem transmembrane regions in the catalytic domain. Nat. Commun. 9, 405-(2018).
- Tanigawa J, Mimatsu H, Mizuno S, Okamoto N, Fukushi D, Tominaga K, Kidokoro H, Muramatsu Y, Nishi E, Nakamura S, Motooka D, Nomura N, Hayasaka K, Niihori T, Aoki Y, Nabatame S, Hayakawa M, Natsume J, Ozono K, Kinoshita T, Wakamatsu N & Murakami Y. Phenotype-genotype correlations of PIGO deficiency with variable phenotypes from infantile lethality to mild learning difficulties. Hum. Mutat. 38, 805-815 (2017).
- Lee G-H, Fujita M, Takaoka K, Murakami Y, Fujihara Y, Kanzawa N, Murakami K, Kajikawa E, Takada Y, Saito K, Ikawa M, Hamada H, Maeda Y & Kinoshita T. A GPI processing phospholipase A2, PGAP6, modulates Nodal signaling in embryos by shedding CRIPTO. J. Cell Biol. 215, 705-718 (2016).
- Makrythanasis P, Kato M, Zaki M, Saitsu H, Nakamura K, Santoni F, Miyatake S, Nakashima M, Issa MY, Guipponi M, Letourneau A, Logan C, Roberts N, Parry DA, Johnson CA, Matsumoto N, Hamamy H, Sheridan E, Kinoshita T, Antonarakis SE & Murakami Y. Pathogenic variants in PIGG cause intellectual disability with seizures and hypotonia. Am. J. Hum. Genet. 98, 615-626 (2016).

# Immunopathology

## Atsushi Kumanogoh, MD/PhD

| Professor | Atsushi Kumanogoh |
|---|---|
| Assistant Professor | **Yoshimitsu Morita** |
| Research Assistant | **5** |
| Support Staff | **5** |

Our research team is involved in two approaches, that is, basic and clinical immunology. As basic aspects of our projects, our proposed study is the regulation of immune cell motility and migratory behavior *in vivo* by soluble and membrane-bound 'immune guidance molecules' such as semaphorins and their receptors. Semaphorins were originally identified as axon-guidance molecules that function during neuronal development. However, cumulative evidence indicates that semaphorins also participate in immune responses, both physiological and pathological, and they are now considered to be potential diagnostic and/or therapeutic targets for a range of diseases. Beyond such basic implications, we are trying to apply the findings from this proposed study into the diagnosis/therapy for human immunological disorders, such as autoimmunity, allergy, immune deficiency, cancer/metastasis, and neurodegenerative diseases. We here focus on the clinical implications of Sema7A in lung adenocarcinoma. Although responses to EGFR tyrosine kinase inhibitors (EGFR-TKIs) are initially positive, 30%-40% of patients with EGFR-mutant tumors do not respond well to EGFR-TKIs, and most lung cancer patients harboring EGFR mutations experience relapse with resistance. Therefore, it is necessary to identify not only the mechanisms underlying EGFR-TKI resistance, but also potentially novel therapeutic targets and/or predictive biomarkers for EGFR-mutant lung adenocarcinoma. We found that the GPI-anchored protein semaphorin 7A (SEMA7A) is highly induced by the EGFR pathway, via mTOR signaling, and that expression levels of SEMA7A in human lung adenocarcinoma specimens were correlated with mTOR activation. Investigations using cell culture and animal models demonstrated that loss or overexpression of SEMA7A made cells less or more resistant to EGFR-TKIs, respectively. The resistance was due to the inhibition of apoptosis by aberrant activation of ERK. The ERK signal was suppressed by knockdown of integrin β1 (ITGB1). Furthermore, in patients with EGFR mutant tumors, higher SEMA7A expression in clinical samples predicted poorer response to EGFR-TKI treatment. Collectively, these data show that the SEMA7A-ITGB1 axis plays pivotal roles in EGFR-TKI resistance mediated by ERK activation and apoptosis inhibition. Moreover, our results reveal the potential utility of SEMA7A not only as a predictive biomarker, but also as a potentially novel therapeutic target in EGFR-mutant lung adenocarcinoma.

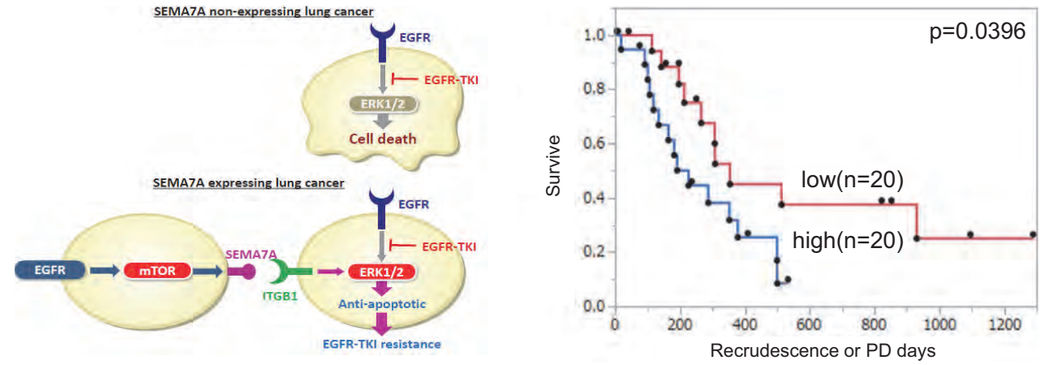



**Figure.**
**Expression of SEMA7A in human lung adenocarcinoma is regulated by the EGFR–mTOR axis and is involved in development of resistance to EGFR-TKIs. Resistance is mediated by inhibition of apoptosis by the SEMA7A–ITGB1–ERK axis.**

## Recent Publications


- Kang S, Nakanishi Y, Kioi Y, Okuzaki D, Kimura T, Takamatsu H, Koyama S, Nojima S, Nishide M, Hayama Y, Kinehara Y, Kato Y, Nakatani T, Shimogori T, Junichi Takagi J, Toyofuku T and Kumanogoh A. Semaphorin 6D reverse signaling controls macrophage lipid metabolism and anti-inflammatory polarization. Nat. Immunol. 19, 561-570 (2018).
- Nishide M and Kumanogoh A. The role of semaphorins in immune responses and autoimmune rheumatic diseases. Nat. Rev. Rheumatol. 14, 19-31 (2018).
- Hosen N, Matsunaga Y, Hasegawa K, Matsuno H, Nakamura Y, Makita M, Watanabe K, Yoshida M, Satoh K, Morimoto S, Fujiki F, Nakajima H, Nakata J, Nishida S, Tsuboi A, Oka Y, Manabe M, Ichihara H, Aoyama Y, Mugitani A, Nakao T, Hino M, Uchibori R, Ozawa K, Baba Y, Terakura S, Wada N, Morii E, Nishimura J, Takeda K, Oji Y, Sugiyama H, Takagi J, and Kumanogoh A. The activated conformation of integrin β7 is a novel multiple myeloma-specific target for CAR T cell therapy. Nat. Med. 23, 1436-1443 (2017).
- Kimura T, Nada S, Takegahara N, Okuno T, Nojima S, Kang S, Ito D, Morimoto K, Hosokawa T, Hayama Y, Mitsui Y, Sakurai N, Sarashina-Kida H, Nishide M, Maeda Y, Takamatsu H, Okuzaki D, Yamada M, Okada M, and Kumanogoh A. Polarization of M2 macrophages requires Lamtor1 that integrates cytokine and amino-acid signals. Nat. Commun. 7, 13130 (2016).

# Immunochemistry

## Hisashi Arase, MD/PhD

| Professor | Hisashi Arase |
|---|---|
| Associate Professor | **Tadahiro Suenaga** |
| Assistant Professor | **Masako Kohyama** |
| Postdoctoral Fellow | **3** |
| Research Assistant | **6** |
| Support Staff | **2** |

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

**Host pathogen interaction mediated by paired receptor**

Paired receptors are composed of activating and inhibitory receptors. PILRα is one of the paired inhibitory receptors that are expressed on various immune cells. We have shown that PILRα plays an important role in the regulation of immune response (Wang et al. *Nat. Immunol*. 2012; Kishida et al. *Int. Immunol*. 2015; Kohyama et al. *Eur. J. Immunol*. 2016). We also 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). Similarly, Siglec-4 (MAG, myelin associated glycoprotein), one of the paired receptors, associates with varicella zoster virus (VZV) gB and mediates VZV infection (Suenaga et al. *Proc. Natl. Acad. Sci. USA*. 2010; Suenaga et al. *J. Biol. Chem*. 2015). These findings suggested that paired receptors are involved in viral infection.

LILR is another type of paired receptor family. We found that activating LILRA2 recognizes abnormal immunoglobulins cleaved by microbial proteases but not normal immunoglobulins. LILRA2 seems to be a sensor to detect immunoglobulin abnormalities in microbial infection (Hirayasu et al. *Nature Microbiology* 2016). On the other hand, we found that RIFINs, products of multigene family of *Plasmodium falciparum*, bind to inhibitory LILRB1 and downregulate immune response. Furthermore, expression of RIFINs was associated with severe malaria. These findings suggest that binding of RIFIN to LILRB1 plays an important role in immune evasion by *Plasmodium falciparum* (Figure 1. Saito et al. *Nature* 2017).

**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; Shimizu et al. *Int, Immunol*. 2019). In addition, we could detect autoantibodies against the β2GPI/HLA class II complex in the patients with refractory cutaneous ulcers (Arase et a. *Br. J. Dermatol*. 2017). Similarly, we also found that the myeloperoxidase/HLA class II complex is a target for autoantibodies in ANCA-associated vasculitis (Hiwa et al. *Arthritis. Rheumatol*. 2017). Autoantibody binding to misfolded proteins transported to the cell surface by MHC class II molecules was strongly correlated with susceptibility to autoimmune disease. This suggested that misfolded proteins, which normally would not be exposed to the immune system, can be targets for autoantibodies as 'neo self' antigens, which is involved in the pathogenicity of autoimmune diseases (Figure 2).

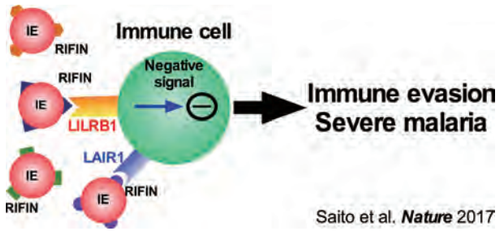



**Figure 1.**
**Immune evasion of Plasmodium falciparum by RIFIN via inhibitory receptors.**
***Plasmodium falciparum* induces the expression of RIFINs on the surface of infected erythrocytes. Individual RIFINs may have evolved to target host inhibitory receptors, thus facilitating escape from host immune systems, which may lead to inefficient development of immunity against malaria parasites (Satio et al. *Nature* 2016).**

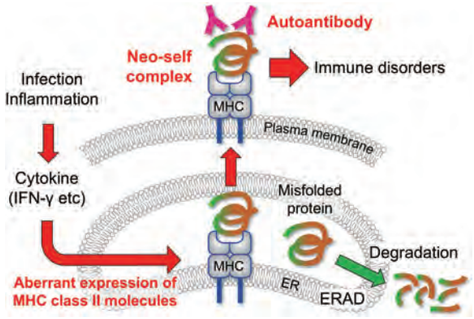



**Figure 2.**
**Misfolded proteins transported to the cell surface by MHC class II molecules are targets for autoantibodies.**
**Cellular misfolded proteins are generally degraded in the cells and are not transported to outside the cells. Therefore, misfolded proteins transported to the cell surface by MHC class II molecules may be recognized as 'neo-self' antigens by the immune system, which might initiate on aberrant immune response to self-antigens (Jiang et al. *Int. Immunol*. 2013; Jin et al. *Proc. Natl. Acad. Sci. USA*. 2014; Tanimura et al. *Blood* 2015, Arase *Adv. Immunol*. 2016; Arase et al. *Br. J. Dermatol*. 2017; Hiwa et al. *Arthritis Rheumatol*. 2017, Shimizu et al. *Int Immunol*. 2019).**

## Recent Publications


- Shimizu Y, Kohyama M, Yorifuji H, Jin H, Arase N, Suenaga T, Arase H. FcγRIIIA-mediated activation of NK cells by IgG heavy chain complexed with MHC class II molecules. Int. Immunol. (2019) in press.
- Saito F, Hirayasu K, Satoh T, Wang CW, Lusingu J, Arimori T, Shida K, Palacpac NMQ, Itagaki S, Iwanaga S, Takashima E, Tsuboi T, Kohyama M, Suenaga T, Colonna M, Takagi J, Lavstsen T, Horii T, Arase H. Immune evasion of Plasmodium falciparum by RIFIN via inhibitory receptors. Nature 552, 101-105 (2017).
- Hiwa R, Ohmura K, Arase N, Jin H, Hirayasu K, Kohyama M, Suenaga T, Saito F, Terao C, Atsumi T, Iwatani H, Mimori T, Arase H. Myeloperoxidase/HLA Class II Complexes Recognized by Autoantibodies in Microscopic Polyangiitis. Arthritis. Rheumatol. 69, 2069-2080 (2017).
- Hirayasu K, Saito F, Suenaga T, Shida K, Arase N, Oikawa K, Yamaoka T, Murota H, Chibana H, Nagai H, Nakamura Y, Katayama I, Colonna M, Arase H. LILRA2 is an innate immune sensor for microbially cleaved immunoglobulins. Nat. Microbiol. 1, 1-7 (2016).
- Jin H, Arase N, Hirayasu K, Kohyama M, Suenaga T, Saito F, Tanimura K, Matsuoka S, Ebina K, Shi K, Toyama-Sorimachi N, Yasuda S, Horita T, Hiwa R, Takasugi K, Ohmura K, Yoshikawa H, Saito T, Atsumi T, Sasazuki T, Katayama I, Lanier LL and Arase H. Autoantibodies to IgG/HLA-DR complexes are associated with rheumatoid arthritis susceptibility. Proc. Natl. Acad. Sci. USA. 111, 3787-3792 (2014).

# Immune Regulation

## Tadamitsu Kishimoto, MD/PhD

| Professor | Tadamitsu Kishimoto |
|---|---|
| Associate Professor | **Shigeru Hashimoto** |
| Assistant Professor | **Sujin Kang** |
| Postdoctoral Fellow | **3** |
| Research Assistant | **2** |
| Visiting Scientist | **3** |
| Support Staff | **3** |

**Introduction**

Interleukin 6 (IL-6) is a pleiotropic, inflammatory cytokine that plays a role in the host defense mechanism against infections and tissue injury via the stimulation of acute phase reactions, immune responses, and hematopoiesis. However, excessive production of IL-6 is associated with various inflammatory diseases, such as rheumatoid arthritis and Castleman's disease. Blockade of IL-6 signaling using the anti-IL-6 receptor antibody tocilizumab has proven to be an effective treatment for inflammatory disorders. Furthermore, several reports regarding the off-label use of tocilizumab suggest that IL-6 blockade may be a promising therapeutic approach for other inflammatory diseases and cancers. We have shown that AT-rich interactive domain-containing protein 5a (Arid5a) contributes to the augmentation of IL-6 production via post-transcriptional regulation.

**Phosphorylation of a novel site on STAT1 is involved in IL-6 and IL-12p40 expression (ongoing)**

Toll-like receptor 4 (TLR4) is not only fundamental for eliminating bacterial infections but also for driving aberrant inflammation in lethal sepsis. However, the important open question of how TLR4 endocytosis is involved in the expression of proinflammatory cytokines remains to be clarified.

We found that TLR4 endocytosis activates the threonine 749 phosphorylation (pT749) of STAT1, but not canonical tyrosine phosphorylation in human macrophages. pT749 STAT1 promotes the expression of IL-6 and IL-12p40 through distinct mechanisms. Regarding IL-6, pT749 induces the expression of *ARID5A*, which subsequently stabilizes IL-6 mRNA. On the other hand, pT749 STAT1 directly augments the transcription of IL-12p40. Our study therefore provides a possible interpretation to the long-standing observation that TLR4 endocytosis is often linked to inflammatory cytokine augmentation.

**Arid5a as a modulator of energy homeostasis (ongoing)**

Several types of immune cells are located in metabolic tissues, providing a framework for the direct regulation of energy homeostasis. However, the precise mechanisms by which the immune system regulates energy homeostasis remain poorly understood. We found that long-term deficiency of Arid5a in mice results in severe adult-onset obesity. In contrast, transgenic mice overexpressing Arid5a are highly resistant to high fat-induced obesity. Molecular studies demonstrated that Arid5a represses the transcription of peroxisome proliferator activated receptor gamma (Ppar-γ). Furthermore, we showed that Arid5a and Ppar-γ are dynamically counter-regulated by each other, hence maintaining homeostasis. Thus, our findings indicated that Arid5a is an important negative regulator of energy metabolism and can be a potential target for metabolic disorders.

**Requirement of Arid5a in immune evasion of pancreatic cancer (ongoing)**

IL-6 has been found to promote several types of tumor progression and perturbation of the tumor microenvironment. However, the functional roles of Arid5a in IL-6-associated malignant tumor progression remain to be elucidated.

We found that Arid5a-deficient pancreatic cancer mouse model cells have decreased tumor formation in immunocompetent mice compared with wild-type cancer cells. On the other hand, both types of cells showed comparable growth in immunodeficient mice. Furthermore, we identified that Arid5a is necessary for acquisition of the mesenchymal phenotype of pancreatic cancer, which are associated with the immunosuppressive ability of malignant tumors. These results suggest that Arid5a plays key roles in pancreatic cancer progression via regulating the immune evasion.

**Positive loop between hyper-IL-6 and vascular endothelial cell activation plays a key role in cytokine storm (ongoing)**

Excessive production of IL-6 leads to an acute severe systemic inflammatory response known as "cytokine storm". However, the precise molecular mechanism for how IL-6 receptor (IL-6R) signaling controls endothelial cell homeostasis remains to be identified.

We identified that upon LPS stimulation, vascular endothelial cells driven IL-6 promptly activates IL-6R trans-signaling pathway in the presence of soluble IL-6R, and this process accelerates the formation of a pro-inflammatory cytokine network including IL-6. Furthermore, treatment with tocilizumab strikingly abolished the generation of this cytokine network LPS-stimulated vascular endothelial cells, suggesting that endothelial cells activated by IL-6 and its receptor signaling form a positive loop during a cytokine storm.

**Establishment of humanized cereblon mice demonstrate two distinct therapeutic pathways of immunomodulatory drugs (published)**

Immunomodulatory drugs (IMiDs), including thalidomide and its derivatives, exert therapeutic effects against several hematopoietic malignancies and inflammatory diseases. However, it is difficult to study the mechanisms of action of IMiDs in murine disease models because murine cereblon (CRBN), which is the substrate of IMiDs, does not mediate some of the therapeutic effects of IMiDs. To overcome this difficulty, we developed humanized cereblon ($CRBN^{I391V}$) mice that mimic the action of IMiDs in human cells.

We found that treating $CRBN^{I391V}$ mice with thalidomide derivatives resulted in the degradation of Cullin4A/$B^{CRBN}$ E3 ligase substrates, such as Ikaros1 and casein kinase-1, whereas wild-type mice remained resistant to the derivatives. In addition, these IMiDs upregulated IL-2 production in $CRBN^{I391V}$ mice but not wild-type mice. Additionally, we used wild-type and $CRBN^{I391V}$ mice with dextran sodium sulfate (DSS)-induced colitis to test the therapeutic effects of IMiDs against DSS-induced colitis. Interestingly, both types of mice treated with DSS responded to IMiDs. These results suggest that whereas the degradative effects of IMiDs on Ikaros1 and casein kinase-1, and the upregulation of IL-2 are dependent on CRBN, the effects of IMiDs on the DSS colitis model are independent of CRBN.

### Recent Publications


- Kang S, Tanaka T, Narazaki M & Kishimoto T. Targeting Interleukin-6 Signaling in Clinic. Immunity 50, 1007-1023 (2019).
- Gemechu Y., et al. Humanized cereblon mice revealed two distinct therapeutic pathways of immunomodulatory drugs. Proc. Natl. Acad. Sci. USA. 115, 11802-11807 (2018).
- Masuda K & Kishimoto T. A Potential Therapeutic Target RNA-binding Protein, Arid5a for the Treatment of Inflammatory Disease Associated with Aberrant Cytokine Expression. Curr. Pharm. Des. 24, 1766-1771 (2018).
- Tanaka T, Narazaki M & Kishimoto T. Interleukin (IL-6) Immunotherapy. Cold Spring Harb Perspect Biol. 10, a028456 (2018).

# Mucosal Immunology

## Kiyoshi Takeda, MD/PhD

| Professor | Kiyoshi Takeda |
|---|---|
| Associate Professor | **Eiji Umemoto** |
| Assistant Professor | **Hisako Kayama** |
| | **Ryu Okumura** |
| | **Mari Murakami** |
| Research Assistant | **5** |
| Support Staff | **1** |

We have been focusing on the mechanisms regulating intestinal homeostasis to reveal the pathogenesis of inflammatory bowel disease (IBD), represented by Crohn's diseases and ulcerative colitis. Recent evidence indicates that both immune cells and epithelial cells in the intestine play a critical role for pathogenesis. In addition, environmental factors such as microbiota contribute to the development of IBD. Therefore, we are analyzing (1) intestinal immune cells, which include innate myeloid cell populations residing in the intestinal lamina propria and possessing unique regulatory functions, (2) intestinal epithelial cells, which mediate barrier functions by separating intestinal immunity and intestinal environmental factors, and (3) intestinal environmental factors, which influence host immunity. These include dietary components, intestinal microbiota, and metabolites produced by microbiota.

**GPR31-dependent dendrite protrusion of intestinal CX3CR1$^+$ cells by bacterial metabolites pyruvate and lactate**

The small intestine harbors a variety of myeloid subsets. In particular, mononuclear cells expressing a chemokine receptor CX3CR1 (CX3CR1$^+$ cells) are the major mononuclear phagocyte population and regulate intestinal homeostasis. They reside underneath epithelial layers and penetrate epithelium by extending transepithelial dendrites to capture luminal microorganisms and dietary antigens. However, it remains unclear how dendrite protrusion by CX3CR1$^+$ cells is induced in the intestine. We found that a methanol-soluble fraction prepared from the small intestinal contents induced dendrite extension of intestinal CX3CR1$^+$ cells and that the fraction activated the cultured cells expressing G-protein-coupled receptor GPR31, which was highly and selectively expressed in CX3CR1$^+$ cells in the small intestine. In GPR31-deficient mice, CX3CR1$^+$ cells in the small intestine showed defective dendrite protrusions and impaired uptake of orally administered *Salmonella* Typhimurium. In line with these observations, GPR31-deficient mice showed reduced uptake of the bacteria in the small intestine, mesenteric lymph node, and spleen. They also showed decreased concentration of serum IgG specific to *S.* Typhimurium and reduced resistance to oral infection with pathogenic *S.* Typhimurium, indicating that GPR31 is required for immune response to enteric bacteria.

We next intended to identify the particular luminal products that activate GPR31. We purified a GPR31-activating product in the methanol-soluble fraction of the small intestine step-by-step, and identified lactic acid. Lactic acid and pyruvic acid, which is structurally related to lactic acid, activated GPR31, with relatively high affinity of pyruvic acid to GPR31. Concentrations of D-lactate, L-lactate, and pyruvate were severely reduced in the small intestinal luminal contents of germ-free mice compared with SPF mice, indicating that both compounds were produced in a bacteria-dependent manner in the intestine. Among several *Lactobacillus* strains, *L. helveticus* secreted lactate and high amounts of pyruvate.

We next analyzed whether lactic acid and pyruvic acid induce dendrite protrusion of intestinal CX3CR1$^+$ cells. Both lactic acid and pyruvic acid induced dendrite extension of CX3CR1$^+$ cells of wild-type mice, but not GPR31-deficient mice in vitro. Oral administration of lactate or pyruvate enhanced dendrite protrusion of CX3CR1$^+$ cells in the small intestine of wild-type mice, but not GPR31-deficient mice. Furthermore, when treated with pyruvate or lactate, wild-type mice showed enhanced immune responses and high resistance to intestinal S. Typhimurium infection. Previous reports showed that the CX3CL1/CX3CR1 axis mediates dendrite protrusion of CX3CR1$^+$ cells. Expression of GPR31 in intestinal CX3CR1$^+$ cells was severely decreased in CX3CR1-deficient or CX3CL1-deficient mice, and that CX3CL1 treatment induced GPR31 expression in intestinal CX3CR1$^+$ cells of CX3CL1-deficient mice, indicating that the CX3CL1/CX3CR1 axis regulates GPR31 expression. These findings demonstrate that lactate and pyruvate, which are produced in the intestinal lumen in a bacteria-dependent manner, contribute to enhanced immune responses by inducing GPR31-mediated dendrite protrusion of intestinal CX3CR1$^+$ cells (Figure).

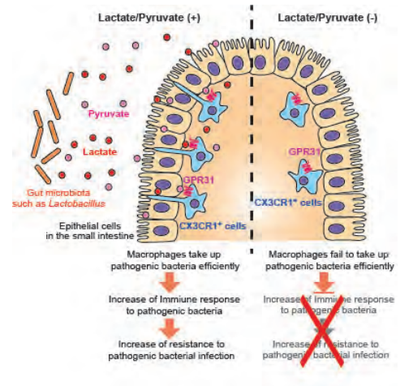



**Figure.**
**The pyruvate/lactate-GPR31 axis induces dendrite protrusion of intestinal CX3CR1$^+$ cells.**
**In the intestinal lumen, commensal bacteria such as *Lactobacillus* species produce pyruvate and lactate, and these bacterial metabolites bind to GPR31 expressed by intestinal CX3CR1$^+$ cells. The GPR31-mediated signaling induces dendrite protrusion of CX3CR1$^+$ cells, allowing them to take up pathogenic bacteria efficiently. Thus the pyruvate/lactate-GPR31 axis contributes to effective immune responses against pathogenic bacteria infection.**

## Recent Publications


- Morita N, Umemoto E, Fujita S, Hayashi A, Kikuta J, Kimura I, Haneda T, Imai T, Inoue A, Mimuro H, Maeda Y, Kayama H, Okumura R, Aoki J, Okada N, Kida T, Ishii M, Nabeshima R, Takeda K. Nature 566, 110-114 (2019).
- Kayama H, Kohyama M, Okuzaki D, Motooka D, Barman S, Okumura R, Muneta M, Hoshino K, Sasaki I, Ise W, Matsuno H, Nishimura J, Kurosaki T, Nakamura S, Arase H, Kaisho T, Takeda K. Heme ameliorates dextran sodium sulfate-induced colitis through providing intestinal macrophages with noninflammatory profiles. Proc. Natl. Acad. Sci. USA. 115, 8418-8423 (2018).
- Ikeda K, Kinoshita M, Kayama H, Nagamori S, Kongpracha P, Umemoto E, Okumura R, Kurakawa T, Murakami M, Mikami N, Shintani Y, Ueno S, Andou A, Ito M, Tsumura H, Yasutomo K, Ozono K, Takashima S, Sakaguchi S, Kanai Y, Takeda K. Slc3a2 Mediates Branched-Chain Amino-Acid-Dependent Maintenance of Regulatory T Cells. Cell Rep. 21, 1824-1838 (2017).
- Kitada S, Kayama H, Okuzaki D, Koga R, Kobayashi M, Arima Y, Kumanogoh A, Murakami M, Ikawa M, Takeda K. BATF2 inhibits immunopathological Th17 responses by suppressing Il23a expression during Trypanosoma cruzi infection. J. Exp. Med. 214, 1313-1331 (2017).
- Okumura R, Kurakawa T, Nakano T, Kayama H, Kinoshita M, Motooka D, Gotoh K, Kimura T, Kamiyama N, Kusu T, Ueda Y, Wu H, Iijima H, Barman S, Osawa H, Matsuno H, Nishimura J, Ohba Y, Nakamura S, Iida T, Yamamoto M, Umemoto E, Sano K, Takeda K. Lypd8 promotes the segregation of flagellated microbiota and colonic epithelia. Nature 532, 117-21 (2016).

# Immune Regulation

## Hitoshi Kikutani, MD/PhD

| Professor | Hitoshi Kikutani |
|---|---|
| Assistant Professor | **Shuhei Sakakibara** |
| Postdoctoral Fellow | 1 |
| Research Assistant | 1 |
| Visiting Scientist | 2 |
| Support Staff | 1 |

**Allergic conversion of protective anti-bacterial responses in chronic rhinosinusitis with nasal polyposis (CRSwNP)**

CRSwNP is characterized by eosinophilic inflammation and nasal polyposis with unknown etiology. In this study, we attempted to identify the causative allergen of CRSwNP. By using single cell antibody cloning and expression, we investigated the reactivity of IgE produced in nasal polyps (NPs) of CRSwNP patients. The majority of isolated monoclonal IgE antibodies appeared to target nasal resident bacteria such as *Streptococcus pyogenes, Staphylococcus aureus, Haemophillus influenzae,* and *Moraxella catarrhalis*. Peripheral mononuclear cells from CRSwNP patients contained increased frequencies of Th2 memory cells specific to *S. pyogenes*, indicating that nasal bacteria are causative allergens for the disease. To understand the development pathway of such bacteria-specific IgE, high-throughput sequencing of NP-associated BCR repertoires was performed. This analysis showed that major clonal lineages of IgE were frequently found in IgG or $IgA_1$ clonal lineages, indicative of sequential class switch from these Ig isotypes to IgE in NPs. Taken together, CRSwNP is a chronic allergy, which is aberrantly converted from a protective immune response against nasal bacteria (Figure 1) (Takeda K et al., 2019).

**Bystander inhibition of humoral immune responses by Epstain-Barr virus LMP1**

Epstein-Barr virus (EBV) infects over 95% of adults and persists throughout the lifespan. EBV establishes latent infection in B cells with expression of limited viral genes. Latent membrane protein 1 (LMP1), one of the EBV genes expressed in the latently infected B cells, is an integral plasma membrane protein and provides constitutive activation of signaling pathways including NF-κB, p38 and c-Jun. To elucidate the role of LMP1 in B cell function in vivo, we generated transgenic mice expressing LMP1 under the control of the CD19 promoter or the activation-induced cytidine deaminase (AID) promoter. We found that LMP1 expression in antigen-committed B cells severely impaired humoral immune responses not only through direct inhibition of germinal center B cell differentiation, but also indirect suppression of the neighboring B cells (Figure 2). Our study further demonstrated that LMP1-expressing B cells overexpress indoleamine 2,3-dioxygenase 1 (IDO1), and therefore tryptophan deprivation and harmful metabolites cause the indirect suppression. This bystander inhibition by LMP1+ B cells through IDO1 represents a novel mechanism for immune evasion of EBV (Tsai C-Y et al., 2018).

**Characterization of low-affinity progenitor B cells for pathogenic anti-dsDNA antibody-producing cells derived from SLE**

From our previous study for monoclonal anti-dsDNA antibodies isolated from acute SLE patients (Sakakibara S et al., 2017), we hypothesized that low-affinity anti-ssDNA B cells can acquire high-affinity to both ss- and dsDNA by only one or two mutations (Figure 3). This raises the questions of how low-affinity anti-ssDNA precursor B cells escape from tolerance checkpoints and in what conditions they undergo clonal expansion and affinity mutation to differentiate into high affinity anti-dsDNA antibody-producing cells. To address these questions, we generated a site-directed knock-in (KI) mouse line, G9gl, which carries unmutated IgH and L chains derived from one of the SLE anti-DNA antibody clones. The numbers of G9gl-expressing B cells was reduced in blood and lymphoid organs of the KI mice. Although $G9gl^+$ B cells displayed reduced levels of surface BCR, they were not functionally anergic and could respond efficiently to LPS, anti-CD40, or BCR crosslinking. Moreover, ssDNA could induce vigorous activation and proliferation in G9gl B cells. A fraction of G9gl mice exhibited high titer of serum autoantibodies such as anti-DNA IgG with spontaneous germinal center formation in the spleen. Taken together, low affinity precursors of SLE-derived anti-DNA B cells can escape from a tolerance check point during early B cell development and retain the capability to trigger self-reactive germinal center reaction.

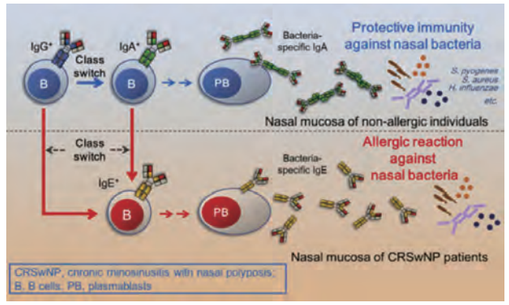



**Figure 1.**
**The underlying pathology of CRSwNP is allergic conversion of protective immunity against bacteria in nasal mucosa.**

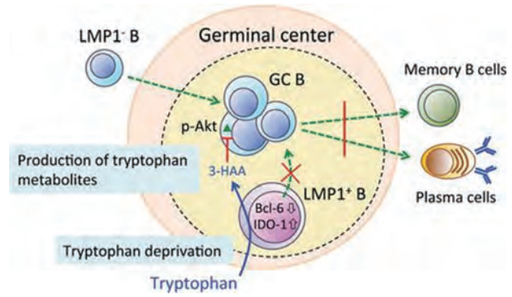



**Figure 2.**
**EBV LMP1-expressing B cells inhibit humoral immune responses by direct germinal center B cell differentiation and by stander suppression of $LMP1^-$ B cells through IDO1 overexpression.**

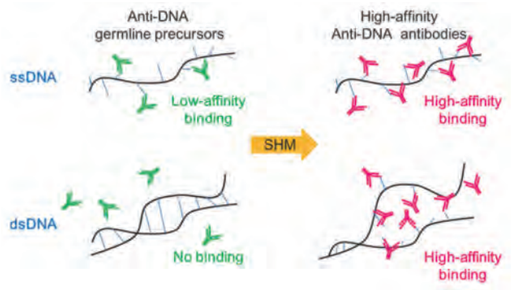



**Figure 3.**
**Unmutated low-affinity anti-ssDNA precursors cannot bind to dsDNA. After SHM, high-affinity anti-DNA clones acquire binding capability to both ss and dsDNA at high affinity.**

## Recent Publications


- Takeda K., et al. Allergic conversion of protective mucosal immunity against nasal bacteria in chronic rhinosinusitis with polyposis. J. Allergy Clin. Immunol. 143, 1163-1175 (2019).
- Tsai C.-Y., et al. Bystander inhibition of humoral immune responses by Epstein-Barr virus LMP1. Int. Immunol. 30, 579-590 (2018).
- Sakakibara S., et al. Clonal evolution and antigen recognition of anti-nuclear antibodies in acute systemic lupus erythematosus. Scientific Reports 7, 16428 (2017).

# Experimental Immunology

## Shimon Sakaguchi, MD/PhD

| Professor | Shimon Sakaguchi |
|---|---|
| Associate Professor | **James Badger Wing** |
| Assistant Professor | **Kenji Ichiyama** |
| Postdoctoral Fellow | **3** |
| Research Assistant | **4** |
| Support Staff | **4** |

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

Treg cells, which specifically express the transcription factor Foxp3, are actively engaged in the maintenance of immunological self-tolerance and homeostasis. This year, we have explored the roles of Treg cells in tumor immunity, in particular, possible contribution of CTLA-4 and PD-1 highly expressed by tumor-infiltrating Treg cells.

Anti-CTLA4 monoclonal antibody (mAb) is efficacious in enhancing tumor immunity in humans. CTLA-4 is expressed by conventional T cells upon activation and also by naturally occurring FOXP3$^{+}$CD4$^{+}$ regulatory T (Treg) cells constitutively, raising a question of how anti-CTLA-4 mAb can differentially control these functionally opposing T-cell populations in tumor immunity. We showed that FOXP3$^{high}$ potently suppressive effector Treg cells were abundant in melanoma tissues, expressing CTLA-4 at higher levels than tumor-infiltrating CD8$^{+}$ T cells. Upon in vitro tumor-antigen stimulation of peripheral blood mononuclear cells from healthy individuals or melanoma patients, Fc-region-modified anti-CTLA-4 mAb with high antibody-dependent cell-mediated cytotoxicity (ADCC) and/or cellular phagocytosis (ADCP) activity selectively depleted CTLA-4$^{+}$FOXP3$^{+}$ Treg cells and consequently expanded tumor-antigen-specific CD8$^{+}$ T cells. Importantly, the expansion occurred only when antigen stimulation was delayed several days from the antibody treatment to spare CTLA-4$^{+}$ activated effector CD8$^{+}$ T cells from being killed by the mAb. Similarly, in tumor-bearing mice, high-ADCC/ADCP anti-CTLA-4 mAb treatment and tumor antigen vaccination several days later significantly prolonged their survival and markedly elevated cytokine production by tumor-infiltrating CD8$^{+}$ T cells, whereas antibody treatment concurrent with vaccination did not. Anti-CTLA-4 mAb modified to exhibit a lesser or no Fc-binding activity failed to show such timing-dependent in vitro and in vivo immune enhancement. Thus, high ADCC anti-CTLA-4 mAb is able to selectively deplete effector Treg cells and evoke tumor immunity depending on the CTLA-4-expressing status of effector CD8$^{+}$ T cells.

PD-1 blockade is another cancer immunotherapy effective in various types of cancer. In a fraction of treated patients, however, it causes rapid cancer progression called hyper-progressive disease (HPD). With our observation of HPD in ~10% of anti-PD-1 mAb-treated advanced gastric cancer (GC) patients, we explored how anti-PD-1 mAb caused HPD in these patients and how HPD could be treated and prevented. In the majority of GC patients, tumor-infiltrating FoxP3$^{high}$CD45RA$^{-}$CD4$^{+}$ T cells [effector Treg (eTreg) cells], which were abundant and highly suppressive in tumors, expressed PD-1 at equivalent levels as tumor-infiltrating CD4$^{+}$ or CD8$^{+}$ effector/memory T cells and at much higher levels than circulating eTreg cells. Comparison of GC tissue samples before and after anti-PD-1 mAb therapy revealed that the treatment markedly increased tumor-infiltrating proliferative (Ki67$^{+}$) eTreg cells in HPD patients, contrasting with their reduction in non-HPD patients. Functionally, circulating and tumor-infiltrating PD-1$^{+}$ eTreg cells were highly activated, showing higher expression of CTLA-4 than PD-1$^{-}$ eTreg cells. PD-1 blockade significantly enhanced *in vitro* Treg-cell suppressive activity. Similarly, in mice, genetic ablation or antibody-mediated blockade of PD-1 in Treg cells increased their proliferation and suppression of anti-tumor immune responses. Taken together, PD-1 blockade may facilitate the proliferation of highly suppressive PD-1$^{+}$ eTreg cells in HPDs, resulting in inhibition of anti-tumor immunity. The presence of actively proliferating PD-1$^{+}$ eTreg cells in tumors is therefore a reliable marker for HPD. Depletion of eTreg cells in tumor tissues would be effective in treating and preventing HPD in PD-1 blockade cancer immunotherapy.

These findings on CTLA-4 and PD-1 expressed by Treg cells are instrumental in designing cancer immunotherapy with mAbs targeting the molecules commonly expressed by FOXP3$^{+}$ Treg cells and tumor-reactive effector T cells.

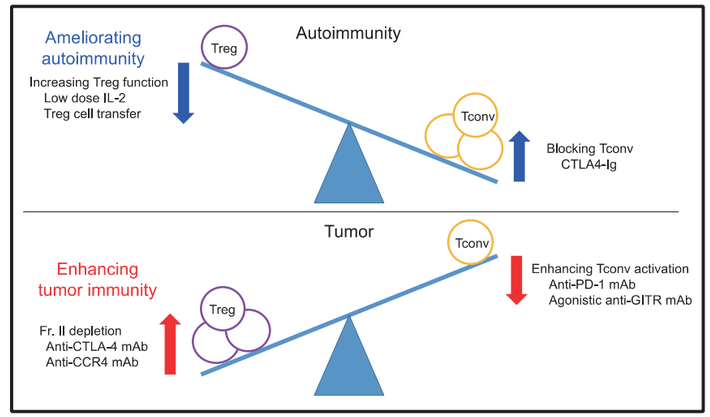



**Figure.**
**Balance of Treg cells in autoimmunity and anti-tumor immunity.**
**Approaches to prevent autoimmunity or enhance anti-tumor immunity. Depletion of Treg cells, their destabilization or enhancement of Tconv activation will tip the balance from prevention of autoimmunity to enhancing anti-tumor immunity. Conversely, expanding Treg numbers, enhancing their suppressive activity, or direct suppression of Tconv activation will tip the balance in the opposite direction. Adapted from Wing et al. *Immunity*. 2019.**

## Recent Publications


- Wing JB, Tanaka A, Sakaguchi S. Human FOXP3$^{+}$ Regulatory T Cell Heterogeneity and Function in Autoimmunity and Cancer. Immunity 50, 302-316 (2019).
- Kamada T, Togashi Y, Tay C, Ha D, Sasaki A, Nakamura Y, Sato E, Fukuoka S, Tada Y, Tanaka A, Morikawa H, Kawazoe A, Kinoshita T, Shitara K, Sakaguchi S, Nishikawa H. PD-1$^{+}$ regulatory T cells amplified by PD-1 blockade promote hyperprogression of cancer. Proc. Natl. Acad. Sci. USA. 116, 9999-10008 (2019).
- Yasuda K, Kitagawa Y, Kawakami R, Isaka Y, Watanabe H, Kondoh G, Kohwi-Shigematsu T, Sakaguchi S, Hirota K. Satb1 regulates the effector program of encephalitogenic tissue Th17 cells in chronic inflammation. Nat. Commun. 10, 549 (2019).
- Ha D, Tanaka T, Kibayashi T, Tanemura A, Sugiyama D, Wing JB, Lim EL, Tang K, Adeegbe D, Newell EW, Katayama I, Nishikawa H, and Sakaguchi S. Differential control of human Treg and effector T cells in tumor immunity by Fc-engineered anti-CTLA-4 antibody. Proc. Natl. Acad. Sci. USA. 116, 609-618 (2019).
- Hirota K, Hashimoto M, Ito Y, Matsuura M, Ito H, Tanaka, M, Watanabe H, Kondoh G, Tanaka A, Yasuda K, Kopf M, Potocnik AJ, Stockinger B, Sakaguchi N, Sakaguchi S. Autoimmune Th17 cells induced synovial stromal and innate lymphoid cells secretion of the cytokine GM-CSF to initiate and augment autoimmune arthritis. Immunity 48, 1220-1232 (2018).

# Cell Signaling


## Takashi Saito, PhD

| | |
|---|---|
| Professor | Takashi Saito |



**Summary**

The objective of our laboratory is to determine the molecular mechanisms of T cell activation, differentiation, and function. Toward this goal, we have studied basic mechanisms such as antigen recognition, T cell activation and differentiation, and regulation of function from a signaling perspective.

Our finding that TCR-microclusters (MC) initiate T cell activation led us to analyze the dynamic recruitment/assembly of signaling molecules at the immune synapse. Using approaches similar to those used in our studies of CTLA4 and PD-1, the dynamic regulation of other inhibitory co-stimulation receptors such as LAG3 and TIGIT are being analyzed.

We have analyzed several molecules that are highly expressed upon T cell activation, as possible targets to modulate T cell activation and function. CRTAM was originally cloned as an adhesion receptor, but is now found to play a critical role in determining the $CD4^+$ CTL lineage. The TCR downstream signaling adaptor CIN85 is now found to mediate negative regulation of T cell activation (below, Figure). The function of the innate-sensor STING in T cells was analyzed since it is highly expressed in T cells. STING activation induced growth inhibition and type I-IFN production in T cells.

We ultimately aim to modulate T cell function/activation to prevent autoimmunity and allergic inflammation. We have analyzed the function of autoimmune-related phosphatases PTPN22/2. The KO mice showed enhanced activation and an increase in effector/memory T cells. Imaging analysis and MS analysis of PTPN-associated molecules will identify the inhibitory mechanisms.


**Function of adaptors CIN85/CD2AP in T cell function**

Adaptor molecules play critical roles to mediate activation signals and serve as branch points for the direction of signals. CIN85 has been shown to mediate negative functions by receptor internalization in non-immune cells while it contributes to NF-κB activation in B cells. We have analyzed the role of CIN85 and another family member CD2AP by generating T cell-specific deficient mice. CIN85 deficiency resulted in increased CD4 and CD8 T cells in the thymus and periphery, particularly the effector/memory T cells. Stimulation of the KO T cells induced increased phosphorylation of TCR proximal signaling molecules such as ZAP-70, PLCγ, and Erk, indicating increased phosphorylation downstream of ZAP-70. Ag stimulation induced increased IL-2 production and proliferation. There was no obvious role for TCR internalization upon stimulation. To clarify the mechanism of CIN85-mediated inhibitory function, we first defined the domains mediating negative regulation as SH3/PR regions and then identified molecules binding to these domains by MS analysis. We found that the phosphatase Sts-2 binds to CIN85 upon activation. Since Sts-2 has been shown to inhibit T cell activation by targeting ZAP-70, CIN85 mediates negative regulation by assembly with the phosphatase Sts-2 to inhibit phosphorylation downstream of ZAP70. In contrast, and in spite of a previous report that CD2AP has inhibitory functions in T cells, there was no obvious abnormality in T cell signaling and function in mice with a specific deficiency of CD2AP in T cells. These results indicate that CIN85-Sts2 is a new inhibitory axis for T cell signaling and could be a therapeutic target to augment T cell function (Figure).

**Innate signal regulation of T cell function**

We have analyzed modulation of T cell function by innate signals. Indeed, we have shown that nucleic acids induce co-stimulation of T cell activation and differentiation of Th2 cells. We further explored functional aspects upon cytosolic sensing of DNA in T cells and found that T cells express high levels of STING. Stimulation of T cells by STING ligands cGAMP surprisingly induced the production of type I interferon (IFN-I). The amount of IFN-I produced by activated T cells is much higher than by innate cells such as DCs. TCR co-stimulation is required for IFN-I production because TCR signaling induces sustained activation of IRF3. Concomitantly, stimulation by cGAMP strongly induces cell cycle arrest and inhibits T cell proliferation; the inhibition of proliferation is mediated by blocking mTORC1 activation. Thus, STING-dependent cytosolic DNA-sensing in T cells induces both inflammatory responses to induce IFN-I production, and inhibition of cell growth. We also demonstrated that STING stimulation in T cells is important for anti-tumor responses *in vivo*.

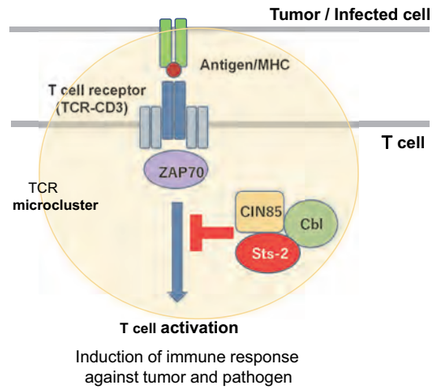
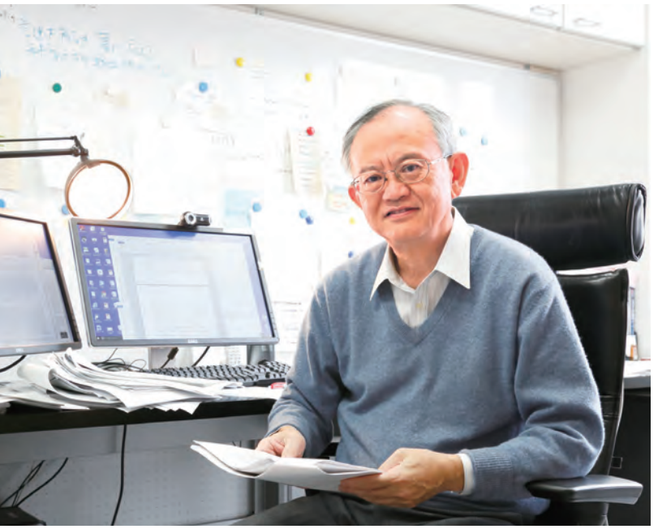



**Figure.**
**The adaptor protein CIN85 mediates inhibition of T cell activation by recruitment of the phosphatase Sts-2 and the ubiquitin ligase Cbl into the TCR microcluster upon TCR stimulation.**

## Recent Publications


- Nemeth T, Futosi K, Szabo M, Aradi P, Saito T, Mocsai A & Jakus Z. Importance of Fc receptor γ-chain ITAM tyrosines in neutrophil activation and in vivo autoimmune arthritis. Front. Immunol. 10, 252 (2019).
- Kong MS, Hashimoto-Tane A, Kawashima Y, Sakuma M, Yokosuka T, Kometani K, Onishi R, Carpino N, Ohara O, Kurosaki T, Phua KK & Saito T. Inhibition of T cell activation and function by the CIN85 adaptor protein. Sci. Signal. 12, eaav4373 (2019).
- Imanishi T, Unno M, Kobayashi W, Yoneda N, Matsuda S, Ikeda K, Hoshii T, Hirao A, Miyake K, Barber GN, Arita M, Ishii KJ, Akira S & Saito T. Reciprocal regulation of STING and TCR signaling by mTORC1 for T cell activation and function. Life Sci. Alliance 2, e201800282 (2019).
- Yamamoto T, Masuta Y, Momota M, Kanekiyo M, Kanuma T, Takahama S, Moriishi E, Yasutomi Y, Saito T, Graham BS, Takahashi Y & Ishii KJ. A unique nanoparticulate TLR9 agonist enables a HA split vaccine to confer FcγR-mediated protection against heterologous lethal influenza virus infection. Int. Immunol. 3, 81-90 (2019).
- Fujimoto H, Saito Y, Ohuchida K, Kawakami E, Fujiki S, Watanabe T, Ono R, Kaneko A, Takagi S, Najima Y, Hijikata A, Cui L, Ueki T, Oda Y, Hori S, Ohara O, Nakamura M, Saito T & Ishikawa F. Deregulated Mucosal Immune Surveillance through Gut-Associated Regulatory T Cells and PD-1+ T Cells in Human Colorectal Cancer. J. Immunol. 200, 3291-3303 (2018).

# Lymphocyte Differentiation



## Tomohiro Kurosaki, MD/PhD

| Professor | Tomohiro Kurosaki |
|---|---|
| Associate Professor | Wataru Ise |
| | Takeshi Inoue |
| Assistant Professor | Ryo Shinnakasu |
| Research Assistant | 2 |
| Visiting Scientist | 1 |
| Support Staff | 4 |

**Requirement for memory B cell activation in protection from heterologous influenza virus reinfection**

Long-lived plasma cells (LLPCs) constitutively produce antibodies (Ab) and neutralize invading antigens immediately upon re-infection, whereas memory B cells require re-stimulation by specific antigens for their differentiation into Ab-secreting plasma cells. Since serum Ab titers are known to correlate with vaccine efficacy, the importance of LLPCs is well appreciated. In contrast, the importance of memory B cells in conferring protection to reinfection has been controversial.

We and others have recently demonstrated that germinal center (GC) B cells with relatively low affinities were preferentially recruited into the memory B cell compartment. In addition, it has been shown that memory B cells form at an early stage during GC reaction. These two lines of evidence suggest that such mechanisms may prevent the memory B cell population from becoming overly committed to the initial immunizing antigen, and instead, contribute to their acquisition of a more diverse repertoire. Hence, it has been proposed that memory B cells may be intrinsically suited for recognition of and protection from secondary infection by antigenically variant pathogens. To test this proposal, we used a mouse model of drifted viral infection with the pandemic H1N1 (Narita) virus then the H1N1 (PR8) virus. We demonstrate that GC-experienced anti-HA stem-specific memory B cells are generated during primary infection with the Narita virus, and are activated upon re-infection with the PR8 virus, thereby contributing to host protection.

**Stability of Tfh-GC B cell contacts is key for plasma cell-prone GC cell formation**

During GC reaction, higher-affinity B cells are directed to the plasma cell fate, whereas lower-affinity cells enter into the recycling GC cell pool. In regard to selection mechanisms towards recycling GC or plasma cell fates, it has been postulated that precursor cells for such fates already become committed in the GC. To test this model, we first identified a small population of $Bcl6^{lo}CD69^{hi}$ light zone (LZ) GC cells with higher-affinity BCRs and that express IRF4, which favors the plasma cell fate over GC recycling. In contrast, $Bcl6^{hi}CD69^{hi}$ population with lower-affinity BCRs favored GC recycling. Mechanistically, the generation of $Bcl6^{lo}CD69^{hi}$ cells relied on CD40 in a dose-dependent manner. Moreover, we found that ICAMI and SLAM levels on LZ GC cells were upregulated by CD40 stimulation. Consequently, $Bcl6^{lo}CD69^{hi}$ cells expressed higher levels of these adhesion molecules than $Bcl6^{hi}CD69^{hi}$ cells, thereby affording more stable GC B-Tfh contacts. Thus, we propose a precursor model in which the duration of the Tfh-GC B interaction is a key decisive factor for formation of plasma precursor versus GC recycling precursor cells.

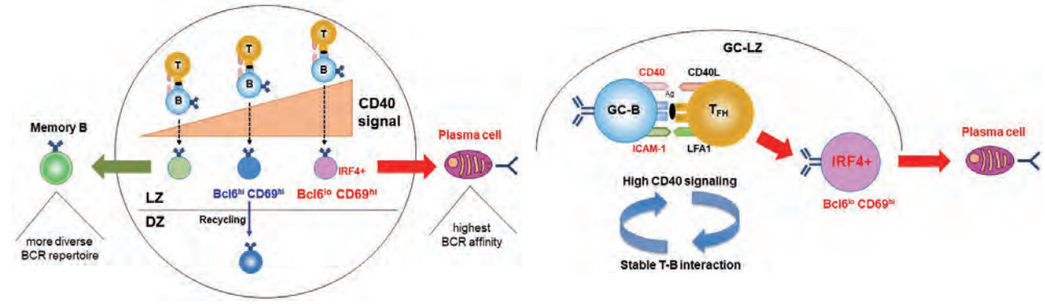



**Figure.**
**Figure: Development of plasma cell precursors in the germinal center**
**(Left) A population of $Bcl6^{lo}CD69^{hi}$ light zone(LZ)GC cells with IRF4 and higher affinity BCR favors the plasma cell fate. In contrast, $Bcl6^{hi}CD69^{hi}$ cells with lower affinity BCRs favor GC recycling.CD40 acts as a dose-dependent regulator for plasma cell precursor formation. (Right) CD40 signaling upregulates adhesion molecules, such as ICAM-1 or SLAM, thereby affording more stable GC B-$T_{FH}$ contacts. The consequence of strong interaction with $T_{FH}$ is the formation of $IRF4^{+}Bcl6^{lo}$ plasma cell-prone GC cells.**

## Recent Publications


- Ise W, Kurosaki T. Plasma cell differentiation during the germinal center reaction. Immunol. Rev. 288(1), 64-74. (2019).
- Ise W, Fujii K, Shiroguchi K, Ito A, Kometani K, Takeda K, Kawakami E, Yamashita K, Suzuki K, Okada T, Kurosaki T. T Follicular Helper Cell-Germinal Center B Cell Interaction Strength Regulates Entry into Plasma Cell or Recycling Germinal Center Cell Fate. Immunity 48(4), 702-715.e4. (2018).
- Herndler-Brandstetter D, Ishigame H, Shinnakasu R, Plajer V, Stecher C, Zhao J, Lietzenmayer M, Kroehling L, Takumi A, Kometani K, Inoue T, Kluger Y, Kaech SM, Kurosaki T, Okada T, Flavell RA. $KLRG1_{+}$ Effector $CD8_{+}$ T Cells Lose KLRG1, Differentiate into All Memory T Cell Lineages, and Convey Enhanced Protective Immunity. Immunity 48(4), 716-729.e8. (2018).
- Inoue T, Moran I, Shinnakasu R, Phan TG, Kurosaki T. Generation of memory B cells and their reactivation. Immunol. Rev. 283(1), 138-149. (2018).
- Inoue T, Shinnakasu R, Ise W, Kawai C, Egawa T, Kurosaki T. The transcription factor Foxo1 controls germinal center B cell proliferation in response to T cell help. J. Exp. Med. 214(4), 1181-1198 (2017).

# Malaria Immunology

## Cevayir Coban, MD

| Professor | Cevayir Coban |
|---|---|
| Assistant Professor | **Michelle Sue Jann LEE** |
| Postdoctoral Fellow | **1** |
| Visiting Scientist | **1** |
| Support Staff | **1** |

Malaria is caused by the protozoan parasite *Plasmodium spp.* that often leads to severe complications and death. With over 200 million infection cases per year, we are still far from global malaria eradication. Growing evidence suggests that millions of lives in endemic regions suffer from asymptomatic- or post-malaria complications. Therefore, collective elimination programs and/or reduction of the burden of malaria are equally important efforts. In our lab, we focus on the elucidation of the mechanisms involved in the host and *Plasmodium* parasites interactions. We have recently gained advanced understanding of the disease pathology by using experimental mouse malaria models which led us to reach to the conclusion that *Plasmodium* parasite presence in different organs should be considered in a tissue-specific context (*Coban et al., Nature Review Immunology, 2018*).

We have used several imaging technologies such as high-field MRI, multi-photon microscopy, and micro-CT to understand immunopathology of malaria in tissues and organs in mice models. Depending on our research needs we have developed additional new techniques/methods to study immune responses to, or immunopathology caused by, these parasites.

Neutrophils are an immediate host defense cells that respond to invading pathogens mainly via releasing their extracellular traps (NETs). However, there is a lack of information on the role of neutrophils and NETs during malaria infection. Thus, we recently developed an automated analysis method to rapidly acquire and characterize NETs by using imaging flow cytometry (*Lelliott et al.,Cytometry A, 2019*). The image analysis algorithm was established based on morphological data showing the extrusion of DNA. This method was successfully applied to measure NETs in whole blood during infection of mice with the malaria parasite *Plasmodium yoelii* NL (Figure 1). We believe this method will be used to study NETs not only during malaria but in other infectious and non-infectious diseases.

We finally aim to translate our understanding of host-*Plasmodium* interactions into the vaccines or drugs to prevent/treat malaria. We have developed a new adjuvant called synthetic hemozoin, a synthetic analog of *Plasmodium*-produced hemozoin, and completed its preliminary GLP non-clinical safety and toxicology studies in several animals and infection models (*Lee et al., Vaccine, 2016*). Very recently, we have delineated the mode of action of synthetic hemozoin adjuvant (*Lee et al., European J. Immunology, 2019*). We showed that B cell-intrinsic MyD88 signaling is involved in the mode of action of certain particulate adjuvants such as synthetic hemozoin and this may enhance our specific understanding of how adjuvants and vaccines work (Figure 2).

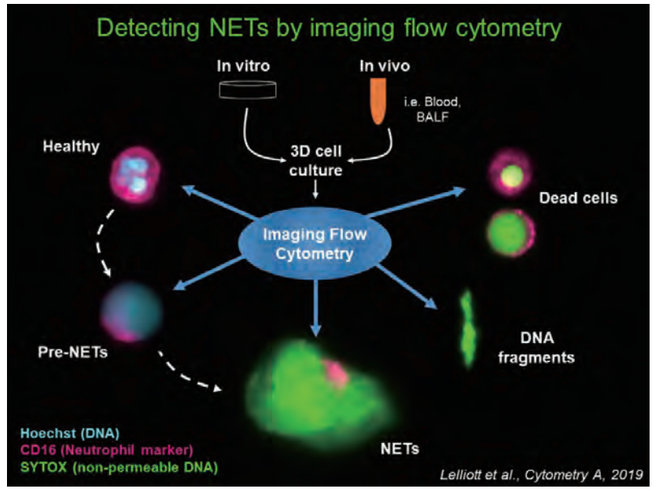



**Figure 1.**
**Detecting NETs by imaging flow cytometry.**
**Special preparation and handling of three-dimensional cell culture and neutrophil stimulation allow us to image key characteristics and image features of NETs by using imaging flow cytometry.**

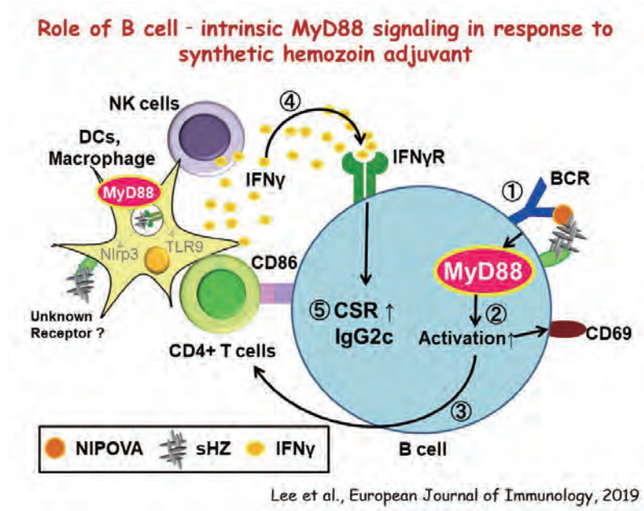



**Figure 2.**
**Cell-intrinsic MyD88 plays differential roles in the modes of action of vaccine adjuvants.**
**Using synthetic hemozoin particulate adjuvant, we found that B cell intrinsic MyD88 is required for IFNγ induction in T cells, NK cells and dendritic cells to induce IgG2c class switching in a TLR/IL1/Inflammasome-independent manner.**

## Recent Publications


- Lee MSJ, Natsume-Kitatani Y, Temizoz B, Igari Y, Tsukui T, Kobiyama K, Kuroda E, Ise W, Inoue T, Kurosaki T, Mizuguchi K, Akira S, Ishii KJ, Coban C. B cell-intrinsic MyD88 signaling controls IFNγ-mediated early IgG2c class switching in response to a particulate adjuvant. Eur. J. Immunol. (2019) in press.
- Lelliott PM, Momota M, Lee MSJ, Kuroda E, Iijima N, Ishii KJ, Coban C. Rapid quantification of NETs in vitro and in whole blood samples by imaging flow cytometry. Cytometry A doi: 10.1002/cyto.a.23767 (2019).
- Coban C, Lee MSJ, Ishii KJ. Tissue-specific immunopathology during malaria infection. Nat. Rev. Immun. doi:10.1038/nri.2017.138 (2018).
- Lee MSJ, Maruyama K, Fujita Y, Konishi A, Lelliott PM, Itagaki S, Horii T, Lin JW, Khan SM, Kuroda E, Akira S, Ishii KJ, Coban C. *Plasmodium* products persist in the bone marrow and promote chronic bone loss. Sci. Immunol. June 2, 2 (12), pii: eaam8093 (2017).
- Zhao H et al. Olfactory Plays a Key Role in Spatiotemporal Pathogenesis of Cerebral Malaria. Cell Host Microbe 15(5), 551-63 (2014).

# Vaccine Science

## Ken J. Ishii, MD/PhD

| Professor | Ken J. Ishii |
|---|---|
| Associate Professor | **Etsushi Kuroda** |
| | **Norifumi Iijima** |
| Assistant Professor | **Natsuko Kishishita** |
| | **Burcu Temizoz** |
| Postdoctoral Fellow | **3** |
| Research Assistant | **3** |
| Visiting Scientist | **12** |
| Support Staff | **1** |

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

**A unique nanoparticulate TLR9 agonist enables an HA split vaccine to confer FcγR-mediated protection against heterologous lethal influenza virus infections**

The development of a universal influenza vaccine that can provide a robust and long-lasting protection against a broader range of influenza virus strains is a global public health priority. One approach to improve vaccine efficacy is to use an adjuvant to boost immune responses to the target antigens; nevertheless, the role of adjuvants in the context of influenza vaccines is not fully understood.

We have previously developed the K3-schizophyllan(SPG) adjuvant, which is composed of nanoparticulated oligodeoxynucleotides K3, a TLR9 agonist with SPG. SPG is a non-agonistic β-glucan ligand of Dectin-1. In this study, K3-SPG given with conventional influenza hemagglutinin (HA) split vaccine (K3-SPG HA) conferred protection against an antigenically mismatched heterologous virus challenge. While K3-SPG HA elicited robust cross-reactive HA-specific IgG2c and CD8 T-cell responses, CD8 T-cell depletion had no impact on this cross-protection. In contrast, K3-SPG HA was not able to confer protection against the heterologous virus challenge in FcRγ-deficient mice. Our results indicated that FcγR-mediated antibody responses induced by the HA antigen and K3-SPG adjuvant were important for potent protection against antigenically mismatched influenza virus infections. Thus, we demonstrated that the K3-SPG-adjuvanted vaccine strategy broadens protective immunity against influenza and provides a basis for the development of next-generation influenza vaccines.

**Combination and inducible adjuvants targeting nucleic acid sensors**

Innate immune sensing of nucleic acids derived from invading pathogens or tumor cells via pattern recognition receptors is crucial for mounting protective immune responses against infectious disease and cancer. Recently, discovery of tremendous amounts of nucleic acid sensors as well as identification of natural and synthetic ligands for these receptors revealed the potential of adjuvants targeting nucleic acid sensing pathways for designing efficacious vaccines. Especially, current data indicated that unique adjuvants targeting TLR9 and stimulator of interferon genes (STING)-dependent cytosolic nucleic acid sensing pathways along with the combinations of already existing adjuvants are promising candidates for this purpose. Here, we review current vaccine adjuvants targeting nucleic acid sensors and their modes of action. (Figure 1, 2)

**An Antigen-Free, Plasmacytoid Dendritic Cell-Targeting Immunotherapy To Bolster Memory CD8+ T Cells in Nonhuman Primates**

The priming, boosting, and restoration of memory cytotoxic CD8+ T lymphocytes by vaccination or immunotherapy in vivo is an area of active research. Particularly, nucleic acid-based compounds have attracted attention due to their ability to elicit strong Ag-specific CTL responses as a vaccine adjuvant. Nucleic acid-based compounds have been shown to act as anticancer monotherapeutic agents even without coadministration of cancer Ag(s); however, so far they have lacked efficacy in clinical trials. We recently developed a second-generation TLR9 agonist, which is a humanized CpG DNA(K3) complexed with schizophyllan (SPG). This is called K3-SPG, which is a nonagonistic Dectin-1 ligand. K3-SPG was previously shown to act as a potent monoimmunotherapeutic agent against established tumors in mice in vivo. In this study we extend the monoimmunotherapeutic potential of K3-SPG to a nonhuman primate model. K3-SPG activated monkey plasmacytoid dendritic cells to produce both IFN-α and IL-12/23 p40 in vitro and in vivo. A single injection s.c. or i.v. of K3-SPG significantly increased the frequencies of activated memory CD8+ T cells in circulation, including Ag-specific memory CTLs, in cynomolgus macaques.

This increase did not occur in macaques injected with free CpG K3 or polyinosinic-polycytidylic acid. Injection of 2 mg K3-SPG induced mild systemic inflammation, however, levels of proinflammatory serum cytokines and circulating neutrophil influx were lower than those induced by the same dose of polyinosinic-polycytidylic acid. Therefore, even in the absence of specific Ags, we show that K3-SPG has potent Ag-specific memory CTL response-boosting capabilities, highlighting its potential as a monoimmunotherapeutic agent for chronic infectious diseases and cancer.

**Future prospect**

Vaccine target diseases are now not only restricted to a framework of infectious diseases but include a broad range of diseases such as cancer, allergy, Alzheimer's disease, and many other lifestyle-related diseases. As we move the main lab to the Institute of Medical Science, The University of Tokyo, we will continue our 'innovative' research and development of vaccines against these diseases while working closely together and actively exchanging researchers with the National Institute of Biomedical Innovation, Health and Nutrition (NIBIOHN).

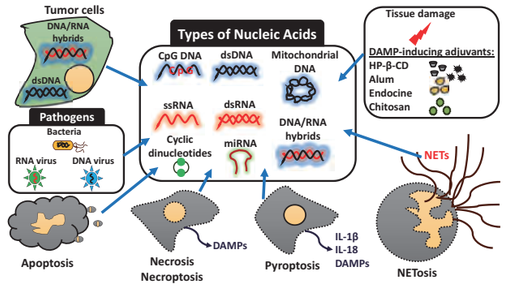



**Figure 1.**
Sources and types of nucleic acid adjuvants differentially modulating immune responses.

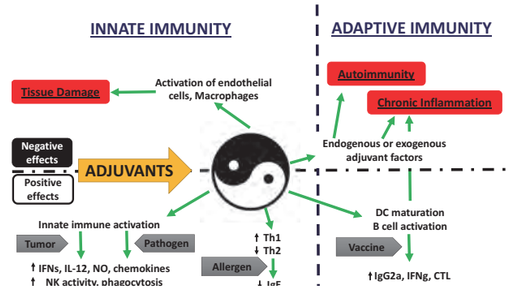



**Figure 2.**
The yin and yang of adjuvants in health and disease.

## Recent Publications


- Yamamoto T, Masuta Y, Momota M, Kanekiyo M, Kanuma T, Takahama S, Moriishi E, Yasutomi Y, Saito T, Graham BS, Takahashi Y, Ishii KJ. A unique nanoparticulate TLR9 agonist enables a HA split vaccine to confer FcγR-mediated protection against heterologous lethal influenza virus infection. Int Immunol. 15, 31(2), 81-90 (2019).
- Temizoz B, Kuroda E, Ishii KJ. Combination and inducible adjuvants targeting nucleic acid sensors. Curr. Opin. Pharmacol. 41, 104-113 (2018).
- Masuta Y, Yamamoto T, Natsume-Kitatani Y, Kanuma T, Moriishi E, Kobiyama K, Mizuguchi K, Yasutomi Y, Ishii KJ. An Antigen-Free, Plasmacytoid Dendritic Cell-Targeting Immunotherapy To Bolster Memory CD8(+) T Cells in Nonhuman Primates. J Immunol. 200(6), 2067-2075 (2018).
- Coban C, Lee MSJ, Ishii KJ. Tissue-specific immunopathology during malaria infection. Nat Rev Immunol. 18(4), 266-278 (2018).

# Immunoparasitology

## Masahiro Yamamoto, PhD

| Professor | Masahiro Yamamoto |
|---|---|
| Associate Professor | **Miwa Sasai** |
| Assistant Professor | **Ji Su Ma** |
| | **Youngae Lee** |
| Postdoctoral Fellow | **1** |
| Research Assistant | **1** |
| Visiting Scientist | **1** |
| Support Staff | **1** |

*Toxoplasma gondii* is an obligatory protozoan parasite that can infect nearly all warm-blooded animals, including humans. It is estimated that one-third of the world's population is infected with *T. gondii*; notably, most infections are asymptomatic. *T. gondii*, however, also causes toxoplasmosis in immunocompromised individuals; the clinical signs of toxoplasmosis comprise encephalitis, hepatitis, and myocarditis. Individuals with increased susceptibility to toxoplasmosis include those with acquired immunodeficiency syndrome (AIDS), those undergoing chemotherapy, fetuses with congenital diseases, and newborn babies of women who initially contracted the infection during pregnancy. *T. gondii* is ranked among the top five human pathogens that cause economic loss and life impairment via food-borne illness in the United States. Thus, *T. gondii* is an important pathogen of both humans and animals.

*T. gondii* secretes various effector molecules into host cells upon infection, to promote efficient parasite growth and dissemination *in vivo*. The effector mechanisms used by the parasite to subvert host immune responses have been extensively analyzed in mouse models. The proteins ROP5, ROP16, ROP17, ROP18, GRA7, and TgIST are secreted from rhoptries or dense granules to suppress anti-*T. gondii* cell-autonomous immune responses; this results in increased parasite virulence in mice. GRA6, a dense granule protein, activates the host transcription factor NFAT4 to induce chemokines and recruit neutrophils to sites of infection, thereby promoting parasite dissemination and maximizing parasite virulence. GRA15, another dense granule protein, is secreted into host cells to activate another host transcription factor, NF-κB, in both mouse and human cells. Similar to GRA6, GRA15 activates host immune responses and mediates IL-1 production via activation of the NLRP3 inflammasome. Lack of GRA15 in *T. gondii* parasites promotes *in vivo* parasite proliferation in mice. Given that GRA15-deficient *T. gondii* is more virulent than wild-type *T. gondii* in mice, GRA15 might assist host survival by limiting parasite replication; hence, it may play an anti-parasitic role in mice. However, the significance of GRA15 as a virulence factor in humans is not well understood.

The mechanisms underlying host resistance to *T. gondii* rely on innate and adaptive immunity, and involve various immune/non-immune cells and cytokines. Among these contributing factors, interferon-γ (IFN-γ), which is the most important host cytokine that targets *T. gondii*, is largely produced by $CD4^+$ T cells and natural killer cells; it stimulates cell-autonomous responses in immune cells, including macrophages and dendritic cells, or non-immune cells (e.g., fibroblasts). IFN-γ activates the STAT1 transcription factor and induces the expression of hundreds of genes. IFN-γ-inducible GTPases and nitric oxide (NO) mediate parasite clearance and growth inhibition in mice, respectively. On the other hand, IFN-γ-dependent nutrient deprivation and cell death have been established as anti-*T. gondii* responses in human cells. IFN-γ stimulates the expression of indoleamine 2,3-dioxygenase (IDO) to degrade tryptophan, an essential nutritional amino acid for the intracellular growth of *T. gondii* in human cells.

The anti-*T. gondii* role for inducible NO synthase (iNOS), an IFN-γ-inducible protein, has been established in mice. Deletion or pharmacological inhibition of the iNOS gene in mouse macrophages results in profoundly reduced NO production in response to IFN-γ, along with concomitant parasite growth. However, blocking iNOS activity does not affect the IFN-γ-induced anti-parasite response in human macrophages or monocytes. Thus, although iNOS may play different roles in mice and humans, its precise role in the IFN-γ-mediated interplay between humans and *T. gondii* remains unknown.

In FY2018, we have reported a novel virulence strategy for *T. gondii*, whereby the pathogen utilizes the GRA15 effector protein and the host co-factor iNOS to suppress the IFN-γ-induced IDO-dependent cell-autonomous immune response in human cells.

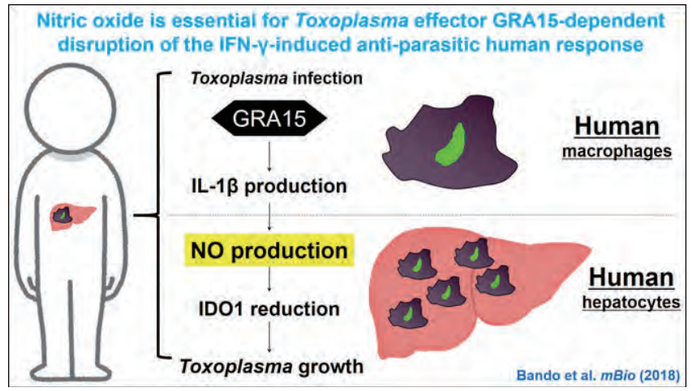
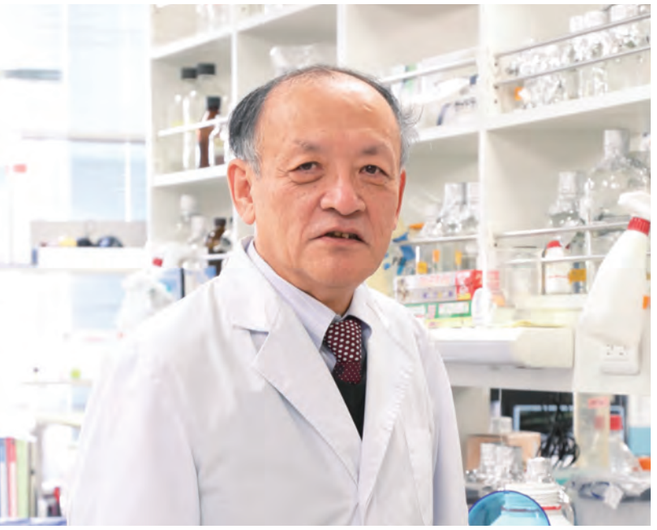



**Figure.**
**In human cells, Toxoplasma secretes a virulence factor GRA15 to produce IL-1β by activating NLRP3 inflammasome, eventually inhibiting IDO1 expression through indirect induction of iNOS and NO.**

## Recent Publications


- Bando H, Lee Y, Sakaguchi N, Pradipta A, Ma JS, Tanaka S, Cai Y, Liu J, Shen J, Nishikawa Y, Sasai M, Yamamoto M. Inducible Nitric Oxide Synthase Is a Key Host Factor for *Toxoplasma* GRA15-Dependent Disruption of the Gamma Interferon-Induced Antiparasitic Human Response. MBio. 2018 Oct 9;9(5). pii: e01738-18. doi: 10.1128/mBio.01738-18.
- Bando H, Sakaguchi N, Lee Y, Pradipta A, Ma JS, Tanaka S, Lai DH, Liu J, Lun ZR, Nishikawa Y, Sasai M, Yamamoto M. *Toxoplasma* Effector TgIST Targets Host IDO1 to Antagonize the IFN-γ-Induced Anti-parasitic Response in Human Cells. Front Immunol. (2018) 9:2073. doi: 10.3389/fimmu.2018.02073.
- Sasai M, Sakaguchi N, Ma JS, Nakamura S, Kawabata T, Bando H, Lee Y, Saitoh T, Akira S, Iwasaki A, Standley DM, Yoshimori T, Yamamoto M. Essential role for GABARAP autophagy proteins in interferon-inducible GTPase-mediated host defense. Nat. Immunol. (2017) 18:899-910. doi: 10.1038/ni.3767.
- 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 Rep. (2015) 13:223-233. doi: 10.1016/j.celrep.2015.09.005.
- 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. (2015) 112:E4581-4590. doi: 10.1073/pnas.1510031112.

# Biochemistry and Immunology



## Shigekazu Nagata, PhD

| Professor | Shigekazu Nagata |
|---|---|
| Associate Professor | **Katsumori Segawa** |
| Postdoctoral Fellow | **2** |
| Research Assistant | **2** |
| Visiting Scientist | **1** |
| Support Staff | **1** |

Phospholipids are asymmetrically distributed between inner and outer leaflets of plasma membranes. 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 ATP-dependent phospholipid flippases that translocate PtdSer from outer to inner leaflets (Figure 1). When cells undergo apoptosis, the asymmetrical distribution of phospholipids is disrupted by a scramblase(s) that non-specifically scrambles phospholipids between the two leaflets, leading to the PtdSer-exposure. The PtdSer, thus exposed to the cell surface, works as an "eat me" signal of apoptotic cells for macrophages. In addition to apoptotic cells, the PtdSer-exposure is observed in activated platelets, lymphocytes and mast cells, capacitated sperm, aged erythrocytes, exosomes, and enveloped virus.

We identified two P4-type ATPases (ATP11A and 11C) and their subunit CDC50A as flippases at plasma membranes. The CDC50A works as a chaperone for ATP11A and 11C to escort them from ER to plasma membranes, and is required for their flippase activity at plasma membranes. ATP11A and 11C contain caspase-recognition sites in the middle of molecules, and their flippase activity is destroyed during apoptosis. ATP11C-null mice suffer from B cell deficiency. We recently found that B cell progenitors express ATP11C but not ATP11A. Thus, B cells in ATP11C-null mice have no flippase at plasma membranes, causing sustained PtdSer-exposure, and are engulfed alive by macrophages (entosis or cell cannibalism).

There are two families of membrane proteins carrying 10 transmembrane regions that support non-specific scrambling of phospholipids at plasma membranes. Five members (TMEM16C, 16D, 16F, 16G and 16J) of the TMEM16 family function as $Ca^{2+}$-dependent scramblases. With a microarray system of membrane bilayers in which phospholipids are asymmetrically distributed, we recently showed that a single dimeric molecule of TMEM16F can scramble phospholipids. TMEM16F is ubiquitously expressed in various cells, while other members are expressed in specific tissues. The TMEM16F$^{-/-}$ platelets cannot expose PtdSer, leading to the reduced ability to produce thrombin for blood clotting. In fact, human patients of Scott syndrome, a congenital bleeding disorder, were found to carry a loss of function mutation in the TMEM16F gene.

Three members (Xkr4, Xkr8 and Xkr9) of the Xkr family enhance the scrambling phospholipids during apoptosis. Xkr8 is expressed ubiquitously, while Xkr4 and Xkr9 are expressed in a tissue-specific manner, in the brain or intestine, respectively. These XKR members contain a caspase-recognition site in the C-terminal tail region, and are cleaved by caspase during apoptosis to function as a scramblase. Thus, in apoptotic cells, caspase cleaves and irreversibly inactivates ATP11A and ATP11C flippases, while it cleaves and activates the Xkr8 scramblase, to quickly and irreversibly expose PtdSer (Figure 2). The PtdSer, thus on the dead cell's surface, is recognized by macrophages for engulfment. Lymphocytes and neutrophils express only Xkr8, and a lack of Xkr8 prevents the PtdSer-exposure and engulfment by macrophages. We found that the inefficient engulfment of apoptotic cells due to the Xkr8 mutation activates the autoimmunity. It is likely that unengulfed dead cells undergo secondary necrosis, release cellular components, and activate the immunity, leading to an SLE-type autoimmune disease.

As mentioned above, PtdSer is exposed in various biological processes. We recently found that Xkr8 can be activated by phosphorylation (Figure 2). This phosphorylation-induced activation of Xkr8 was independent from the caspase-mediated activation. We will study which kinase(s) is responsible for activating Xkr8, and which biological process activates Xkr8. We are also interested in the molecular mechanism of how flippases flip phospholipids, and of how scramblases scramble phospholipids at plasma membranes.

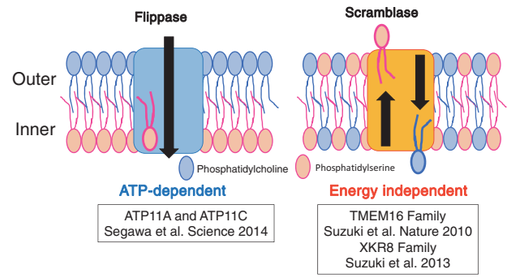



**Figure 1.**
**Flippase and Scramblase.**
**Plasma membranes in eukaryotic cells are comprised of outer and inner leaflets, in which phosphatidylserine (PtdSer) and phosphatidylcholine are located exclusively at inner or outer leaflets, respectively. The asymmetrical distribution of PtdSer is maintained by an ATP dependent flippase. We showed that ATP11A and 11C are flippases at plasma membranes. In various biological processes, the asymmetrical distribution of phospholipids is disrupted by the action of scramblase. We identified two family of proteins (TMEM16 and Xkr) as $Ca^{2+}$- and caspase-dependent scramblases, respectively.**

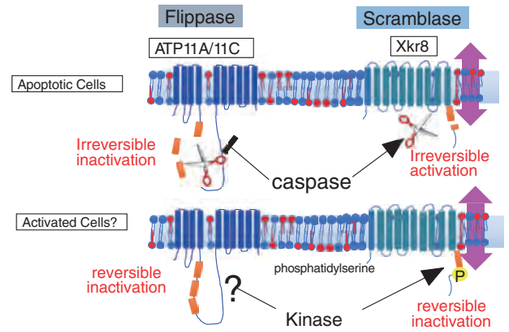



**Figure 2.**
**Activation of Xkr8's scramblase and inactivation of flippases by two independent mechanisms.**
**In apoptosis, caspase cleaves Xkr8 to activate scramblase while it cleaves flippases (ATP11A and 11C) to inactivate, causing irreversible exposure of PtdSer. Under activated conditions, unidentified kinase(s) phosphorylates Xkr8 to activate scramblase. At the same time, it seems that flippases are phosphorylated to inactivate their flippase activity.**

## Recent Publications


- Sakuragi T, Kosako H and Nagata S.: Phosphorylation-mediated activation of mouse Xkr8 scramblase for phosphatidylserine exposure. Proc. Natl. Acad. Sci. USA. 116, 2907-2912 (2019).
- Segawa K, Yanagihashi Y, Yamada K, Suzuki C, Uchiyama Y and Nagata S. Phospholipid flippases enable precursor B cells to flee engulfment by macrophages. Proc. Natl. Acad. Sci. USA. 115, 12212-12217 (2018).
- Nagata S. Apoptosis and clearance of apoptotic cells. Annu. Rev. Immunol. 36, 489-517 (2018).
- Kawano M and Nagata S. Lupus-like autoimmune disease caused by a lack of Xkr8, a caspase-dependent phospholipid scramblase. Proc. Natl. Acad. Sci. USA. 115, 2132-2137 (2018).
- Nagata S and Tanaka M. Programmed cell death and the immune system. Nat. Rev. Immunol. 17, 333-340 (2017).

# Molecular Neuroscience

## Toshihide Yamashita, MD/PhD

| Professor | Toshihide Yamashita |
|---|---|
| Associate Professor | Yuki Fujita |
| | Takahide Itokazu |
| Assistant Professor | Hiroshi Nakagawa |
| | Oluwaseun Fatoba |
| | Hiroshi Tsujioka |
| | Yosuke Ohtake |
| | Kana Harada |
| Postdoctoral Fellow | 2 |
| Research Assistant | 4 |
| Support Staff | 4 |

Disorders of the central nervous system, such as cerebrovascular diseases, cerebrospinal trauma, and encephalomyelitis, often cause spatiotemporal changes in the nervous system and in various biological systems, such as the immune system and vascular system. We have analyzed disorders of the neural networks in the central nervous system and the subsequent restoration process from the perspective of the functional network of biological systems (Figure 1). Further, we have analyzed the mechanism by which the spatiotemporal dynamics in those biological systems control a series of processes (Figure 2). Particularly, the ultimate goal of this study is to elucidate the control mechanism exerted by the associations among the nervous system, immune system, and vascular system. Additionally, we aim to elucidate the principles involved in the operation of living organisms with neural network disorders within the central nervous system by observing such disorders and their functional recovery process with respect to the dynamics of the entire biological system and by conducting a comprehensive analysis of the association between each system.

We perceive the central nervous system as a single organ within a biological system; further, studies from the perspective of the involvement of the entire biological system in disorders and recovery of neural networks are scarce. By perceiving disorders in the neural networks and the biological reactions during the subsequent recovery process as a "scrap-and-build" strategy, we aim to elucidate the mechanisms behind a series of reactions as well as their significance that may potentially create a new and never-before-seen trend in Life Sciences.

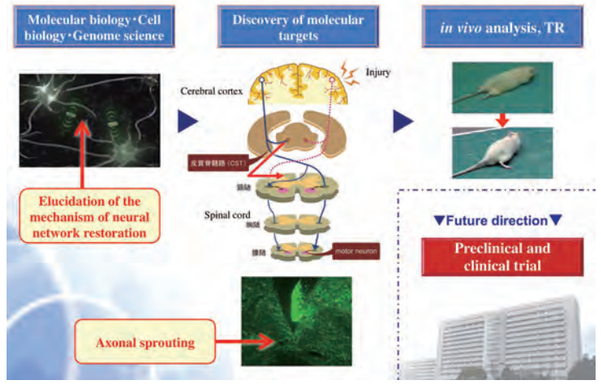



**Figure 1.**
**The mechanism of spontaneous functional recovery.**

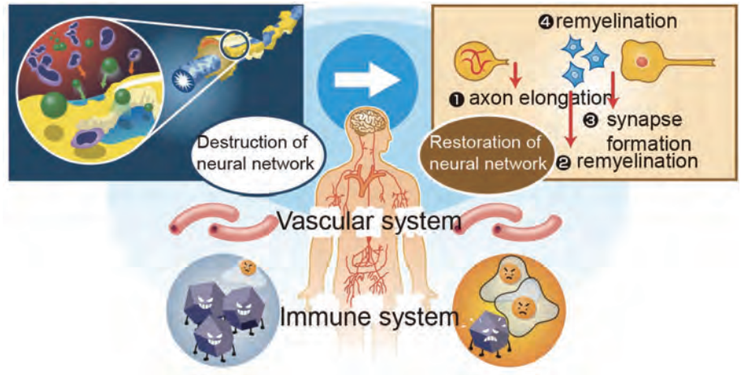



**Figure 2.**
**Biological systems that regulate rewiring of neural network after CNS injury.**

## Recent Publications


- Tanabe S & Yamashita T. B-1a lymphocytes promote oligodendrogenesis during brain development. Nat. Neurosci. 21, 506-516 (2018).
- Kuroda M, Muramatsu R, Maedera N, Koyama Y, Hamaguchi M, Fujishima H, Yoshida M, Konishi M, Itoh N, Mochizuki H & Yamashita T. Promotion of central nervous system remyelination by peripheral FGF21. J. Clin. Invest. 127, 3496-3509 (2017).
- Fujita Y, Masuda K, Nakato R, Katou Y, Tanaka T, Nakayama M, Takao K, Miyakawa T, Shirahige K & Yamashita T. Cohesin regulates formation of neuronal networks in the brain. J. Exp. Med. 214, 1431-1452 (2017).
- Fujitani M, Zhang S, Fujiki R, Fujihara Y & Yamashita T. A chromosome 16p13.11 microduplication causes hyperactivity through dysregulation of miR-484/protocadherin-19 signaling. Mol. Psychiatry 22, 364-374 (2017).
- Hayano Y, Takasu K, Koyama Y, Ogawa K, Minami K, Asaki T, Kitada K, Kuwabara S & Yamashita T. Dorsal horn interneuron-derived Netrin-4 contributes to spinal sensitization in chronic pain via Unc5B. J. Exp. Med. 213, 2949-2966 (2016).

# Molecular Immunology

## Sho Yamasaki, PhD

| Professor | Sho Yamasaki |
|---|---|
| Assistant Professor | **Eri Ishikawa** |
| | **Chihiro Motozono** |
| Postdoctoral Fellow | **2** |
| Research Assistant | **6** |
| Visiting Scientist | **2** |
| Support Staff | **2** |

Our bodies are continuously exposed to various insults caused by infections and stresses, most of which are primarily sensed by immune receptors to maintain tissue homeostasis. However, the molecular mechanism by which these receptors discriminate diverse insults to elicit suitable immune responses remains elusive. To illustrate the molecular basis behind this regulation, our laboratory focuses on the following areas; 1) immune sensing of internal and external insults via C-type lectin receptors, 2) T cell responses induced by self-peptides, and 3) innate T cell subsets related to autoimmune diseases.

**Spontaneous Development of Tfh Cell and Type 2 Immune Disorders by $Ca^{2+}$ deficiency**

Appropriate T cell responses are controlled by strict balance between activatory and inhibitory pathways downstream of TCR. Although mice or humans with impaired TCR signaling develop autoimmunity, the precise molecular mechanisms linking reduced TCR signaling to autoimmunity are not fully understood. Engagement of TCR activates $Ca^{2+}$ signaling mainly through store- operated $Ca^{2+}$ entry activated by stromal interaction molecule (Stim) 1 and Stim2. Despite defective T cell activation, mice deficient in both Stim1 and Stim2 in T cells (conditional double knockout [cDKO]) developed lymphoproliferative disorders and skin inflammation with a concomitant increase in serum IgG1 and IgE levels. In cDKO mice, follicular helper T (Tfh) cells were dramatically increased in number, and they produced IL-4 spontaneously. These inflammatory symptoms were abolished by the deletion of IL-4 in cDKO mice. Tfh development and inflammatory symptoms in cDKO mice were abrogated by further deletion of NFAT2 in T cells. These findings suggest that Tfh cells are spontaneously developed in the absence of $Ca^{2+}$ signaling and caused unregulated type 2 responses.

**Identification of antagonistic ligands for CLRs in invasive bacteria**

Group A *Streptococcus* (GAS) causes invasive streptococcal infections in humans, resulting in high mortality. Thus, GAS is also known as "killer bacteria" or "flesh-eating bacteria." The mechanism by which the immune system recognizes this potent pathogen remains elusive. In this study, we showed that the innate immune receptor Mincle (macrophage inducible C-type lectin) plays pivotal roles against invasive GAS infection through the recognition of monoglucosyldiacylglycerol (MGDG), a component of the lipoteichoic acid anchor. MGDG induced proinflammatory cytokines, ROS, and NO production in a Mincle-dependent manner. In an invasive GAS infection model, Mincle-deficient mice exhibited severe bacteremia and rapid lethality. GAS also possesses another Mincle ligand, diglucosyldiacylglycerol; however, this glycolipid interfered with MGDG-induced activation. These results indicate that Mincle plays a central role in protective immunity against acute GAS infection.

**Discovery of immunostimulatory glycolipids in Salmonella reveals functional convergence with mycobacteria**

Salmonella species are among the world's most prevalent pathogens. Because the cell wall interfaces with the host, we designed a lipidomics approach to reveal pathogen-specific cell wall compounds. Among the molecules differentially expressed between *Salmonella Paratyphi* and *S. Typhi*, we focused on lipids that are enriched in *S. Typhi*, because it causes typhoid fever. We discovered a previously unknown family of trehalose phospholipids, 6,6′-diphosphatidyltrehalose (diPT) and 6-phosphatidyltrehalose (PT). Cardiolipin synthase B (ClsB) is essential for PT and diPT, but not for cardiolipin biosynthesis. Chemotyping outperformed clsB homology analysis in evaluating synthesis of diPT. DiPT is restricted to a subset of Gram-negative bacteria: large amounts are produced by *S. Typhi*, lower amounts by other pathogens, and variable amounts by *Escherichia coli* strains. DiPT activates Mincle, a macrophage activating receptor that also recognizes mycobacterial cord factor (6,6′-trehalose dimycolate). Thus, Gram-negative bacteria show convergent function with mycobacteria. Overall, we discovered a previously unknown immunostimulant that is selectively expressed among medically important bacterial species.

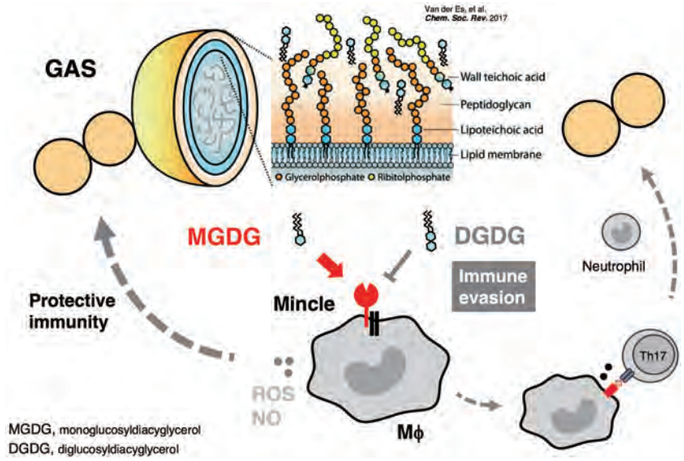
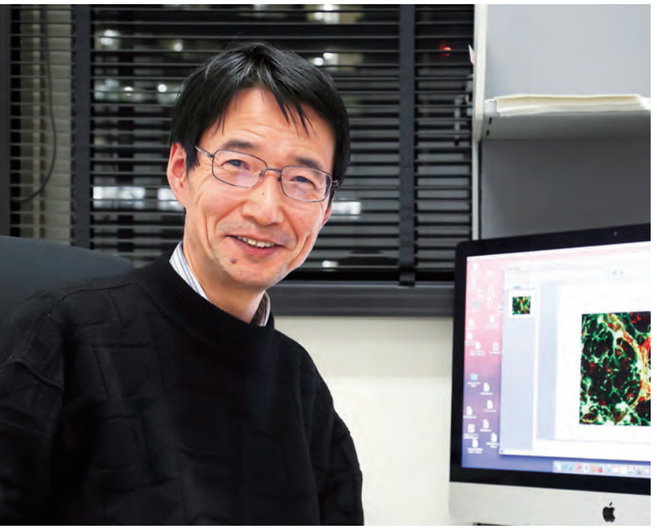



**Figure.**
**GAS generates antagonistic glycolipids to evade host immunity.**

## Recent Publications


- Nagata M, Izumi Y, Ishikawa E, Kiyotake R, Doi R, Iwai S, Omahdi Z, Yamaji T, Miyamoto T, Bamba T, Yamasaki S. Intracellular metabolite β-glucosylceramide is an endogenous Mincle ligand possessing immunostimulatory activity. Proc. Natl. Acad. Sci. USA.114, E3285-E3294 (2017).
- Imai T, Matsumura T, Mayer-Lambertz S, Wells CA, Ishikawa E, Butcher SK, Barnett TC, Walker MJ, Imamura A, Ishida H, Ikebe T, Miyamoto T, Ato M, Ohga S, Lepenies B, van Sorge NM, Yamasaki S. Lipoteichoic acid anchor triggers Mincle to drive protective immunity against invasive group A Streptococcus infection. Proc. Natl. Acad. Sci. USA. 115, E10662-E10671 (2018).
- Toyonaga K, Torigoe S, Motomura Y, Kamichi T, Hayashi JM, Morita YS, Noguchi N, Chuma Y, Kiyohara H, Matsuo K, Tanaka H, Nakagawa Y, Sakuma T, Ohmuraya M, Yamamoto T, Umemura M, Matsuzaki G, Yoshikai Y, Yano I, Miyamoto T, Yamasaki S. C-Type Lectin Receptor DCAR Recognizes Mycobacterial Phosphatidyl-Inositol Mannosides to Promote a Th1 Response during Infection. Immunity 45, 1245-57 (2016).
- Ishikawa E, Kosako H, Yasuda T, Ohmuraya M, Araki K, Kurosaki T, Saito T, Yamasaki S. Protein kinase D regulates positive selection of CD4(+) thymocytes through phosphorylation of SHP-1. Nat. Commun. 7, 12756 (2016).
- Yonekawa A, Saijo S, Hoshino Y, Miyake Y, Ishikawa E, Suzukawa M, Inoue H, Tanaka M, Yoneyama M, Oh-Hora M, Akashi K, Yamasaki S. Dectin-2 is a direct receptor for mannose-capped lipoarabinomannan of mycobacteria. Immunity 41, 402-13 (2014).

# Stem Cell Biology and Developmental Immunology



## Takashi Nagasawa, MD/PhD

| Professor | Takashi Nagasawa |
|---|---|
| Associate Professor | Tatsuki Sugiyama |
| Assistant Professor | Yoshiki omatsu |
| | Masanari Seike |
| Support Staff | 1 |

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

Additionally, we identified a population of reticular cells expressing CXCL12 at high levels, termed CXCL12-abundant reticular (CAR) cells within bone marrow (*Immunity* 2006), and indicated that CAR cells are adipo-osteogenic progenitors and the major producer of CXCL12 and SCF, creating the special microenvironment (niche) for HSCs and B cells (*Immunity* 2010). We found that the transcription factor Foxc1 was preferentially expressed in CAR cells in the marrow, enhancing CXCL12 and SCF expression and was essential for inhibiting adipogenic processes in CAR cell progenitors, and development and maintenance of niches for HSCs and immune cells (*Nature* 2014). Subsequently, we found that the transcription factor Ebf3 was also preferentially expressed in CAR cells in the bone marrow. We generated mice lacking both Ebf3 and Ebf1 and found that CXCL12 and SCF expression and hematopoietic stem cell and progenitor cell (HSPC) niche function of CAR cells were markedly impaired with depleted HSCs in infant marrow of the mutants, and the mutants became progressively more osteosclerotic, leading to the complete occlusion of marrow cavities in early adulthood. Thus, Ebf3/Ebf1 are essential for inhibiting osteoblast differentiation of CAR cells, bone marrow cavity formation, and development and maintenance of niches for HSCs and immune cells, enhancing CXCL12 and SCF expression (*Genes Dev* 2018). We are studying the roles of CXCL12-CXCR4 signaling and CAR cells in the spatiotemporal regulation of lymphohematopoiesis during homeostasis and diseases, including chronic myeloid leukemia (CML).

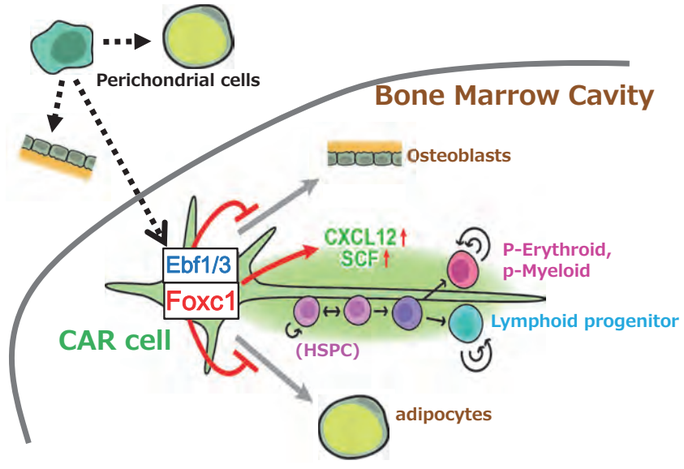



**Figure.**
**The development and functions of CAR cells.**
**The transcription factors Foxc1 and Ebf3, and HSC niche factors CXCL12 and SCF were preferentially and abundantly expressed in CAR cells within the bone marrow.**

## Recent Publications


- Agarwal P, Isringhausen S, Li H, Paterson AJ, He J, Gomariz Á, Nagasawa T, Nombela-Arrieta C and Bhatia R. Mesenchymal Niche-Specific Expression of Cxcl12 Controls Quiescence of Treatment-Resistant Leukemia Stem Cells. Cell Stem Cell. pii: S1934-5909(19)30070-0. 2019 Mar 14.
- Mizuhashi K, Ono W, Matsushita Y, Sakagami N, Takahashi A, Saunders TL, Nagasawa T, Kronenberg HM, and Ono N. Resting zone of the growth plate houses a unique class of skeletal stem cells. Nature. 563(7730), 254-258 (2018).
- Sugiyama T, Omatsu Y and Nagasawa T. Niches for hematopoietic stem cells and immune cell progenitors. Int. Immunol. 31(1), 5-11 (2018).
- Gomariz A, Helbling PM, Isringhausen S, Suessbier U, Becker A, Boss A, Nagasawa T, Paul G, Goksel O, Székely G, Stoma S, Nørrelykke SF, Manz MG and Nombela-Arrieta C. Quantitative spatial analysis of haematopoiesis-regulating stromal cells in the bone marrow microenvironment by 3D microscopy. Nat. Commun. 9(1), 2532 (2018).
- Koga S, Hozumi K, Hirano KI, Yazawa M, Terooatea T, Minoda A, Nagasawa T, Koyasu S and Moro K. Peripheral PDGFRα+gp38+ mesenchymal cells support the differentiation of fetal liver-derived ILC2. J. Exp. Med. 215(6), 1609-1626 (2018).

# Aging Biology

## Eiji Hara, PhD

| Professor | Eiji Hara |
|---|---|
| Associate Professor | Sugiko Watanabe |
| Assistant Professor | Shimpei Kawamoto |
| Postdoctoral Fellow | 3 |
| Research Assistant | 3 |
| Support Staff | 3 |

It has become apparent that aging has a major impact on the incidence of cancers. However, the underlying mechanisms are unclear. We think that cellular senescence plays a key role. In our laboratory, we are aiming to understand the roles and mechanisms of cellular senescence in vivo. We believe that understanding the molecular mechanisms underlying cellular senescence in vivo will provide valuable insight into the development of aging-associated diseases such as cancer, and open up new possibilities for their control.

**Exploring the physiological roles and mechanisms underlying cellular senescence in vivo**

Cellular senescence is the state of irreversible cell cycle arrest that can be induced by a variety of potentially oncogenic stimuli and has, therefore, long been considered to suppress tumorigenesis. We reported that $p16^{INK4a}$ and $p21^{Waf1/Cip1}$, both cyclin-dependent kinase inhibitors, play crucial roles in both the onset and establishment of cellular senescence in cell culture and in mouse models. Recently, we generated transgenic mice expressing firefly luciferase under the control of the $p16^{INK4a}$ or $p21^{Waf1/Cip1}$gene promoters. Using these senescence response reporter mice in combination with knockout mice, we are investigating the timing and, hence, the likely roles and mechanisms, of cellular senescence in vivo.

**Understanding the molecular mechanisms underlying inflammatory diseases induced by senescence-associated secretory phenotypes (SASPs)**

In addition to stable cell cycle arrest, senescent cells also develop senescence-associated secretory phenotypes (SASPs), which contribute both positively and negatively to the onset of inflammatory diseases such as cancer (depending on the biological context). Despite considerable progress in understanding the biological roles of SASPs, far less is known about how they are induced. Thus, a greater understanding of the underlying molecular mechanisms will lead to novel therapeutic strategies for various aging-associated diseases, including cancer. Similar to aging, obesity is associated with cancer. However, the underlying mechanisms are not well understood. Recently, we traced the association between obesity and increased cancer risk to gut microbiota communities that produce DNA-damaging bile acid. We found that DNA-dam aging bile acid promotes development of obesity-associated liver cancer by inducing SASPs in hepatic stellate cells. We are now focusing on the potential clinical implications of these findings.

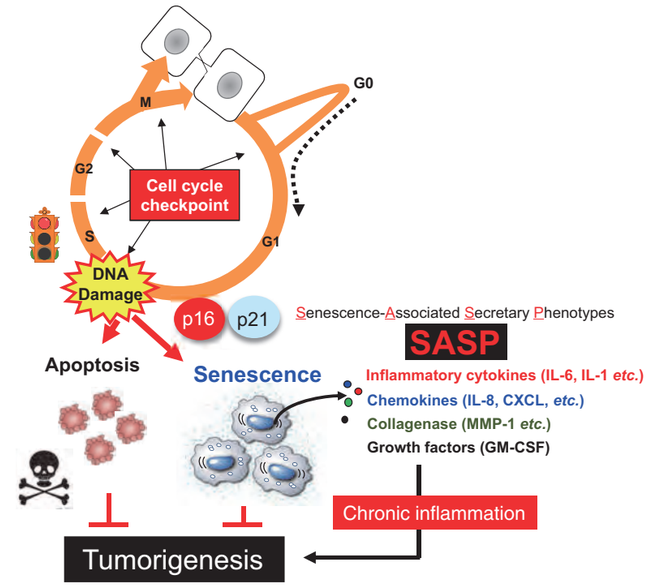



**Figure 1.**
**Cellular senescence initially inhibits proliferation of damaged cells, thereby acting as a fail-safe mechanism. However, in the long term, senescent cells may eventually promote tumorigenesis via SASPs.**

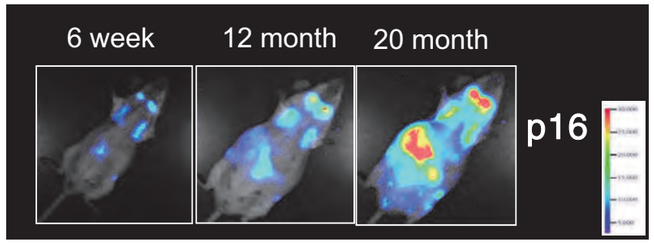
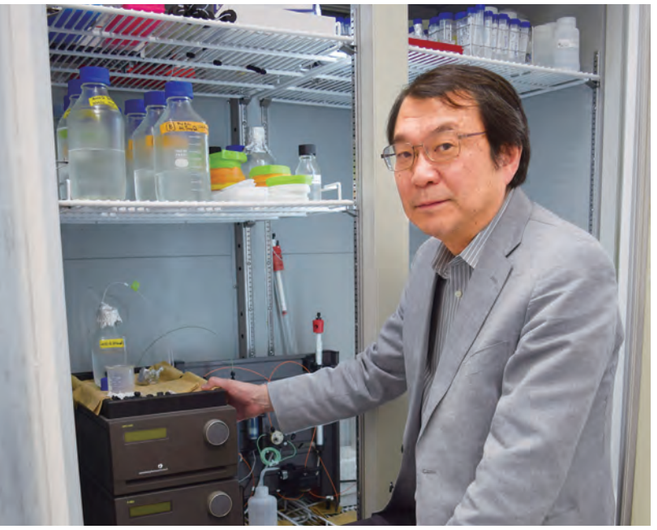



**Figure 2.**
**Real-time bioluminescence imaging of $p16^{INK4a}$ gene expression during aging. (*Journal of Cell Biology* 186, 393-407. 2009).**

## Recent Publications


- Takahashi A., et al. Downregulation of cytoplasmic DNases is implicated in cytoplasmic DNA accumulation and SASP in senescent cells. Nat. Commun. 9, 1249 (2018).
- Okuma A, Hanyu A, Watanabe S, Hara E. $p16^{Ink4a}$ and $p21^{Cip1/Waf1}$ promote tumour growth by enhancing myeloid-derived suppressor cells chemotaxis. Nat. Commun. 8, 2050 (2017).
- Takahashi A., et al. Exosomes maintain cellular homeostasis by excreting harmful DNA from cells. Nat Commun. 8, 15287 (2017).
- Takasugi M., et al. Small extracellular vesicles secreted from senescent cells promote cancer cell proliferation through EphA2. Nat. Commun. 8, 15728 (2017).

# Oncogene Research



## Masato Okada, PhD

| Professor | Masato Okada |
|---|---|
| Associate Professor | **Shigeyuki Nada** |
| | **Norikazu Yabuta** |
| Assistant Professor | **Kentaro Kajiwara** |
| | **(S.A.) Tetsuya Kimura** |
| Postdoctoral Fellow | **1** |
| Research Assistant | **3** |
| Support Staff | **1** |

**Role of Src tyrosine kinase in tumor progression**

We have investigated the role of Src tyrosine kinase in tumor progression. Src is the first-identified oncogenic tyrosine kinase, but no significant mutation of the *SRC* gene occurs in any type of human cancer. Nonetheless, the function of Src is frequently upregulated in various malignant cancers, and it is appreciated that upregulated Src plays a crucial role in tumor progression, particularly in the acquisition of invasive and metastatic features. To elucidate the molecular mechanisms underlying the upregulation of Src, we investigated the regulatory mechanism of *SRC* gene expression and searched for Src-activating factors. We found that TGF-β treatment directly induces *SRC* gene expression via the Smad pathway in some epithelial cells, and determined the promoter and enhancer regions located upstream of the *SRC* gene. The upregulation of Src contributes to the progression of TGF-β–induced epithelial-mesenchymal transition. We also found that TGF-β induces Src activation even in mesenchymal cells, such as trabecular meshwork cells and fibroblasts, by promoting the expression of Src scaffold proteins, such as CasL. In addition, we identified a Src-activating membrane glycoprotein (temporarily named Rsp1) in lipid rafts of epithelial cells. Upregulation of Rsp1 induces prominent activation of Src and the STAT3 pathway, which promotes the invasive activity of epithelial cells. We also found that ablation of Rsp1 inhibits HGF-induced morphological changes and cell growth, and attenuates membrane presentation of MET, resulting in inhibition of invasive activity induced by HGF. These findings suggest that Rsp1 is a co-receptor of MET and thereby stabilizes its signaling (Figure 1). Furthermore, we have recently found that ablation of Rsp1 suppresses the compensatory renal hypertrophy, indicating that Rsp1 is required for the HGF-MET signaling even in vivo. Rsp1 and MET are crucial for promoting cancer cell invasion; therefore, we expect this study to identify a potential therapeutic target in some types of cancer.

**Role of p18 in the regulation of mTORC1 nutrient signaling**

During analysis of the function of Src in the regulation of growth factor signaling, we identified a new Src substrate termed p18/Lamtor1, which exclusively localizes to lipid rafts of lysosomes. Subsequent analysis revealed that p18 functions by forming a hetero-heptamer complex (Ragulator), consisting of p18, p14, MP1, HBXIP, p10, and RagA/C, and it is required for activation of mTORC1 on lysosomes. Conditional KO of p18 in the epidermis showed that p18-mTORC1 is crucial not only for anabolism of bio-materials, but also for catabolism via autophagy, indicating that p18 is tightly associated with the regulation of mTORC1 nutrient signaling in vivo. Furthermore, collaborative studies of the immune system revealed that p18 is required for polarization of M2 macrophages by integrating cytokine and amino acid signaling, and for proliferation of $CD4^+$ T cells and the suppressive function of regulatory T cells. These findings underscore the critical role of p18 in the regulation of metabolic homeostasis in various tissues and cells. We are now analyzing the role of p18 in the intestinal tract by generating epithelia-specific p18 KO mice. To further analyze the regulation of the p18 complex at the molecular level, we recently determined the crystal structure of Ragulator. This revealed that p18 wraps around the other components of Ragulator and provided significant insights into the role of p18-mediated regulation of mTORC1 on lysosomes. Recent analysis using p18 KO cells that lack regulatory components of Rag GTPase, such as GATOR1 and FLCN, showed that p18-Ragulator complex provides a regulatory platform that is indispensable for amino acid-dependent regulation of mTORC1 (Figure 2). These findings identified the interacting molecular surface as a potential therapeutic target in lifestyle diseases, such as diabetes mellitus and cancer, both of which are linked to dysfunction of the mTORC1 pathway.

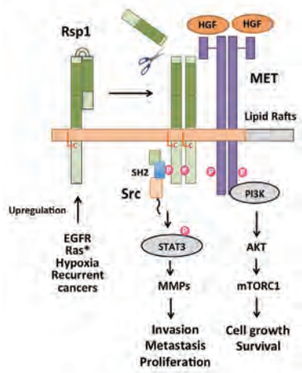



**Figure 1.**
**Role of Rsp1 in the Src-mediated growth factor signaling.**

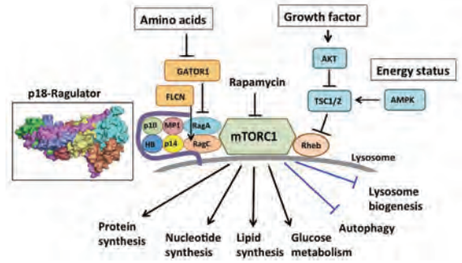
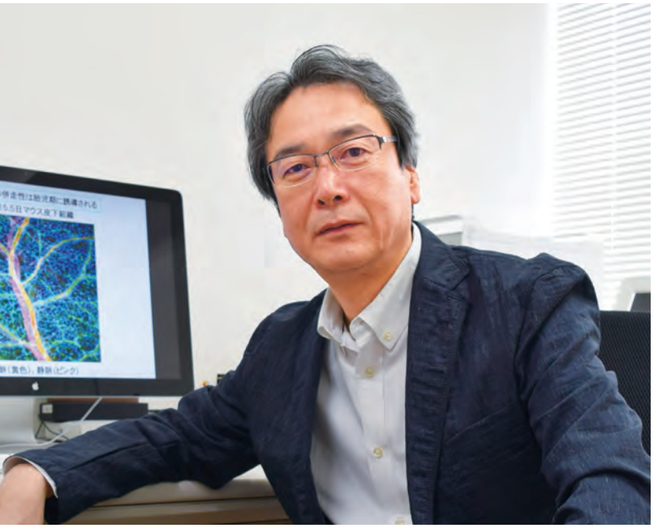



**Figure 2.**
**Molecular basis for the regulation of mTORC1 on lysosomes.**

## Recent Publications


- Tsukamoto T, Kajiwara K, Nada S, Okada M, Src mediates TGF-β-induced intraocular pressure elevation in glaucoma. J. Cell Physiol. 234, 1730-1744 (2019).
- Kimura T, Kumanogoh A, Okada M. Roles of Lamtor1 in Macrophages, CD4+ T-cells, and Regulatory T-cells. Crit. Rev. Immunol. 38, 403-414 (2018).
- Hayama Y, Kimura T, Takeda Y, Nada S, Koyama S, Takamatsu H, Kang S, Ito D, Maeda Y, Nishide M, Nojima S, Sarashina-Kida H, Hosokawa T, Kinehara Y, Kato Y, Nakatani T, Nakanishi Y, Tsuda T, Koba T, Okada M, Kumanogoh A. Lysosomal Protein Lamtor1 Controls Innate Immune Responses via Nuclear Translocation of Transcription Factor EB. J. Immunol. 200, 3790-3800 (2018).
- Yonehara R, Nada S, Nakai T, Nakai M, Kitamura A, Ogawa A, Nakatsumi H, Nakayama KI, Li S, Standley DM, Yamashita E, Nakagawa A, Okada M. Structural basis for the assembly of the Ragulator-Rag GTPase complex. Nat. Commun. 8, 1625 (2017).
- Kimura T, Nada S, Takegahara N, Okuno T, Nojima S, Kang S, Ito D, Morimoto K, Hosokawa T, Hayama Y, Mitsui Y, Sakurai N, Sarashina-Kida H, Nishide M, Maeda Y, Takamatsu H, Okuzaki D, Yamada M, Okada M, Kumanogoh A. Polarization of M2 macrophages requires Lamtor1 that integrates cytokine and amino-acid signals. Nat. Commun. 7, 13130 (2016).

# Signal Transduction



## Nobuyuki Takakura, PhD

| Professor | Nobuyuki Takakura |
|---|---|
| Associate Professor | **Hisamichi Naito** |
| Assistant Professor | **Hiroyasu Kidoya** |
| Postdoctoral Fellow | **3** |
| Research Assistant | **4** |
| Support Staff | **1** |

Our research team is involved in studies in terms of vascular biology and stem cell biology. Among our achievements in 2018, two interesting results are shown here.

**How hematopoietic stem cells undergo dormant status in bone marrow**

The balance between self-renewal and differentiation of hematopoietic stem and progenitor cells (HSPCs) maintains hematopoietic homeostasis, failure of which can lead to hematopoietic disorder. The fate of HSPCs is controlled by signals from the bone marrow niche resulting in alteration of the stem cell transcription network. Regnase-1, a member of the CCCH zinc finger protein family possessing RNAse activity, mediates post-transcriptional regulatory activity through degradation of target mRNAs. The precise function of Regnase-1 has been explored in inflammation-related cytokine expression but its function in hematopoiesis has not been elucidated. Here, we found that Regnase-1 regulates self-renewal of HSPCs through modulating the stability of *Gata2* and *Tal1* mRNA. In addition, we clarified that dysfunction of Regnase-1 leads to the rapid onset of abnormal hematopoiesis. Thus, our data reveal that Regnase-1-mediated post-transcriptional regulation is required for HSPC maintenance and suggest that it represents a leukemia tumor suppressor (Figure 1; Kidoya et al. Nature Commun. 2019).

**How we prevent vascular damage from toxic factor developed by microbiota**

TNFα is a pleiotropic cytokine which has potential to induce apoptosis under inflammation. How endothelial cells (ECs) are spared from this fate in inflammatory environments where TNFα is present is not known. Here, we found that TGFβ-activated kinase 1 (TAK1) ensures EC survival and maintains vascular integrity upon TNFα stimulation. Endothelial-specific TAK1 knockout mice exhibited intestinal and liver hemorrhage due to EC apoptosis, leading to vascular destruction and rapid death. This EC apoptosis was induced by TNFα from myeloid cells (possibly macrophages) responding to intestinal microbiota. TNFα secretion associated with inflammation also induced vascular defects in inflamed organs. Additionally, we determined that TAK1 deletion in tumor ECs resulted in blood vessel and hence tumor regression. Our results illuminate mechanisms ensuring survival of intestinal and liver ECs under physiological conditions and ECs of other organs under inflammatory conditions that could be exploited for anti-angiogenic therapy to treat cancer (Figure 2; Naito et al. Dev Cell 2019).

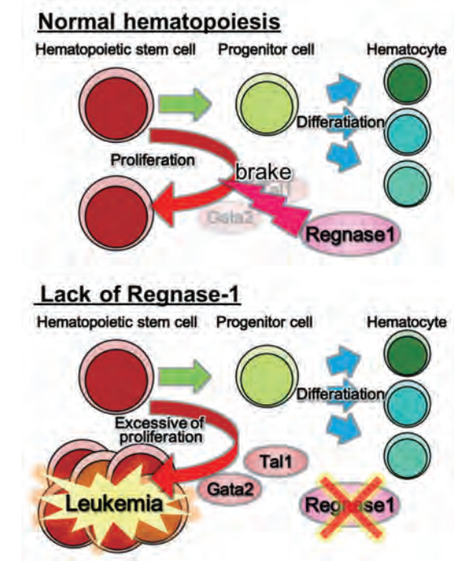



**Figure 1.**
**Involvement of Regnase-1 in the dormancy of hematopoietic stem cells.**

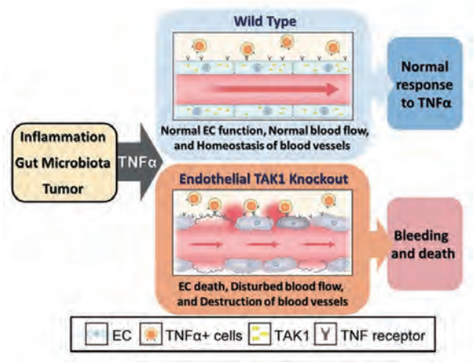



**Figure 2.**
**TAK1 inhibits apoptosis of endothelial cells.**

## Recent Publications


- Kidoya H, Muramatsu F, Shimamura T, Jia W, Satoh T, Hayashi Y, Naito H, Kunisaki Y, Arai F, Seki M, Suzuki Y, Osawa T, Akira S, Takakura N. Regnase-1-mediated post-transcriptional regulation is essential for hematopoietic stem and progenitor cell homeostasis. Nat. Commun. 10, 1072 (2019).
- Naito H, Iba T, Wakabayashi T, Tai-Nagara I, Suehiro JI, Jia W, Eino D, Sakimoto S, Muramatsu F, Kidoya H, Sakurai H, Satoh T, Akira S, Kubota Y, Takakura N. TAK1 prevents endothelial apoptosis and maintains vascular integrity. Dev. Cell 48, 151-166 (2019).
- Eino D, Tsukada Y, Naito H, Kanemura Y, Iba T, Wakabayashi T, Muramatsu F, Kidoya H, Arita H, Kagawa N, Fujimoto Y, Takara K, Kishima H, Takakura N. LPA4-mediated vascular network formation increases the efficacy of anti-PD-1 therapy against brain tumors. Cancer Res. 78, 6607-6620 (2018).
- Wakabayashi T, Naito H, Suehiro JI, Lin Y, Kawaji H, Iba T, Kouno T, Ishikawa-Kato S, Furuno M, Takara K, Muramatsu F, Weizhen J, Kidoya H, Ishihara K, Hayashizaki Y, Nishida K, Yoder MC, Takakura N. CD157 marks tissue-resident endothelial stem cells with homeostatic and regenerative properties. Cell Stem Cell 22, 384-397 (2018).
- Takara K, Eino D, Ando K, Yasuda D, Naito H, Tsukada Y, Iba T, Wakabayashi T, Muramatsu F, Kidoya H, Fukuhara S, Mochizuki N, Ishii S, Kishima H, Takakura N. Lysophosphatidic acid receptor 4 activation augments drug delivery in tumors by tightening endothelial cell-cell contact. Cell Rep. 20, 2072-2086 (2017).

# Single Molecule Imaging

**Toshio Yanagida, PhD**
**Ben Seymour, MD/PhD**

| Professor | Toshio Yanagida<br>Ben Seymour |
|---|---|
| Research Assistant | 1 |

**Recent advances in imaging methodologies provide a unique opportunity to interrogate biological process at multiple levels. Our goal is to integrate imaging methods across levels and species, with new intelligent approaches to data analysis, to provide translational insight into the multi-system interactions in the body that support physiological and pathophysiological functions.**

Experimentally, our research integrates hypothesis-driven and data-driven approaches to understanding how the brain responds to injury and inflammation. In our core hypothesis-driven models of the brain, we have recently showed how the ability to predict some sort of pain or injury can generalize to a range of situations – a process that we believe underlies the development of anxiety in clinical situations (Norbury et al, 2018). And in our data-driven work, we have been able to build prediction models of depression, based on brain network imaging and modelling, that span a broad range of clinical causes (depression is associated with multiple psychiatric and clinical medical disorders, not least chronic inflammation)(Yamashita et al, 2018). Together, both approaches allow us to start to build a bigger picture of depression and anxiety that can be applied across the clinical spectrum.

We used this to develop new and sophisticated models of how the brains ability for organism-level defense is organized at a whole-brain level. This has yielded the first brain-wide model of the pain system, which spans basic homeostatic responding to cognitive awareness (Seymour, 2019; Figure 1). Based on this, we are now in a position to start to propose how this system is susceptible to different causes of pain, in particular inflammatory pain.

Given the complexity of the brain, we have argued that this integrated experimental-theoretical approach requires a new, inter-disciplinary framework, spanning bioscience and engineering science, especially artificial intelligence and robotics (Figure 2). In a pair of papers, we have set out the details of how this can proceed (Lee and Seymour, 2019; Lee et al, 2019). In particular, we highlight the use of sophisticated simulation and hardware platforms to model the development of symptoms (such as fatigue and depression) and diseases (such as chronic pain after inflammation). We are now starting to take this approach to our own clinical data in patients with inflammatory joint disease.

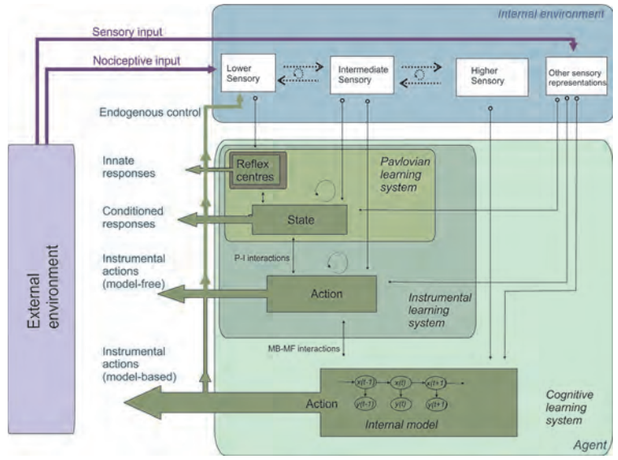



**Figure 1.**
**Model of the Pain system in the brain, showing how multiple, integrated control loops provide a robust but flexible solution to the problem of responding to pain and injury, and learning about it to provide pre-emptive responding wherever possible. This provides a sophisticated homeostatic mechanism that spans basic physiological responses to behaviour (from Seymour, 2019).**

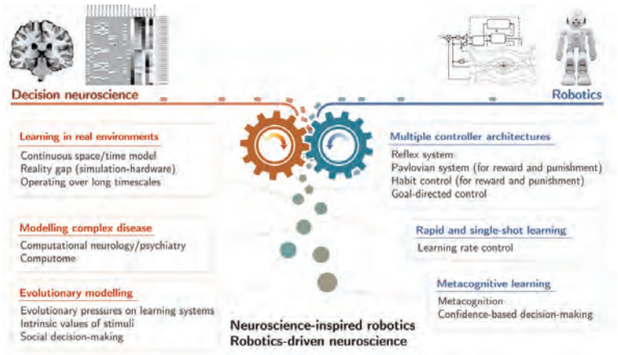
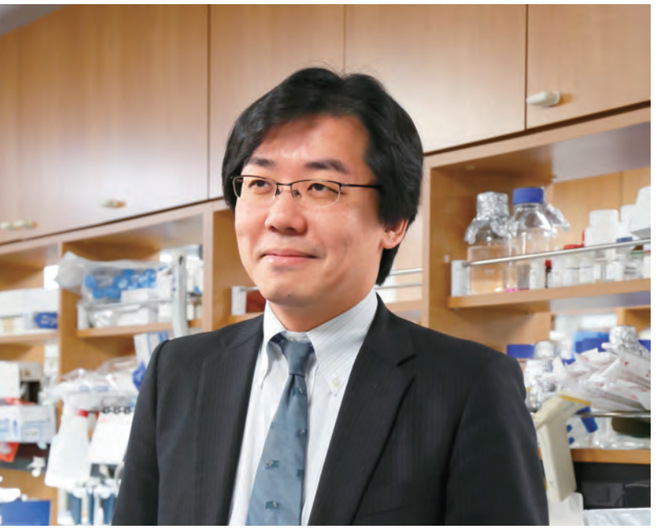



**Figure 2.**
**An overview of an inter-disciplinary approach to understanding complex decision-systems in the brain, utilizing insight from artificial intelligence and robotics (from Lee and Seymour, 2019).**

## Recent Publications


- Seymour B. Pain: A Precision Signal for Reinforcement Learning and Control. Neuron 101(6), 1029-1041 (2019).
- Lee SW, Seymour B. Decision-making in brains and robots—the case for an interdisciplinary approach. Current Opinion in Behavioral Sciences 26, 137-45 (2019).
- Abouleila Y, Onidani K, Ali A, Shoji H, Kawai T, Lim CT, Kumar V, Okaya S, Kato K, Hiyama E, Yanagida T, Masujima T, Shimizu Y, Honda K. Live single cell mass spectrometry reveals cancer-specific metabolic profiles of circulating tumor cells. Cancer Sci. 110(2), 697-706 (2019).
- Yamashita et al. A prediction model of working memory across health and psychiatric disease using whole-brain functional connectivity. eLife 7. e38844 (2018).
- Norbury A, Robbins T.W. and Seymour B. Value generalization in human avoidance learning. eLife 7, e34779 (2018).

# Immunology and Cell Biology



## Masaru Ishii, MD/PhD

| Professor | Masaru Ishii |
|---|---|
| Associate Professor | **Keizo Nishikawa** |
| Assistant Professor | **Junichi Kikuta** |
| | **Hiroki Mizuno** |
| | **Takao Sudo** |
| | **Yutaka Uchida** |
| Research Assistant | 2 |
| Visiting Scientist | 1 |
| Support Staff | 2 |

**Intravital imaging of bone cell dynamics *in vivo***

We have originally developed a novel imaging system for visualizing inside bones by using intravital multiphoton microscopy. We have succeeded in visualizing the *in vivo* behaviors of bone-resorbing macrophage, osteoclast, *i.e.*, the migration and positioning of their precursor macrophages (*Nature*, 2009, *J. Exp. Med*., 2010), their mode of bone-resorbing function *in vivo* (*J. Clin. Invest*., 2013), and the functional and physical coupling with bone-forming osteoblasts (*Nat. Commun*., 2018). Our study identified two distinct mature osteoclast (mOCs) functional states; i.e., bone-resorbing (R) mOCs firmly adhering to bones and devouring the bone matrix by secreting acids, and non-resorbing (N) mOCs relatively loosely attached and wriggling along the bone surface. In order to further analyze the actual event of bone resorption *in vivo*, in collaboration with Dr. Kikuchi, we have developed a new chemical probe for detecting proton secretion in bone resorption by mOCs (*Nat. Chem. Biol*., 2016).

We could detect an *in vivo* mode of dynamic communication between mature osteoblasts (mOBs) and mOCs, and found that the mOBs and mOCs were distributed mainly in a segregated fashion, although some direct cell-to-cell contact was detected between mOBs and mOCs in spatiotemporally limited areas. A pH-sensing fluorescence probe revealed that mOCs secreted protons for bone resorption when they were not in contact with mOBs, whereas mOCs contacting mOBs were non-resorptive, suggesting that mOBs could inhibit the bone resorption activity of mOCs by direct cell–cell contact. This study is the first to use intravital imaging techniques to reveal spatiotemporal intercellular interactions between mOBs and mOCs, thus contributing to our understanding of bone homeostasis *in vivo*.

**Intravital imaging-based investigation of pharmacological effects**

We have shown that our intravital imaging technology turned out to be a powerful tool for dissecting in vivo pharmacological actions of various drugs. Regarding the biologic agents used in clinics for treating rheumatoid arthritis, anti-IL-6R and anti-TNFα mAbs affected mOCs and switched bone-resorbing mOCs to non-resorbing cells while CTLA4-Ig had no action on mOCs but mobilized osteoclast precursors, eliminating their firm attachment to bone surfaces. Intravital imaging revealed that various biologic DMARDs acted at specific therapeutic time-points during osteoclastic bone destruction, with different efficacies. These results enable us to grasp the real modes of action of drugs, optimizing the usage of drug regimens (*Ann. Rheum*. Dis., 2018).

In terms of bisphosphonate agents used in clinics for treating osteoporosis, we investigated the short-term effect of three types of bisphosphonates, risedronate, alendronate and minodronate on controlling the bone resorptive activity of mOCs using intravital multiphoton microscopy with pH-sensing fluorescence probe. Risedronate was the most effective at increasing osteoclast motility and changing the localization of proton pumps, which led to an inhibition of bone resorption (*JBMR plus*, 2018). Together, these results demonstrate that the intravital imaging system is a useful tool for evaluating the similarities and differences in currently used anti-bone resorptive drugs.

**Application of intravital imaging techniques for dissecting human immunology**

Intravital imaging with multiphoton 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 suitable for analyzing human samples. By collaborating with companies (supported by AMED) we are developing a new microscopy system for applying human tissues and organs in vivo. Currently, we have succeeded in visualizing non-labelled normal and cancerous human tissues, which can be used for differential diagnosis (*Sci. Rep*., 2017), and will enableus to dissect human immunology in future.

Thus, the aim of our laboratory is to understand the fundamental principle controlling cellular dynamics in various kinds of tissues and organs *in vivo* (see the Figure). By means of our advanced imaging techniques, we have investigated the dynamic nature of different cell types in a time-dependent manner, in addition to the spatial and structural information.

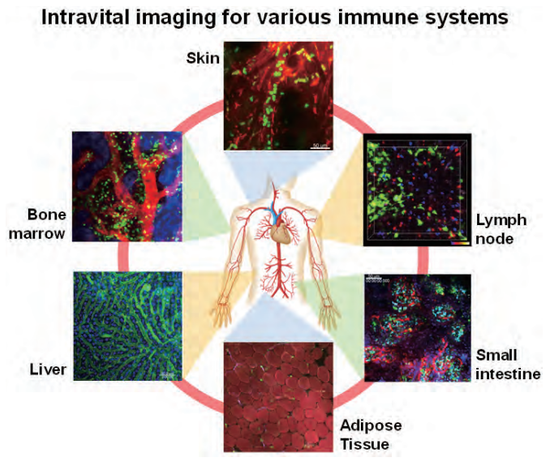



**Figure.**
**Intravital imaging for various immune systems.**
**Immune cells are high dynamic and interconnecting various tissues and organs, by forming a 'soft-wired' network. We are elucidating the basic principle controlling the dynamic nature of immune cells by visualizing *in vivo* behaviors with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

## Recent Publications


- Kikuta J., et al. Dynamic analyses of the short-term effects of different bisphosphonates using intravital two-photon microscopy. JBMR plus 22, 362-6 (2018).
- Matsuura Y., et al. *In vivo* visualization of different modes of action of biologic DMARDs inhibiting osteoclastic bone resorption. Ann. Rheum. Dis. 77, 1219-25 (2018).
- Furuya M., et al. Direct cell-cell contact between mature osteoblasts and osteoclasts dynamically controls their functions in vivo. Nat. Commun. 9, 300 (2018).
- 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. 12, 579-85 (2016).
- Iwamoto Y., et al. Intercellular communication between keratinocytes and fibroblasts induces local osteoclast differentiation: a mechanism underlying cholesteatoma-induced bone destruction. Mol. Cell Biol. 36(11), 1610-20, (2016).

# Nuclear Medicine


## Jun Hatazawa, MD/PhD

| Professor | Jun Hatazawa |
|---|---|
| Associate Professor | **Hiroki Kato** |
| Assistant Professor | **Tadashi Watabe** |
| Research Assistant | **2** |
| Support Staff | **2** |

**Background and objective**

Neuroinflammation is known to be one of the major pathological abnormalities of epilepsy. Translocator protein (TSPO) ligand PET imaging has been suggested as a functional imaging for detection of epileptogenic foci in patients with intractable epilepsy. Especially the second-generation TSPO radioligand like C-11 DPA713 is known to have high specific binding than prototypical agent PK11195. The utility of PET by such agents, however, has never be examined for pediatric patients with epilepsy. Although the quantitative parameters like distribution volume of the tracer are usually used as an index of specific binding, invasive arterial sampling and long duration scanning are necessary to calculate the parameter. The purpose of this study is to suggest the minimum invasive scan protocol of C-11 DPA713 PET to identify the lesions in pediatric patients with intractable epilepsy.

**Methods**

Ten pediatric patients (age: 13±6.4 years old, male/female: 7/3) with intractable epilepsy whose foci or cause had already been diagnosed were included in this study. (Table 1) For five patients, the epileptogenic lesions were surgically resected. All of them were proven to be seizure-free after follow-up observation of 6 months to 1.5 year. Although the remaining five patients have not undergone focus resection yet for various reasons, locations of epileptogenic zones have already been identified by surface electrical encephalography (EEG), magnetic encephalography (MEG), or other kinds of imaging.

For all patients, C-11 DPA was intravenously injected over 30 seconds by the infusion pump at the start of the scan. Administration dose of the tracer was around 7 MBq / kg. The PET images were acquired for 60 minutes by Eminence SOPHIA SET-3000 BCT/X (Shimadzu Co, Kyoto, Japan) in the three-dimensional acquisition mode. Before emission scan, transmission data using a rotating Cs-137 point source for attenuation correction was acquired. The difference in the time activity curve between the ipsilateral and contralateral volume of interest was evaluated as the asymmetry index (AI).

**Results and Summary**

As a result, all of the positive cases in F-18 FDG PET were also positive in C-11 DPA713 PET. In some cases, the epileptogenic foci were evident in C-11 DPA713 PET, while F-18 FDG PET could not visualize them clearly. We used the AI between ipsi- and contralateral areas as the distribution volume ratio for quantitative measure. The AI increased over 60 minutes after the injection in all the patients irrespective of pathological features. In the five patients, the AI reached a plateau around 60 min after the RI administration. As far as pediatric patients with epilepsy are concerned, low radiation exposure and short scan duration are required. Static scanning of C-11 DPA713 PET around 60 minutes after injection seemed to be appropriate to visualize the relative distribution volume of the PET ligand in the epileptogenic foci in pediatric cases.

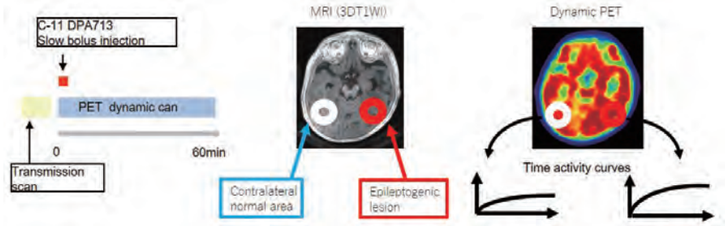



**Figure 1.**
**PET scanning and evaluation of time activity curve in the brain.**

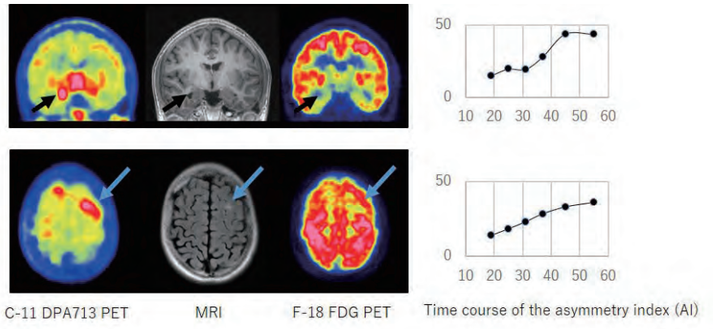



**Figure 2.**
**C-11 DPA713 PET, F-18 FDG PET, and MRI of the representative cases.**

## Recent Publications


- Watabe T, Kaneda-Nakashima K, Liu Y, Shirakami Y, Ooe K, Toyoshima A, Shimosegawa E, Fukuda M, Shinohara A, Hatazawa J. Enhancement of astatine-211 uptake via the sodium iodide symporter by the addition of ascorbic acid in targeted alpha therapy of thyroid cancer. J. Nucl. Med. pii: jnumed.118.222638 (2019).
- Kanai Y, Miyake Y, Shimosegawa E, Hatazawa J. Radiosynthesis of (11)C-phenytoin Using a DEGDEE Solvent for Clinical PET Studies. Asia Ocean J. Nucl Med. Biol. Spring 6(2), 149-154 (2018). doi: 10.22038/aojnmb.2018.10846.
- Ikeda H, Hayashi Y, Takahashi N, Watabe T, Kanai Y, Shinohara A, Kato, H,Watabe H, Shimosegawa E, Hatazawa J. Application of astatine-210: Evaluation ofastatine distribution and effect of pre-injected iodide in whole body of normalrats. Appl. Radiat. Isot. 139, 251-255 (2018). doi:10.1016/j.apradiso.2018.05.021.
- Hatazawa J. (11)C-acetate PET/CT: a potential alternative of transcranialbiopsy for grading cerebral gliomas. Eur. J. Nucl. Med. Mol. Imaging. 45(6), 1011 (2018). doi: 10.1007/s00259-018-3950-2.
- Kato H, Isohashi K, Shimosegawa E, Hatazawa J. Increase in extraction ofl-123 iomazenil in patients with chronic cerebral ischemia. PLoS One. 13(1):e0190720 (2018). doi: 10.1371/journal.pone.0190720.

# Chemical Imaging Techniques


## Kazuya Kikuchi, PhD

| Professor | Kazuya Kikuchi |
|---|---|
| Associate Professor | **Yuichiro Hori** |
| Assistant Professor | **Masafumi Minoshima** |
| Postdoctoral Fellow | **2** |
| Research Assistant | **2** |
| Support Staff | **1** |


### Perfluorocarbon-based $^{19}$F MRI Nanoprobes for In Vivo Multicolor Imaging

Multicolor imaging methods are of great importance to reveal molecular interactions in living cells or animals. The most common technique is multicolor fluorescence imaging by utilizing spectrally distinct reporters such as fluorescent proteins or fluorescent dyes. However, due to the limited light penetration, it is difficult to noninvasively visualize tissues at a depth beyond submillimeter. On the other hand, multicolor magnetic resonance imaging (MRI) probes have been developed because MRI noninvasively provides images of deep tissues with excellent soft-tissue contrast and superior spatial resolution. Especially, $^{19}$F MRI has received considerable attentions as a promising imaging modality that provides no endogenous background signal in animal bodies and the broad chemical shift range (>350 ppm) in $^{19}$F NMR spectroscopy. Recently, we developed a multifunctional PFC-based silica nanoparticle, termed FLAME (FLuorine Accumulated silica nanoparticle for MRI contrast Enhancement) as a $^{19}$F MRI contrast agent. FLAME is made up of a liquid PFC core and a stable silica shell, which can be modified with various functional groups such as small molecules or peptides in organic solvents. Here we report five types of PFC-encapsulated silica nanoparticles that show $^{19}$F NMR peaks with different chemical shifts. A series of multicolor PFC-encapsulated silica nanoparticles enabled the $^{19}$F MR imaging with triple colors in vivo.

To achieve in vivo multicolor $^{19}$F MRI based on FLAMEs, fluorine compounds should fulfill the following requirements: (1) the $^{19}$F NMR peaks of fluorine compounds do not overlap each other; (2) PFCs exist as a liquid state at the measurement temperature because $T_2$ of liquid state substances are much longer than those of solid state substances; (3) PFCs are not volatile or hydrophilic for the formulation of emulsions. In addition, the following properties are important for the ideal system: (4) PFCs exhibit the single $^{19}$F NMR peak for discrimination from the peaks of other fluorine compounds and (5) PFCs have long transverse relaxation time ($T_2$) for sensitive imaging. In this study, we prepared the following fluorine compounds: perfluoro-[15] crown-5 ether (PFCE), perfluorooctylbromide (PFOB), perfluorodichlorooctane (PFDCO), perfluorotributylamine (PFTBA), and 1,1,1-tris(perfluoro-tert-butoxymethyl)ethane (TPFBME). Considering the above properties from the $^{19}$F NMR spectra, we selected PFCE@$SiO_2$, TPFBME@$SiO_2$, and PFTBA@$SiO_2$ as the nanoprobes for triple color $^{19}$F MRI (Figure 1, left).

Then we conducted the multicolor $^{19}$F MR imaging using PFCE@$SiO_2$, TPFBME@$SiO_2$, and PFTBA@$SiO_2$ in 384-well microplate at the different concentrations. For multicolor $^{19}$F MR imaging, the center frequencies of the TPFBME peak (at approximately $\delta$ = 3.3 ppm), PFTBA peak (at approximately $\delta$ = –53.0 ppm), and PFCE peak (at approximately $\delta$ = –16.4 ppm) were chosen as the frequencies for radiofrequency (RF) output. Then, we acquired the three $^{19}$F MR images by exciting the peaks at the above three chemical shift values, and assigned three pseudocolors to each nanoprobe. To test the multicolor $^{19}$F MRI in vivo, we subcutaneously injected the nanoprobes ($C_{PFC}$ = 10 mM, 25 μL) to indicated sites in a living mouse. Then, the sequential $^{19}$F MR images were acquired using the same frequencies for RF output as the measurements of phantom images. The clear multiplexed $^{19}$F MRI signals of PFCE@$SiO_2$, TPFBME@$SiO_2$, and PFTBA@$SiO_2$ were observed at each injected site on sagittal and coronal MR scans, respectively (Figure 1, middle). This result demonstrates the feasibility of in vivo multicolor $^{19}$F MRI using spectrally distinct PFC-encapsulated nanoprobes.

Furthermore, our in vivo multicolor imaging could be utilized for evaluating the effect of surface functional groups on the hepatic uptake in a mouse (Figure 1, right). These multicolor nanoprobes could be applied to investigate the delivery of nanoparticles with various functional groups not only to liver but also to other organs. In future studies, we will address the development of multicolor PFC-encapsulated silica nanoparticles with OFF/ON-switching ability in response to various stimuli such as enzyme activity, hypoxia, or pH variation. This is expected to be helpful for analyzing the dynamics of multiple enzymes or relationships of enzymes to diverse biological phenomena.

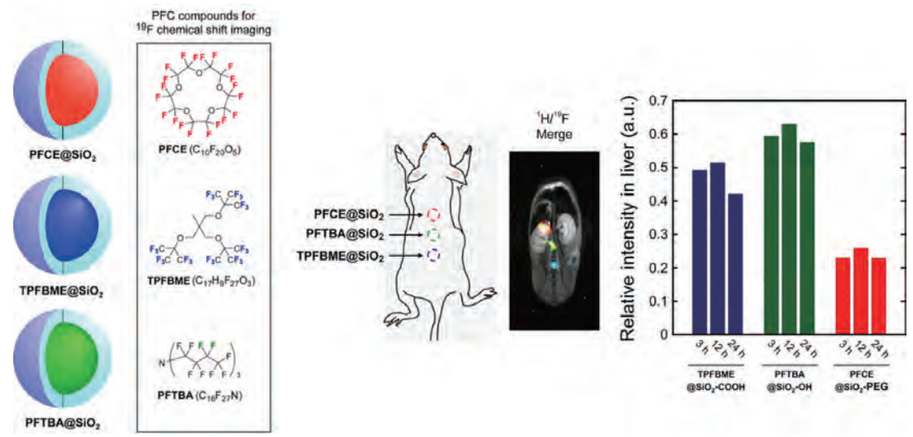
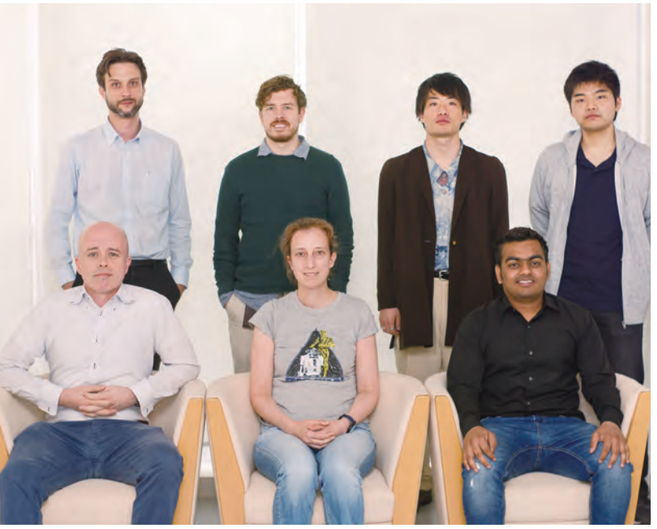



**Figure.**
**(left) Diagram of PFCE@$SiO_2$, TPFBME@$SiO_2$, and PFTBA@$SiO_2$ for multicolor $^{19}$F MRI. (middle) $^{19}$F MR image in a living mouse after injection of multicolor $^{19}$F MRI nanoprobes.**
**(right) Evaluation of hepatic uptake in multicolor nanoprobes with different surface modifications (TPFBME@$SiO_2$-COOH, PFTBA@$SiO_2$-OH, and PFCE@$SiO_2$-PEG).**

## Recent Publications


- Akazawa K, Sugihara F, Nakamura T, Matsushita H, Mukai H, Akimoto R, Minoshima M, Mizukami S & Kikuchi K. Perfluorocarbon-Based $^{19}$F MRI Nanoprobes for In Vivo Multicolor Imaging. Angew. Chem. Int. Ed. 57, 16742-16747 (2018).
- Akazawa K, Sugihara F, Minoshima M, Mizukami S & Kikuchi K. Sensing Caspase-1 Activity Using Activatable $^{19}$F MRI Nanoprobes with Improved Turn-on Kinetics. Chem. Commun. 54, 11785-11788 (2018).
- Akazawa K, Sugihara F, Nakamura T, Mizukami S & Kikuchi K. Highly Sensitive Detection of Caspase-3/7 Activity in Living Mice Using Enzyme-responsive $^{19}$F MRI Nanoprobes. Bioconjugate Chem. 29, 1720-1728 (2018).
- Hori Y, Otomura N, Nishida A, Nishiura M, Umeno M, Suetake I & Kikuchi K. Synthetic-Molecule/Protein Hybrid Probe with Fluorogenic Switch for Live-Cell Imaging of DNA Methylation. J. Am. Chem. Soc. 140, 1686-1690 (2018).

# Biophotonics



## Nicholas Isaac Smith, PhD

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

The biophotonics laboratory develops tools for label-free analysis of single cells. Single-cell analysis is a popular target for a large number of researchers, usually pursued by labeling surface markers, by introducing fluorescent dyes into the cell, or by invasive, yet comprehensive, techniques such as single cell RNA sequencing. In contrast, our tools are based on label-free optical methods, which aim to produce some of the same discriminatory capability as the more invasive methods. Additionally, label-free methods are based on endogenous contrasts of the cell, and can also find novel features that can be used to discriminate between cell phenotypes or cell states.

Our optical techniques can be used to evaluate the phenotypes or activation states of T cells, B cells, and macrophage cells. We showed that Raman spectroscopy and quantitative phase imaging can both be used to evaluate whether individual macrophages had been exposed to LPS with a high degree of accuracy (Pavillon et al 2018). The Raman spectroscopic characterization of the cells is based on their endogenous molecular content, which we refer to as the molecular phenotype. The quantitative phase imaging provides an independent, complementary set of cell morphology-related data that can be taken at the same time as the Raman spectroscopic measurements. Autofluorescence signals from the cells are also known to be related to cellular activation/metabolism, and in our methods, autofluorescence images can additionally be taken for each single cell, providing a total of three different independent label-free modes that provide information that can be used to discriminate the activation state. Cells express a variety of molecules on activation, which can be detected by the Raman mode, while cell morphology is observed to change following activation, which can be detected by the quantitative phase imaging. Finally, autofluorescence compounds change in abundance or in distribution throughout the cell, which can be detected in the autofluorescence image data. The results from these developments showed that it is possible to use this label-free information to determine the activation states in macrophages with an accuracy that appears higher than some of the gold standard methods such as labeling of iNOS signals, and without perturbing the cells.

We also found that during inhibition of the macrophage LPS response, macrophages inhibited by progesterone showed different (instead of simply weaker) responses to LPS when studied by Raman analysis. This indicates that the Raman measurements have the ability to detect not only the strength of activation but also have sensitivity to the type of activation. This points the way towards using Raman spectral analysis to look at features of the immune response, in addition to its use as a tool for discrimination of phenotype and activation state at the single cell level. Figure 1 illustrates this process. Four classes of cells are measured: control, LPS-exposed, progesterone exposed, and LPS/progesterone exposed. The resulting data can be used to show the features that separate the 4 classes in terms of their Raman spectra. We then see features associated with resting macrophages (not shown for clarity), features associated with activation (highlighted in red), and features associated with inhibition (highlighted in green).

During collaboration with the Coban lab, we also found that Raman imaging-based analysis is promising in the study of neutrophil responses, and as a result, Dr Patrick Lelliot has now been granted a post-doctoral position in the Biophotonics lab, where he will work on correlating studies in label-free Raman imaging with imaging flow cytometry.

We also showed that Raman imaging could detect changes that occur in cells during viral infection. MH-S cells infected with encephalomyocarditis virus (EMCV) and time-matched control cells were recorded for a range of timepoints between 2 and 48 hours. The multivariate tool principal component analysis (PCA) was used to assess the cellular changes (either induced by the virus, or as a host cell response) occurring at each time point. Figure 2 shows the comparison across cells measured after 24 hours of infection. The different principal components provide information regarding the components in the cell that are modified or redistributed through the cell in response to the virus. PC1 shows that lipid contents of the infected cells are clearly different from those in control cells, while PC2 (2nd panel) shows significant changes in cell structure. Overall, the Raman imaging and analysis shows that EMCV infection has, by 24 hours, significantly affected the integrity of the MH-S cells. These changes appear in the imaging data without prior assumption, and show particular effects in cytoplasmic lipid content, and changes in overall nuclear integrity, as evidenced by the loss of molecular content (in loadings vectors, data not shown). Interesting questions remain such as whether the Raman-based interpretation of EMCV interactions with these cells are representative of general viral infection or are unique to EMCV.

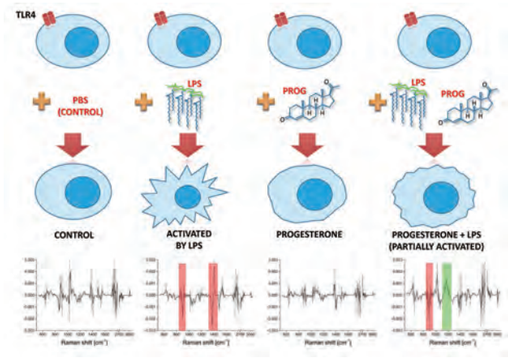



**Figure 1.**
**Raman signatures derived from measurements of single macrophages exposed to PBS, LPS, progesterone, or LPS & progesterone, forming a 4-way classification experiment. Each single cell measurement could be used to accurately classify the state of the cell, and the Raman data shows how each class is distinct, and highlights spectral markers that characterize each class including activation markers (red) and inhibition markers (green).**

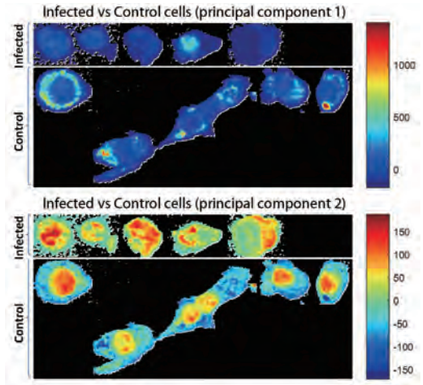



**Figure 2.**
**MH-S cells observed by Raman imaging showing the discrimination of control cells vs MH-S cells infected with EMCV for 24 hours. Principal component analysis highlights spectral features related to the infection, which appear in the strongest principal components PC1 and PC2.**

## Recent Publications


- Pavillon N, Hobro AJ, Akira S and Smith NI. Noninvasive detection of macrophage activation with single-cell resolution through machine learning. Proc. Natl. Acad. Sci. USA. 115(12), E2676-E2685 (2018).
- Hobro AJ and Smith NI. Vibrational spectroscopic imaging of pathogens, microorganisms, and their interactions with host systems. Opt. Commun. 422, 75-84 (2018).
- Pavillon N and Smith NI. Compressed sensing laser scanning microscopy. Opt. Express 24(26), 30038-300052 (2016).
- Hobro AJ and Smith NI. An evaluation of fixation methods: spatial and compositional cellular changes observed by Raman imaging. Vib. Spectrosc. 91, 31-45 (2017).
- Hobro AJ, Kumagai Y, Akira S and Smith NI. Raman spectroscopy as a tool for label-free lymphocyte cell line discrimination. Analyst 141, 3756-3764 (2016).

# Immune Response Dynamics


## Kazuhiro Suzuki, MD/PhD

| Professor | Kazuhiro Suzuki |
|---|---|
| Assistant Professor | **Akiko Nakai** |
| Postdoctoral Fellow | **1** |
| Research Assistant | **2** |
| Support Staff | **2** |


We have been studying the interactions between the nervous and immune systems with a special focus on the roles of adrenergic nerves in the control of adaptive immune responses. Our study revealed a mechanism by which adrenergic nerves control lymphocyte trafficking through lymph nodes. Inputs from adrenergic nerves to the β2-adrenergic receptor expressed on lymphocytes enhance the responsiveness of a specific set of chemokine receptors and inhibit lymphocyte exit from lymph nodes (Nakai et al., J. Exp. Med. 2014). This mechanism was found to generate diurnal variations in lymphocyte numbers in lymph nodes and consequently the magnitude of adaptive immune responses in synchronization with the circadian oscillation of adrenergic nerve activity (Suzuki et al, J. Exp. Med. 2016). In search of factors that mediate the crosstalk of signaling between the two different types of G protein-coupled receptors (GPCRs), the β2-adrenergic receptor and chemokine receptors, we identified a protein complex consisting of copper metabolism MURR1 domain-containing (COMMD) 3 and COMMD8 (COMMD3/8 complex), of which functions had been totally unclear. This year, we have demonstrated that the COMMD3/8 complex plays an important role in GPCR signaling and immune responses (Nakai et al., J. Exp. Med. 2019).

We first identified COMMD8 as a protein that binds to the C-terminal tail of a representative chemokine receptor CXCR4. Additional screening revealed the interaction of COMMD8 with COMMD3. We found that these proteins constitutively formed a complex in the cytosol, but were translocated to the plasma membrane after stimulation of CXCR4. The COMMD3/8 complex also interacted with not only CXCR4 but also other G protein-coupled chemoattractant receptors, including CXCR5, CCR7, and the oxysterol receptor EBI2, after activation of the receptors. Interestingly, COMMD3 and COMMD8 were degraded by the proteasome in the absence of the other, and deficiency of either protein produced the same phenotypes, indicating that both COMMD3 and COMMD8 are required for the stability and functions of their complex. Deficiency of COMMD3 or COMMD8 in B cells reduced their chemotactic responses through the receptors to which the COMMD3/8 complex was recruited. Thus, the COMMD3/8 complex is a positive regulator of chemoattractant receptor signaling.

Agonist binding to GPCRs activates heteromeric G proteins to regulate the generation of second messengers that modulate downstream signaling. Agonist-occupied GPCRs are phosphorylated by GPCR kinases (GRKs) and subsequently recruit β-arrestins that serve as scaffolds to activate signaling molecules, including mitogen-activated protein kinases (MAPKs). The GRK family consists of seven mammalian members, among which GRK2, GRK3, GRK5, and GRK6 are expressed ubiquitously. Different GRKs phosphorylate distinct sites on the C-terminal tail of the receptor, establishing a barcode that dictates the functional consequences of β-arrestin engagement. Thus, specific targeting of GRKs to activated GPCRs is crucial for signal transduction. Our mechanistic analysis for the action of the COMMD3/8 complex demonstrated that this protein complex functions as an adaptor that selectively recruits GRK6 to chemoattractant receptors in a GRK2/3-dependent manner, which promotes MAPK activation and consequently lymphocyte chemotaxis (Fig. 1). It has been suggested that the specificity of GRK recruitment to GPCRs is determined by the relative expression levels of individual GRKs, which vary among cell type, and distinct receptor conformations induced by ligand binding. Our study identifies a GRK-recruiting adaptor, the COMMD3/8 complex, as an additional determinant of GRK specificity for GPCRs.

Consistent with the reduced chemotactic responses of COMMD3- and COMMD8-deficient B cells, the mutant B cells showed multiple defects in their migration in vivo (Fig. 2A). Additionally, deficiency of COMMD3 or COMMD8 in B cells severely impaired humoral immune responses (Fig. 2, B and C). Therefore, the COMMD3/8 complex is essential for proper functioning of the immune system. By exploiting the unique property of the COMMD3/8 complex, it would be possible to degrade and disable the protein complex by pharmacological disruption of the interaction between COMMD3 and COMMD8. Development of such drugs would provide a novel approach for immune regulation, which may be applicable to the treatment of immune disorders.

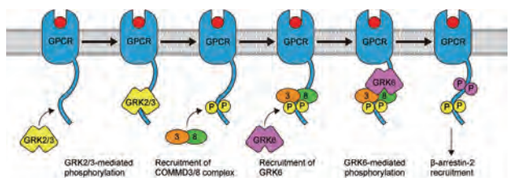



**Figure 1.**
**Proposed role of the COMMD3/8 complex in GPCR signaling.**
**The COMMD3/8 complex recruits GRK6 to GPCRs through a stepwise mechanism: (i) the C-terminal tail of activated GPCR is phosphorylated by GRK2 and GRK3. (ii) the COMMD3/8 complex is associated with the receptor tail through electrostatic interactions with the phosphorylated residues. (iii) GRK6 is recruited to the receptor through the interaction with the COMMD3/8 complex and phosphorylates the C-terminal tail.**

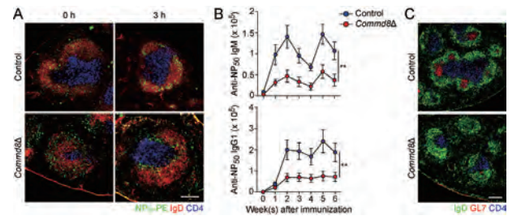



**Figure 2.**
**Deficiency of the COMMD3/8 complex impairs B cell migration and humoral immune responses.**
**(A) COMMD8-deficient (*Commd8*Δ) B cells show a defect in migration toward the outer follicle at 3 h after immunization. (B and C) B cell-specific deficiency of COMMD8 severely impairs the antibody response (B) and generation of germinal center B cells (C). Scale bars, 200 μm.**

## Recent Publications


- Nakai A, Fujimoto J, Miyata H, Stumm R, Narazaki M, Schulz S, Baba Y, Kumanogoh A and Suzuki K. The COMMD3/8 complex is a determinant of GRK6 specificity for chemoattractant receptors. J. Exp. Med. (2019) in press.
- Suzuki K and Nakai A. Immune modulation by neuronal electric shock waves. J. Allergy Clin. Immunol. 141, 2022-2023 (2018).
- Suzuki K and Nakai A. Control of lymphocyte trafficking and adaptive immunity by adrenergic nerves. Clin. Exp. Neuroimmunol. 8, 15-22 (2017).
- Suzuki K, Hayano Y, Nakai A, Furuta F and Noda M. Adrenergic control of the adaptive immune response by diurnal lymphocyte recirculation through lymph nodes. J. Exp. Med. 213, 2567-2574 (2016).
- Nakai A, Hayano Y, Furuta F, Noda M and Suzuki K. Control of lymphocyte egress from lymph nodes through β2-adrenergic receptors. J. Exp. Med. 211, 2583-2598 (2014).

# Systems Immunology

## Daron M Standley, PhD

| Professor | Daron M Standley |
|---|---|
| Associate Professor | **Kazutaka Katoh** |
| | **Shunsuke Teraguchi** |
| Assistant Professor | **Songling Li** |
| | **Floris Van Eerden** |
| Postdoctoral Fellow | **2** |
| Research Assistant | **2** |
| Support Staff | **2** |

In 2018 our lab continued to pursue functional analysis of adaptive immune receptors and protein-nucleotide interactions using bioinformatics methods. The methods utilized in these studies include multiple sequence and structural alignment, 3D rendering clustering and molecular dynamics simulations. In addition to such analysis, we have begun to carry out single cell sequencing of T cells in order to determine their TCRs and to predict TCR-epitope-MHC interactions.

**Structural modeling of adaptive immune receptors**

We developed a web-based tool (Repertoire Builder) for modeling BCR and TCR structures from sequence (Schritt, D. and Li, S. et al. MDSE, in press.) To our knowledge, Repertoire Builder is the most accurate tool available for BCR and TCR modeling. Moreover, Repertoire Builder can typically return 10,000 models in under 30 minutes.

**Functional analysis of B cell receptors**

The main goals of this project are: (1) to cluster B cells according to their antigen and epitope specificity and (2) to predict the epitope in cases where the antigen is known. The clustering is carried out using a machine learning algorithm that estimates the functional distance between two receptors. In a recent report (Xu, Z. MSDE, in press) we showed that all known experimentally-determined BCR structures could be clustered accurately (AUC 0.981). We further showed that an independent and non-redundant set of 104 anti-HIV BCR sequences could be clustered corresponding to manually-assigned epitopes with a specificity of 99.7% (Figure 1).

**Functional analysis of T cell receptors**

The main goals of this project are: (1) to cluster T cells according to their peptide-MHC (pMHC) specificity and (2) to predict the peptide (epitope) in cases where the MHC is known. To date, much progress has been made on the prediction of pMHC interactions. Now, with the emergence of paired (alpha-beta chain) TCR sequencing methods, there is growing interest in predicting the pMHC targeted by a given TCR. One example where such predictions would be highly valuable is in the discovery of neoantigens targeted by tumor-infiltrating lymphocytes. We have approached this problem by building on our work with BCRs (above). To this end, we first clustered TCRs that target a common pMHC. Next, if we know the MHC and antigen and have identified a cluster containing multiple TCR sequences predicted to target a common pMHC, we predict the targeted peptide. As a proof of concept, we showed that the correct epitope from yellow Fever Virus could be identified from a cluster containing 20 TCRs (Figure 2).

**Multiple sequence alignment**

We are continuously improving the performance and usability of the MAFFT multiple sequence alignment software. In 2018, we developed a database of aligned structural homologs (DASH) that contains domain- and chain-level alignments for every entry in the Protein Data Bank (PDB). MAFFT can communicate with DASH automatically in order to incorporate 3D structural information in multiple sequence alignments (Figure 3).

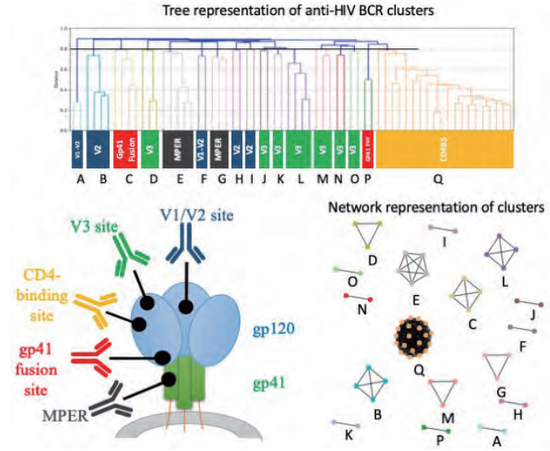



**Figure 1.**
**Relationship between hierarchical clusters and networks.**
The anti-HIV BCR clustering tree was sliced at a cutoff distance (0.796) and clusters were displayed as networks with edge-lengths proportional to their node distance. The sensitivity (True positive/Positive) was 99.7%.

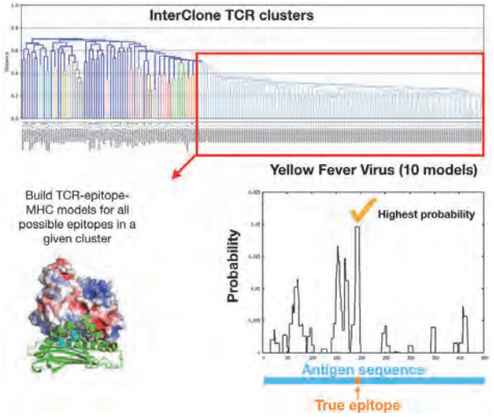



**Figure 2.**
**Predicting the epitope from a cluster of TCRs targeting the Yellow Fever Virus antigen NS1. The correct epitope was predicted by calculating the 3D model of each possible TCR-epitope-MHC triplet, and selecting the epitope with the lowest average binding energy.**

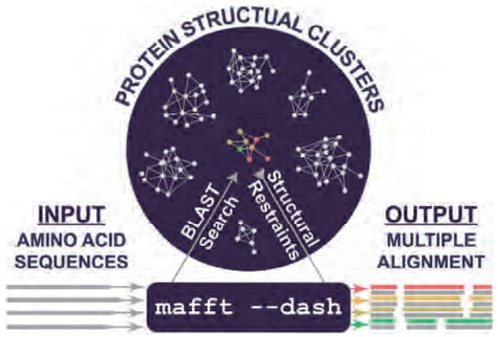



**Figure 3.**
**MAFFT-DASH.**
**The dash option in MAFFT automatically incorporates 3D structural information into multiple sequence alignment calculations, improving their accuracy without additional steps or heavy computational costs.**

## Recent Publications


- Takeda K., et al. Allergic conversion of protective mucosal immunity against nasal bacteria in patients with chronic rhinosinusitis with nasal polyposis. J. Allergy Clin. Immunol. 143, 1163-1175 (2019).
- Li S., et al. Structural modeling of lymphocyte receptors and their antigens. Meth Mol. Biol. (2019) in press.
- Hanieh H., et al. Arid5a stabilizes OX40 mRNA in murine CD4(+) T cells by recognizing a stem-loop structure in its 3'UTR. Eur. J. Immunol. 48, 593-604, doi:10.1002/eji.201747109 (2018).
- Yamasoba D., et al. N4BP1 restricts HIV-1 and its inactivation by MALT1 promotes viral reactivation. Nat. Microbiol. (2019) in press.
- Rozewicki J, Li S, Karlou A, Standley DM & Kazutaka K. MAFFT-DASH: integrated protein sequence and structural alignment. Nucleic Acids Res. (2019) in press.
- Xu Z., et al. Functional clustering of B cell receptors using sequence and structural features. MSDE (2019) in press.

# Statistical Immunology


## Yukinori Okada, MD/PhD


| Professor | Yukinori Okada |
|---|---|
| Research Assistant | 2 |
| Visiting Scientist | 2 |
| Support Staff | 1 |

**Goal of our laboratory**

Genetic backgrounds of individuals have substantial impacts on the risk of a wide range of immune-related diseases. Statistical immunology is a research field that evaluates causality of human genetic variations on immune-related diseases, using statistical and bioinformatics approaches. Recent developments of genome sequencing technologies have provided human genome data of millions of subjects, and successfully identified comprehensive catalogues of genetic risk loci of immune-related diseases. However, little is known regarding how to develop a methodology to integrate large-scale human genome data with diverse biological and immunological resources. The goal of our laboratory is to develop such methods and apply them to the latest large-scale disease genome and multi-layer omics data.

**Elucidation of natural selection pressure in the Japanese population using large-scale whole-genome sequencing**

Footprints of the evolutional history of human beings are embedded in human genome sequences as variation patterns in the population. We conducted a genome-wide scan of natural selection pressure in the Japanese population. By utilizing the large-scale whole-genome sequencing (WGS) data and analyzing positional distributions of extremely rare variants observed among Japanese individuals, we searched selection pressure in the very recent timescale of around 3,000 years ago. We successfully identified multiple genetic loci with significant natural selection pressure, which included the major histocompatibility (MHC) region regulating immune-responses of individuals We then conducted a phenome-wide scan with enrichment of selection pressure, and found that the variants which lowers alcohol consumption dosages were significantly enriched in selection pressure. This results provided novel insights into population genetics and immunology of the Japanese population (Okada Y et al. *Nat Commun* 2018).

**Development of nucleic medicine tool utilizing disease genetics and tissue-specific expression profile of miRNA**

MicroRNAs (miRNAs) modulate the post-transcriptional regulation of target genes and are related to the biology of complex human traits. While miRNAs are considered as promising biomarkers and therapeutic targets, the genetic landscape of miRNAs has been unclear. Considering the strikingly tissue-specific miRNA expression profiles, we developed a bioinformatics tool to quantitatively evaluate enrichment of genome-wide association study (GWAS) signals on miRNA-target gene networks (MIGWAS) according to tissue-specific enrichment (Figure 1). Our novel approach integrates tissue-specific expression profiles of miRNAs in 179 cells with GWAS. We applied MIGWAS to 49 GWASs and successfully identified biologically relevant tissues (e.g., lung for an autoimmune disease of rheumatoid arthritis [RA]). MIGWAS could also point miRNAs as candidate biomarkers of the trait. As clinical validation, we performed differentially expressed miRNA analysis between RA patients and healthy controls, and identified novel biomarker miRNAs (e.g. hsa-miR-762). Our study highlighted that miRNA-target gene network contributes to human disease genetics in a cell type-specific manner, which could yield an efficient screening of miRNAs as promising biomarkers. (Sakaue S et al. *Nucleic Acids Res* 2018).

**Next generation sequencing and machine learning deconvoluted genetic phenotypic landscape of MHC in Japanese**

The MHC region at human chromosome 6 confers strong risk on a variety of human complex traits. The human leukocyte antigen (HLA) gene variants are considered to confer causal risk, while details of their variations have been unclear. We conducted next generation sequencing of 33 HLA and HLA-related genes of 1,120 Japanese individuals, and genotyped high resolution HLA alleles with 6-digit. We applied a non-linear machine learning method of t-distributed stochastic neighbor embedding (tSNE) and classified HLA variations into 11 patterns (Figure 2). A phenome-wide association study of >170,000 Japanese individuals based on the HLA imputation method revealed that more than 50 of the assessed traits conferred significant susceptibility of the MHC variants. Our study provided a novel genetic phenotypic landscape of the MHC region in the Japanese population, which should contribute to understanding the diversity of human immunology and implementing personalized medicine (Hirata J et al. *Nat Genet* 2019).

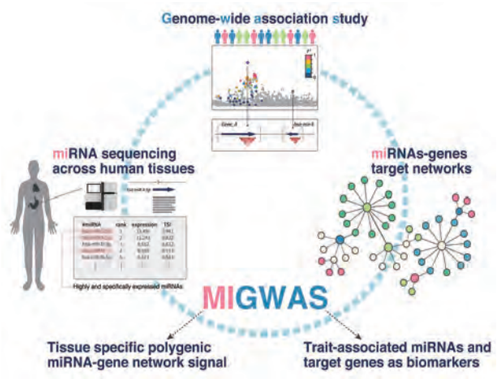



**Figure 1.**
**The scheme of newly developed nucleic medicine bioinformatics tool of MIGWAS (miRNA enrichment analysis of GWAS).**

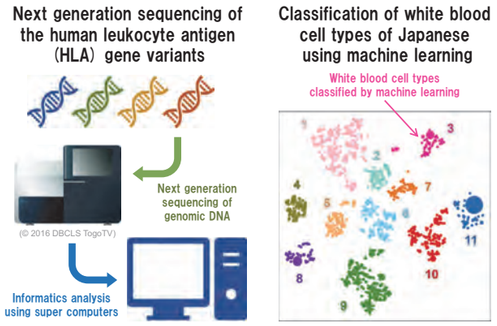



**Figure 2.**
**Next generation sequencing of the HLA genes and pattern classification using the non-linear machine learning method.**

## Recent Publications


- Hirata J., et al. Genetic and phenotypic landscape of the MHC region in the Japanese population. Nat. Genet. 51, 470-480 (2019).
- Suzuki K., et al. Identification of 28 novel susceptibility loci for type 2 diabetes in the Japanese population. Nat. Genet. 51, 379-386 (2019).
- Sakaue S., et al. Integration of genetics and miRNA-target gene network identified disease biology implicated in tissue specificity. Nucleic Acids Res. 46, 11898-11909 (2018).
- Okada Y., et al. Deep whole-genome sequencing reveals recent selection signatures linked to evolution and disease risk of Japanese. Nat. Commun. 9, 1631 (2018).
- Kanai M., et al. Grimon: Graphical interface to visualize multi-omics networks. Bioinformatics 34, 3934-3936 (2018).

# Quantitative Immunology

Associate Professor **Diego Diez**

Our team integrates computational and experimental methods to understand the immune system. We combine next generation sequencing with molecular barcoding techniques to obtain single cell omics information. We develop computational methods to analyze and extract biologically interesting information from this experimental data. We integrate this experimental data with publicly available information into network models of immune regulation. We apply this framework to study gene regulatory networks controlling immune responses, and collaborate with other groups at IFReC to address other immune system questions.

**Development of computational methods**

Single cell omics technologies have changed how we understand cell heterogeneity and identity. However, extracting information from these multi-dimensional datasets remains challenging. One specific area of interest is the identification of cell specific markers. In collaboration with Alexis Vandenbon at Kyoto University, we have developed a method to identify groups of cells with altered expression patterns from multi-dimensional representations of data (e.g. tSNE or PCA plot). Our method, named *haystack*, does not require any previous clustering of the data, providing an alternative approach to identify markers associated with groups of cells. This method is implemented in the R package *singleCellHaystack*, and is available from https://github.com/alexisvdb/singleCellHaystack.

Another difficult task associated with the analysis of single cell omics datasets is the identification of cell types in datasets obtained from unsorted tissues. Due to differences between RNA and protein expression, together with difficulties inherent to single cell omics technologies (e.g. drop out), assigning a cell type identity to each cell in a scRNA-seq experiment is not a simple task. In collaboration with Shunsuke Teraguchi from the Systems Immunology laboratory and Kazuhiko Maeda from the Host Defense laboratory, we have developed a method to quickly assign cell type identities to single cell RNA-seq data. Our method is at the moment tailored towards immune cell types. It uses information from microarray experiments from the ImmGen consortium to generate a dictionary with information about more than 200 different immune cells. This dictionary is used to find correlations between cells in a single cell RNA-seq experiment and the cell types in the dictionary. This method enables to quickly assign cell type identities to hundreds of thousands of cells. The code is implemented in the R package *celltype* and is available from https://github.com/ddiez/celltype.

**Mathematical modeling**

The vast amount of datasets accumulating from single cell omics experiments opens the door to approaches that study the immune system from a more theoretical perspective. We are adopting this perspective and developing methods to model immune transcriptional regulatory networks. Transcriptional regulatory networks are important determinants of cell identity and function. These networks consist of transcriptional activators and repressors, including transcription factors and the target genes they regulate. We model these processes using linear regression methods. We assume that the expression level of each gene depends on the activity of a small number of regulators. Furthermore, regulators contribute in an additive way to the expression level of their target genes. We validate the feasibility of these assumptions using simulated networks resembling different types of real networks. Using the expression level of the regulators as a proxy for their activities we apply these methods to several immune datasets.

**Applications to immunology**

In collaboration with other laboratories at IFReC we apply our methods to address different immune questions. We collaborate with the Host Defense laboratory to study the role of Regnase 1 in T cell development. Using targeted single cell transcriptomics from the BD Rhapsody platform, we analyze the expression of hundreds of genes in tens of thousands of single cell transcriptomes from spleen and thymus in different experimental conditions.

We collaborate with the Experimental Immunology laboratory and the Systems Immunology laboratory to study T cell signaling in a mouse model of rheumatoid arthritis. In this project we use data from 10x Genomics whole genome single cell transcriptomics and VDJ repertoire. We use computational methods to identify T cell signaling pathways driving the development of autoimmunity. Furthermore, we use mathematical models to identify regulatory networks associated with differences in T cell subpopulations.

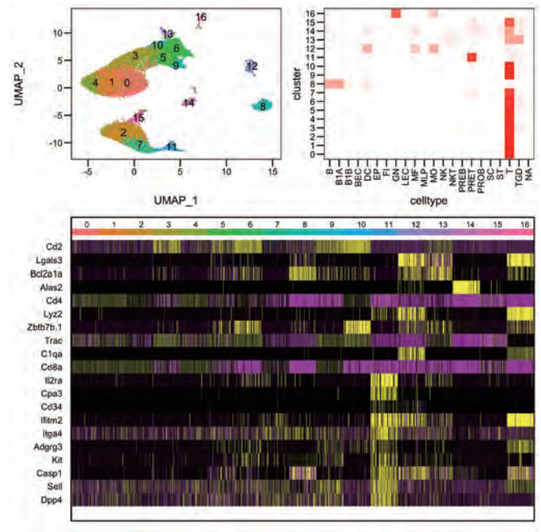



**Figure.**
**Single cell RNA-seq of thousands of cells from the thymus gives insight into the processes driving T cell development.**

## Recent Publications


- Vandenbon A, Diez D. singleCellHaystack: Finding surprising genes in 2-dimensional representations of single cell transcriptome data. bioRxiv 557967; doi: https://doi.org/10.1101/557967 (2019).
- Bahrini I, Song JH, Diez D. & Hanayama R. Neuronal exosomes facilitate synaptic pruning by up-regulating complement factors in microglia. Scientific reports 5, 7989, doi:10.1038/srep07989 (2015).
- Diez D, Agusti A & Wheelock CE. Network analysis in the investigation of chronic respiratory diseases. From basics to application. Am. J. Respir. Crit. Care Med. 190, 981-988, doi:10.1164/rccm.201403-0421PP (2014).

# Events & Outreach Activities

## The 10th International Symposium of IFReC, co-hosted with Cluster Science Days 2018


The 10th International Symposium of IFReC was held on November 5th-6th, 2018 in the Biomedical Center (BMZ) at Venusberg Campus of the University Hospital Bonn. This was concurrently held as Cluster Science Day 2018 through joint organization with ImmunoSensation, University of Bonn. Thirty-five oral and 88 poster presentations including those of seven IFReC PIs were made for an audience of over 300 participants from University of Bonn and vicinal research institutes. IFReC, Research Institute for Microbial Diseases (RIMD) and Graduate School of Frontier Biosciences (FBS) of Osaka University jointly concluded an Academic Exchange Agreement with ImmunoSenation, which is one of the leading institutions in Immunology in Europe. The success of the symposium is expected to promote exchanges of young researchers and international collaboration.

- **Date : November 5-6, 2018**
- **Venue : Biomedical Center (BMZ) at of the University Hospital Bonn, Bonn, Germany**

## Osaka-Heidelberg/Mannheim Symposium on Immune Plasticity

The Osaka-Heidelberg/Mannheim Symposium on Immune Plasticity was held on November 8th, 2018 at International Academic Forum Heidelberg (IWH) of Heidelberg University. A small group of approximately 50 participants permitted intense discussions with immunologists of Heidelberg University on the latest issues in immunology.

- **Date : November 8, 2018**
- **Venue : International Academic Forum Heidelberg, Heidelberg, Germany**

# Next Gen Immunology in Health and Disease


Next Gen Immunology in Health and Disease was held on February 7th and 8th at Osaka International Convention Center, Osaka, Japan. Ten core researchers of top research institutes in Europe, two distinguished researchers in Japanese institutes and seven IFReC researchers were invited as speakers. The symposium had 195 participants including 55 international and 41 from industry or hospitals. The symposium could successfully promote interactions among researchers of IFReC and Europe for future international collaborations and of academia and industry/clinical domains for future collaborations for applied and translational research.


- **Date :** February 7-8, 2019
- **Venue :** Osaka International Convention Center (Grand Cube Osaka), Osaka, Japan

## Day 1

| Speaker | Title |
|---|---|
| **Tadamitsu Kishimoto**<br>IFReC, Osaka University | **Keynote :** A possible therapeutic target, Arid5a for the treatment of inflammatory diseases associated with aberrant cytokine expression |
| **Takashi Nagasawa**<br>IFReC/Graduate School of FBS/Medicine, Osaka University | Bone marrow microenvironmental niches for hematopoietic stem cells and immune cells |
| **Kazuyo Moro**<br>RIKEN IMS, Japan | IL-4 Production of Group 2 Innate Lymphoid Cells |
| Chair : Wataru Ise (IFReC, Osaka University) | |
| **Shimon Sakaguchi**<br>IFReC, Osaka University | Regulatory T cells in common autoimmune diseases |
| **David Klatzmann**<br>Sorbonne University, France | On Treg-based therapies of autoimmune diseases |
| **Federica Sallusto**<br>Universita della Svizzera Italiana/ ETH Zurich, Switzerland | Human Memory T Cell Subsets: from Phenotype to Function |
| **Sjoerd Henricus van der Burg**<br>Leiden University, the Netherlands | Combination treatments to modulate the microenvironment and boost tumor-specific T cells |
| Chair: Kazuhiro Suzuki (IFReC, Osaka University) | |
| **Tomohiro Kurosaki**<br>IFReC, Osaka University/RIKEN IMS, Japan | Selection mechanisms of germinal center cells into the memory B cell compartment |
| **Hisashi Arase**<br>IFReC/RIMD, Osaka University | Paired receptors in host-pathogen interaction |
| **Kiyoshi Takeda**<br>IFReC/Graduate School of Medicine, Osaka University | Regulation of immune responses by intestinal microbiota |
| **Kenya Honda**<br>Keio University/RIKEN IMS, Japan | Gut microbiota-mediated immune modulation |

## Day 2

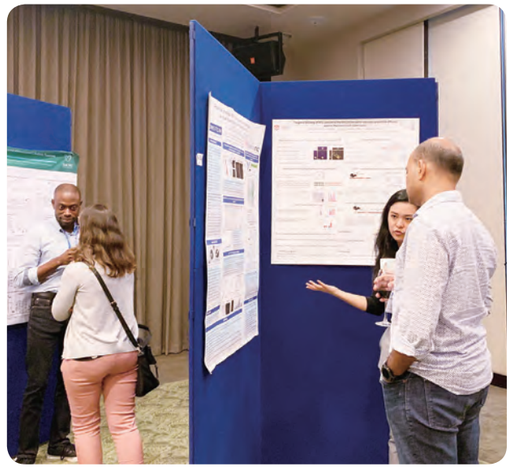
| Speaker | Title |
|---|---|
| Chair : James Badger Wing (IFReC, Osaka University) | |
| **Klaus Rajewsky**<br>Max Delbruck Center for Molecular Medicine, Germany | **Keynote :** Gene targeting: 30 years later |
| **Gioacchino Natoli**<br>Humanitas University, Italy | Access to the genomic regulatory information and the control of inflammatory gene expression |
| **Thomas Weichhart**<br>Medical University of Vienna, Austria | 3M: Macrophages, mTOR and metabolism |
| **Shizuo Akira**<br>IFReC, Osaka University | Towards understanding the mechanism of lung fibrosis |
| Chair : Takashi Satoh (IFReC, Osaka University) | |
| **Ido Amit**<br>Weizmann Institute of Science, Israel | Single-cell genomics: A stepping stone for future immunology discoveries |
| **Anna Katharina (Katja) A Simon**<br>The Kennedy Institute, University of Oxford, UK | Autophagy in the immune system |
| **Eicke Latz**<br>University Hospital Bonn/German Center for Neurodegenerative Diseases /University of Massachusetts, USA | Regulation of inflammasome responses |
| Chair : Masahiro Yamamoto (IFReC, Osaka University) | |
| **Petr Broz**<br>University of Lausanne, Switzerland | Regulation of Gasdermin-D-induced pyroptotic cell death |
| **Sho Yamasaki**<br>IFReC/RIMD, Osaka University | Recognition of tissue damage via C-type lectin receptors |

# The 8th NIF Winter School on Advanced Immunology

The 8th NIF Winter School on Advanced Immunology was held from January 20 to 23, 2019 in Singapore. The NIF Winter School series is organized and held each year alternatively in Singapore and Japan in a collaboration with Singapore Immunology Network (SIgN). The scientific program comprised of 16 guest lectures, participants' short presentations and poster sessions. A group of 47 excellent students including four from IFReC were selected. The enthusiasm of the participants and the high scientific quality of the lectures and the presentations made the NIF Winter School an extremely successful event. The Winter School experience made a strong impact on all participants, by widening and deepening their understanding of immunology, furthering their commitment to excellence in scientific research, and creating many new friendships.


- **Date :** January 20-23, 2019
- **Venue :** Grand Copthorne Waterfront Hotel, Singapore

| Lecturer | Title |
|---|---|
| **Shizuo Akira** (IFReC, Osaka University, Japan) | Understanding the molecular mechanism of lung fibrosis |
| **Veronique Angeli** (National University of Singapore, Singapore) | Hyaluronan receptor LYVE-1-expressing macrophage keeps our artery healthy |
| **Hisashi Arase** (IFReC, Osaka University, Japan) | LILR family receptor in host-pathogen interaction |
| **Marc Bajenoff** (Centre d'Immunologie de Marseille-Luminy, France) | Lymphatic endothelial cells constitute the niche for self-maintaining subcapsular sinus macrophages |
| **Burkhard Becher** (University of Zurich, Switzerland) | The T cell/Phagocyte Interface in Chronic Inflammation |
| **Kenji Kabashima** (SIgN, Singapore) | Cutaneous immune responses to external stimuli |
| **Klaus Karjalainen** (Nanyang Technological University, Singapore) | Maintenance of tissue-resident macrophages |
| **Claudia Kemper** (National heart, Lung and Blood Institute (NIH), USA) | The Force from within: unexpected roles for the complosome in normal cell physiology |
| **Tomohiro Kurosaki** (IFReC, Osaka University, Japan) | Fate decision of germinal center B cells |
| **Claudia Mauri** (University College London, UK) | Cellular and molecular characterization of regulatory B cells |
| **Lai Guan NG** (SIgN, Singapore) | Neutrophils: The Power of Many |
| **Jeff Rathmell** (Vanderbilt Institute of Infection, Immunology, and inflammation, USA) | Fueling T cells in Inflammation and Cancer |
| **Amit Singhal** (SIgN, Singapore) | Harnessing Host Immuno-metabolic circuits For Restricting Mycobacterium tuberculosis |
| **Ashley ST John** (Duke-NUS Medical School, Singapore) | Mast cell responses to virus infection |
| **Sho Yamasaki** (IFReC, Osaka University, Japan) | Recognition of intracellular metabolites through C-type lectin receptors |
| **Simon Yona** (University College London, UK) | Monocytes kinetics in health and disease |



# Visitors to IFReC

## Two directors from Curie Institute

IFReC welcomed Dr. Eliane Piaggio and Dr. Ana-Maria Lennon-Duménil from the Curie Institute, France, in October 2018. IFReC and the Curie Institute confirmed the cooperative relationship in near future. We invited them as speakers for the IFReC seminar on October 29, 2018.


## Courtesy call by Brunei Darussalam

Osaka University welcomed distinguished guests from three universities of Brunei Darussalam in August 2018. The office of Osaka University organized a signing ceremony for an inter-university exchange agreement and the joint symposium. Before the ceremony, IFReC received a courtesy call by the guests from Brunei Darussalam.

## The 23rd Chinese University Student Delegation to Japan Project

Thirty-eight members of the 23rd Chinese University Student Delegation to Japan Project visited IFReC on November 28. The students attended a lecture by Dr. Naganari Ohkura and visited the Experimental Immunology laboratory, MRI imaging facility and the RIMD museum.


# IFReC Seminars


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

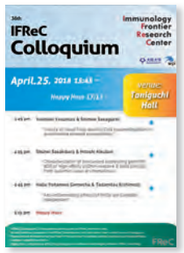
Since its establishment, IFReC has held more than a hundred seminars, which have served as a forum for effective interaction between researchers beyond national borders and academic disciplines. This program has certainly contributed to IFReC's mission of promoting internationalization and interdisciplinary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



| Date | Speaker | Title |
|---|---|---|
| May 21, 2018 | Mahesh Desai (PI, Allergology - Immunology - Inflammation Research Unit, Luxembourg Institute of Health) | Diet-driven interactions of the gut microbiome with the intestinal mucus barrier |
| October 29, 2018 | Eliane Piaggio (Director, Translational Immunotherapy Team, Curie Institute, France) | Targeting Tregs in cancer: a translational approach |
| October 29, 2018 | Ana-Maria Lennon-Dumenil (Director, the Spatio-Temporal Regulation of Antigen Presentation and Cell Migration Team, Curie Institute, France) | Migration of dendritic cells under pressure |
| November 19, 2018 | Mark S. Sundrud (Associate Professor, Department of Immunology & Microbiology, The Scripps Research Institute, USA) | Specialization of T lymphocytes in the ileum |
| February 22, 2019 | Motohiko Kadoki (Massachusetts General Hospital / Broad Institute / Harvard Medical School, USA) | Inter-Organ Dialogues during Vaccination -Lessons from Organismal Systems Immunology- |

# IFReC Colloquia

IFReC colloquia are a series of discussion meetings for IFReC members held once every other month since FY2011. At each colloquium, three speakers from IFReC laboratories give talks about their latest research progress followed by intensive discussion. After the colloquium, a small social gathering is held to further the discussions and encourage the exchange among IFReC members in an informal setting. These events serve as a platform to promote fusion researches and deepen understanding of researches conducted in IFReC.





| | Date | Speaker | Title |
|---|---|---|---|
| 36th | April 25, 2018 | Yoshiaki Yasumizu, Naganari Ohkura, and Shimon Sakaguchi (Experimental Immunology) | Gravity of naïve Treg-specific CpG hypomethylation in autoimmune disease susceptibility |
| | | Shuhei Sakakibara and Hitoshi Kikutani (Immune Regulation) | Characterization of precursors expressing germline BCR of high-affinity dsDNA-reactive B cells derived from systemic lupus erythematosus |
| | | Hailu Yohannes Gemechu and Tadamitsu Kishimoto (Immune Regulation) | Anti-inflammatory effects of IMiDs are Cereblon independent |
| 37th | June 13, 2018 | Takato Kusakabe and Ken Ishii (Vaccine Science) | R&D of Hydroxypropyl-β-Cyclodextrin (HP-β-CD) as a vaccine adjuvant <Hydroxypropyl-β-cyclodexrin (HP-β-CD) is an IL-33 inducer in the lung> |
| | | Rouaa Beshr and Jun Hatazawa (Nuclear Medicine) | 18F-FBPA PET/CT: to distinguish radiation-induced cerebral necrosis from recurrent brain tumor |
| | | Katsumori Segawa and Shigekazu Nagata (Biochemistry & Immunology) | Phospholipid flippases enable precursor B cells to flee entosis |
| 38th | August 29, 2018 | Yukinori Okada (Statistical Immunology) | Genetic and phenotypic landscape of MHC in the Japanese population |
| | | Masanari Seike and Takashi Nagasawa (Stem Cell Biology and Developmental Immunology) | Hematopoietic stem cell niche-specific Ebf3 maintains the bone marrow cavity |
| | | Hiroshi Tsujioka and Toshihide Yamashita (Molecular Neuroscience) | Transcriptomic analysis of spinal cord of axonal sprouting-capable neonatal mice after central nervous system injury |
| 39th | October 24, 2018 | Floris van Eerdan and Daron Standley (Systems Immunology) | Structural modeling of lymphocyte receptors and their antigens |
| | | Hisamichi Naito and Nobuyuki Takakura (Signal Transduction) | The role of endothelial stem cells in vascular regeneration |
| | | Masahiro Nagata and Sho Yamasaki (Molecular Immunology) | Identification of unique bacterial steroids that promote deleterious inflammation |
| 40th | December 19, 2018 | Ben Seymour and Toshio Yanagida (Single Molecule Imaging) | Integrated physiological systems for defence against injury |
| | | Shimpei Kawamoto and Eiji Hara (Aging Biology) | The roles and mechanisms of cellular senescence in aging and cancer |
| | | Tetsuya Kimura and Masato Okada (Oncogene Research) | Prevention of Obesity by Macrophages |

## Science Café

The series of science cafes is a long-standing IFReC outreach activity to promote communication between researchers and the general public. It also enhances people's understanding of immunology researches and the researchers involved . In the two science cafés organized by IFReC in FY2018, about 150 participants in total enjoyed novel topics in immunology in a relaxing atmosphere.

### Science Café on the Edge at Icho Festival 2018

< IGD causes epilepsy and growth delay >

- Speaker : Yoshiko Murakami (Professor, Immunoglycobiology, IFReC/RIMD)
- Date : April 30, 2018
- Venue : TechnoAlliance Hall, Suita Campus, Osaka University


### Science Café on the Edge at Nakanoshima Festival

< Brain tumor and immunity -Mystery of lymphocytes remaining in bone marrow- >

- Speaker : Shohei Koyama (Assistant Professor, Department of Respiratory Medicine and Clinical Immunology, Graduate School of Medicine, Osaka University)
- Date : December 9, 2018
- Venue : Graduate School of Medicine, Osaka University


## Students Visit

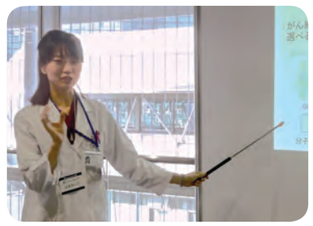
Thirty students visited IFReC on a tour organized by Nara High School, which is designated as a Super Science High School. After the lecture by Assist. Prof. Akiko Nakai (Immune Response Dynamics), the students toured the Laboratory of Host Defense and the Imaging Facility before trying experiments and talking with researchers. Responses from the participants include "a very valuable, unique, and exceptional experience."

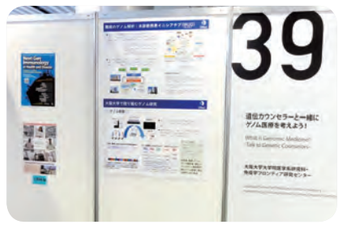
- Date : August 23, 2018

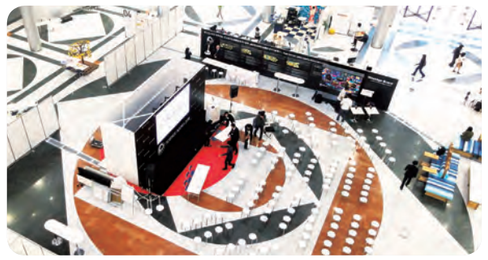
## Science Agora

IFReC participated in Science Agora 2018 held in Tokyo. At the exhibition booth titled "Let's think about genomic medicine with the gene counselors," we organized the screening of an original video depicting the process of genetic counseling, and a question and answer session by guest counselors.

- Date : November 9-11, 2018
- Venue : Telecom Center, Odaiba, Tokyo





# Osaka University Co-Creation Festival 2018

## Co-Creation Festival 2018

IFReC participated in Osaka University Co-Creation Festival 2018 organized by the headquarters office of Osaka University. This event was called “Let's Have Fun with Osaka University!" In the event, IFReC demonstrated the observation of various immune cells through a microscope. Using videos and photos, we also introduced the research of IFReC. The venue was full of visitors throughout the day.

- Date : November 17, 2018
- Venue : LaLaport EXPOCITY, Suita


## Workshop Festival at Grand Front Osaka

Knowledge Capital at GFO (Grand Front Osaka) has regularly held the workshop festival with the aim of utilizing Knowledge Capital as a learning place for students and kids. As part of the program, IFReC organized an event, which introduced immunity to children by making models of blood and immune cells.

- Date : March 16-17, 2019
- Venue : Grand Front Osaka

# Super Science High School Student Fair


Super Science High Schools (SSH) are high schools designated by Japan’s MEXT (Ministry of Education, Culture, Sports, Science and Technology), which promote advanced math/science education and collaborative research projects with universities as well as activities to develop international perspectives.

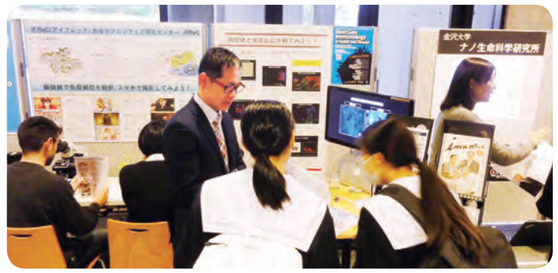
In the SSH Student Fair 2018, more than 200 schools, including over 10 schools from overseas, held booths with posters to present their research achievements. IFReC and other WPI institutes held a collaborative booth and introduced the research activities of each institute using posters, booklets, and demonstrations.


- Date : August 8-9, 2018
- Venue : Kobe International Exhibition Hall
- Host : MEXT and JST
- Support : Boards of Education (Hyogo prefecture and Kobe city)


## "Kagaku Zanmai" in Aichi 2018

"Kagaku Zanmai (addiction to science)" is an advanced initiative called Aichi Model. In this program, the high schools in Aichi prefecture that are actively engaged in science education collaborate with universities and research institutes to conduct research and presentation. At the annual meeting of Kagaku Zanmai 2018, WPI institutes organized a presentation booth to demonstrate top level research in Japan.


- Date： December 27, 2018
- Venue： Toyota Auditorium, Nagoya University
- Host： Okazaki High-school, Aichi prefecture





## The 7th WPI Science Symposium

ITbM and WPI institutes co-organized the 7th WPI Science Symposium in Nagoya. At the booth of IFReC, with the assistance of overseas students, we introduced images and videos obtained through IFReC research, and distributed leaflets of IFReC and WPI.


- Date： December 27, 2018
- Venue： Toyota Auditorium, Nagoya University
- Host： Institute of Transformative Bio-Molecules (WPI- ITbM), Nagoya University
- Support： Nagoya city, Aichi prefecture, MEXT

## AAAS Annual Meeting 2019


The American Association for the Advancement of Science (AAAS), the publisher of Science journal, is the biggest international non-profit organization in the world, and its mission is to "advance science and serve society". The AAAS Annual Meeting assembles diverse participants, including scientists, families, science policymakers, and the media etc., offering symposia, lectures, seminars, poster presentations and exhibitions on a variety of scientific topics.

WPI institutes participated in the AAAS 2019 Annual Meeting in Washington D.C. We held a collaborative booth to introduce the WPI program and the institutes' activities including our approaches for interdisciplinary research and internationalization.

The next AAAS annual meeting will be held in Seattle, WA, USA on Feb. 13-16, 2020.

- Date： February 14-17, 2019
- Venue： Hotel Marriott Wardman Park DC, Washington, D.C., USA
- Host： American Association for the Advancement of Science

## Japanese Language Classes

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

We offer two lecture-style classes, "Class A: Elementary to Pre-intermediate" and "Class B: Intermediate to Advanced." Students are expected to learn basic Japanese grammar including verb and adjective conjugations in Class A, and to learn intermediate/advanced level grammar and vocabulary to improve upon what was learned in Class A as well as kanji in Class B.

The instructor of our Japanese class, Ms. Tajima, who has greatly contributed to our Japanese Class since it launched in 2012, has finished teaching at IFReC. A new instructor, Ms. Tomomune, has succeeded her from FY 2019.

It has been a great pleasure to work with the researchers at IFReC. They were always highly motivated to learn new Japanese grammar and vocabulary despite their busy work schedules. During the lessons, they tried hard to use as much Japanese as possible in discussing their daily lives. As a result, they learned many expressions which were not even in the textbook. Tuesdays and Thursdays have been my favorite days of the week. I will miss them a lot.

Kaori Tajima

I have been teaching Japanese in Japan and the USA to international students from all over the world. In my teaching experience, I am always striving to make my class more interactive so that students can learn independently among themselves, and I also try to have my students experience Japanese customs and culture in my class through hands-on activities such as writing new year greeting cards and introducing Japanese seasonal events. I am also striving to have students develop their communication skills that are essential for daily life in Japan. Going shopping, speaking with classmates, and making friends in Japanese will make their lives more enjoyable.

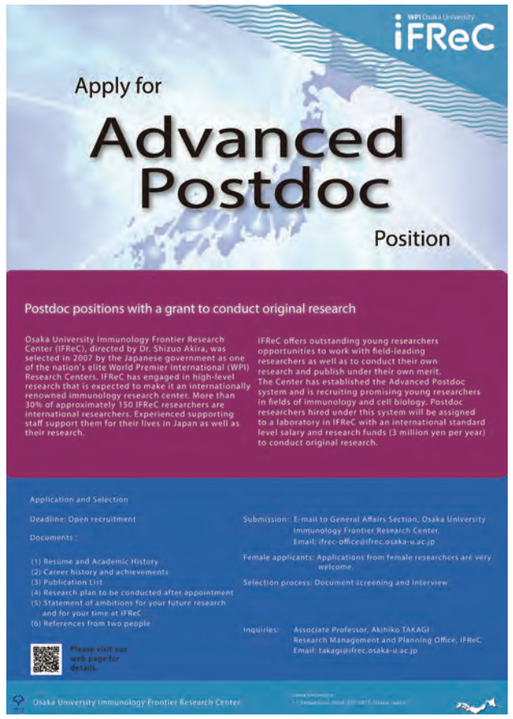
Tomomi Tomomune

# Research Projects

## Advanced Postdoc System

For IFReC, fostering the education of young researchers is a responsibility as a global research institution. It is also vital for the institution to continually incorporate the original ideas of young researchers and to promote international brain circulation in order to further develop IFReC's research. Around the world, leading research institutions are in fierce competition to discover excellent young researchers. In order to employ young researchers who are expected to be internationally active at IFReC and to strengthen IFReC's function as an international hub for career formation (called "brain circulation" by MEXT). This is necessary to improve the conditions for researchers in Japan, to an international standard.

IFReC has, therefore, established the Advanced Postdoc system in 2017. It offers outstanding young researchers in the fields of immunology and cell biology opportunities to work with field-leading researchers in IFReC as well as to conduct their own research and publish under their own merit. IFReC has selected three excellent postdocs for three years under this system out of 171 applicants in 2018. They were assigned to laboratories in IFReC with an international standard level salary and research funds (3 million yen per year) to conduct original research.




## Young Scientist Support Program for Research Abroad

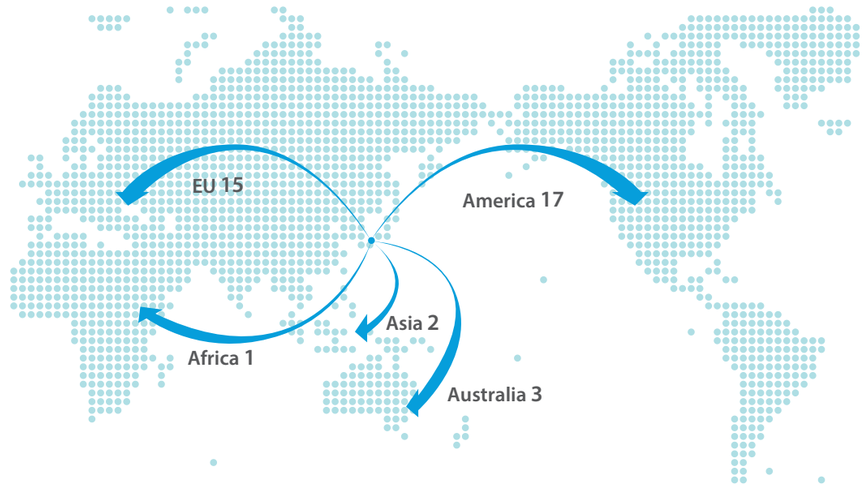
To strengthen our international research network and our basis for international collaborative research, IFReC has provided financial support to young researchers who wish to participate in research activities at overseas institutions.

The program aims to develop the practical skills and abilities of young researchers in international collaborative research and to develop their network with researchers overseas. 2 researchers used this support program in FY2018.

Young Scientist Support Program for Research Abroad

| Name | Country | Conference Attended |
|---|---|---|
| **Alison Jane Hobro** | USA | SCIX Conference / Emory University |
| **Nicolas Pavillon** | USA | SPIE Biomedical Optics conference |

Since the start of this program, IFReC has provided support for 38 visits overseas by young researchers.




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

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

IFReC–RIMD Research Complex at Suita Campus of Osaka University


Photo : S. Higashiyama

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

### Animal Resource Center for Infectious Diseases

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

### Live immuno-imaging facility

- SPF animal experiment facility with 11.7T MRI, in-vivo imager & two-photon microscope.

### Network Administration Office

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

### Core Instrumentation Facility

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

# Kishimoto Foundation Fellowship

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

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

FY2018 Kishimoto Fellowship Recipients

| Position of Recipient | Name (initials) | Nationality | Host researcher | Period |
|---|---|---|---|---|
| Visiting Researcher | P. K. | Turkish | Coban | Mar. 1, 2018 - May 27, 2018 |
| Visiting Researcher | E. E. | Turkish | Coban | Mar. 1, 2018 - May 27, 2018 |
| Visiting Researcher | N. N. | Vietnamese | Kishimoto | Mar. 10, 2018 - Mar. 22, 2018 |
| Visiting Researcher | L. D. | Vietnamese | Kishimoto | Mar. 10, 2018 - May 8, 2018 |
| Visiting Researcher | H. H | Jordanian | Kishimoto | Jun. 1, 2018 - Jul. 20, 2018 |
| Visiting Researcher | G. E | French | Kinoshita | Jul. 2, 2018 - Aug. 10, 2018 |
| Visiting Researcher | F. B | German | Kurosaki | Aug. 11, 2018 - Oct. 11, 2018 |

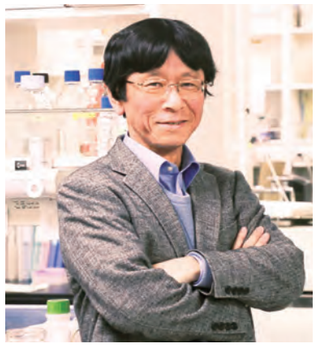
# Major Awards

## Takashi Nagasawa　Japan Academy Prize

Takashi Nagasawa (Stem Cell Biology and Developmental Immunology, IFReC/Graduate School of Frontier Biosciences, Osaka University) won the Japan Academy Prize. The awarded title is “Elucidation of Microenvironments Essential for the Maintenance of Hematopoietic Stem Cells, Hematopoiesis and Bone”.

## Taroh Kinoshita　Medal with Purple Ribbon

Taroh Kinoshita (Immunoglycobiology, IFReC/RIMD) won the Medal with Purple Ribbon, which is awarded to people who have made outstanding contributions in academic fields, arts and sports. Kinoshita and his research group have been trying to reveal how GPI-anchored proteins are synthesized, processed, transported and secreted, and how defects in these processes lead to the onset and pathology of diseases. They have made considerable achievements in this field.



## Kazuya Kikuchi and Miwa Sasai　MEXT Scientists' Prize

Kazuya Kikuchi (Chemical Imaging Techniques, IFReC/Graduate School of Engineering) was awarded by the Minister of Education, Culture, Sports, Science and Technology (MEXT) for his outstanding achievement in "Developments of chemical probes to visualize the functions of the cells and the molecules in living animals". Miwa Sasai (Immunoparasitology, IFReC/RIMD) was given the Young Scientists' Prize of the Commendation for Science and Technology by MEXT. She was awarded for her study on "Pathogen elimination mechanism via intracellular endoplasmic reticulum transport".


## Sho Yamasaki and Sujin Kang　Awarded by JSI

Sho Yamasaki (Molecular Immunology, IFReC/RIMD) won the Japanese Society for Immunology (JSI) Award 2018 for his outstanding achievements in the studies of "mechanisms of pathogen recognition by immune receptors". Sujin Kang (Immune Regulation, IFReC) won the JSI Young Investigator Award 2018. She was recognized for her achievements in immune semaphorins, which involve linking immunity and lipid metabolism.

## Masahiro Yamamoto　Japan Medical R&D Grand Prize/JSPS Prize

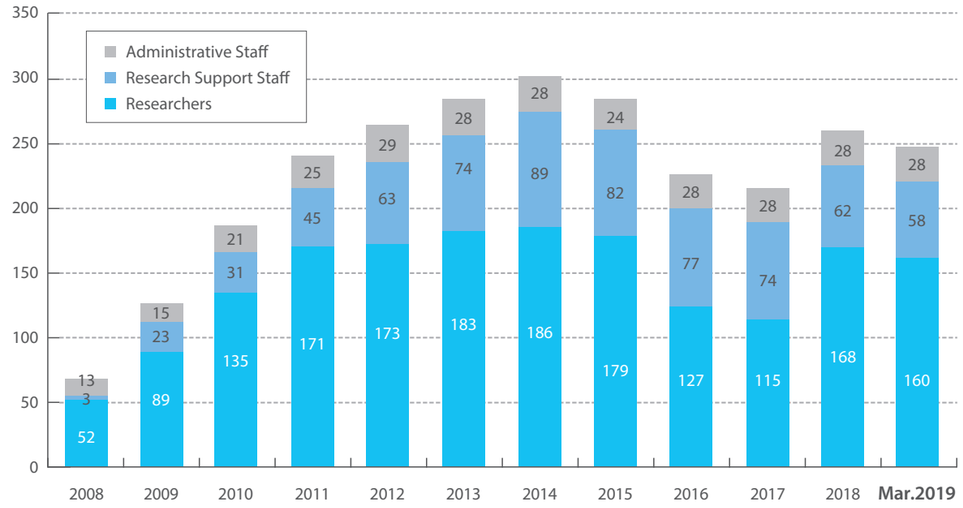
Masahiro Yamamoto (Immunoparasitology, IFReC/RIMD) won the Japan Medical R&D Grand Prize.  The government commented Yamamoto was awarded for his outstanding achievements in the studies of "the elucidation mechanism of host immune system against the pathogenic parasite infections". Using Toxoplasma infection as a model, his group has revealed the ingenious mechanism of the evolved host immune system. Yamamoto also won the JSPS Prize 2018 for his "Analysis of immunological interface between host and intracellular pathogens". A new treatment strategy by the cutting-edge parasitic immunology is widely expected in the world.

## Osaka University and the three companies　Awarded by MEXT at JOIP

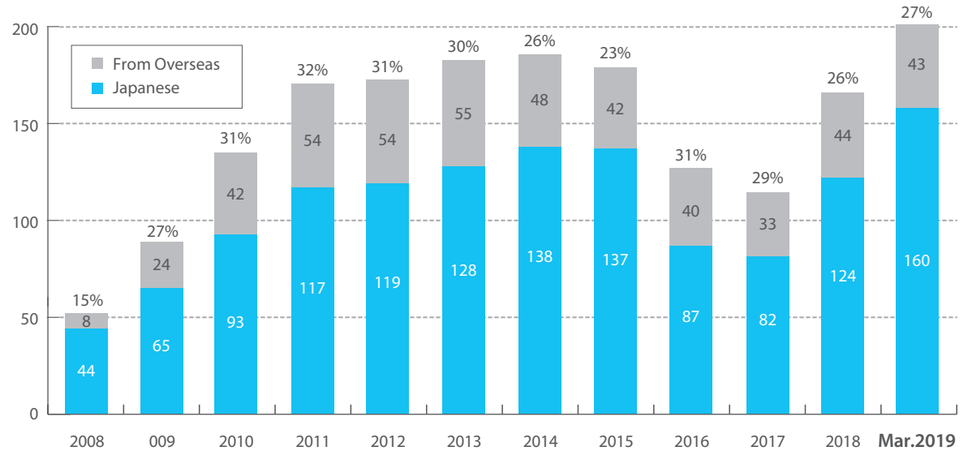
The Japan Open Innovation Prize (JOIP) was launched with the aim of appreciating the most leading and original initiatives expected to be used as future role models to further promote open innovations in Japan. In February, 2019, Osaka University, Chugai Pharmaceutical Co., Ltd., Otsuka Pharmaceutical Co., Ltd., and Daikin Industries, Ltd. won the MEXT Award at the 1st JOIP with "University-Industry Co-creation from the Basic Research Stage -Collaboration between Organizations". IFReC has greatly contributed to the contracts between Osaka Universisty and both pharmaceutical firms.

## Composition

Number of IFReC Staff

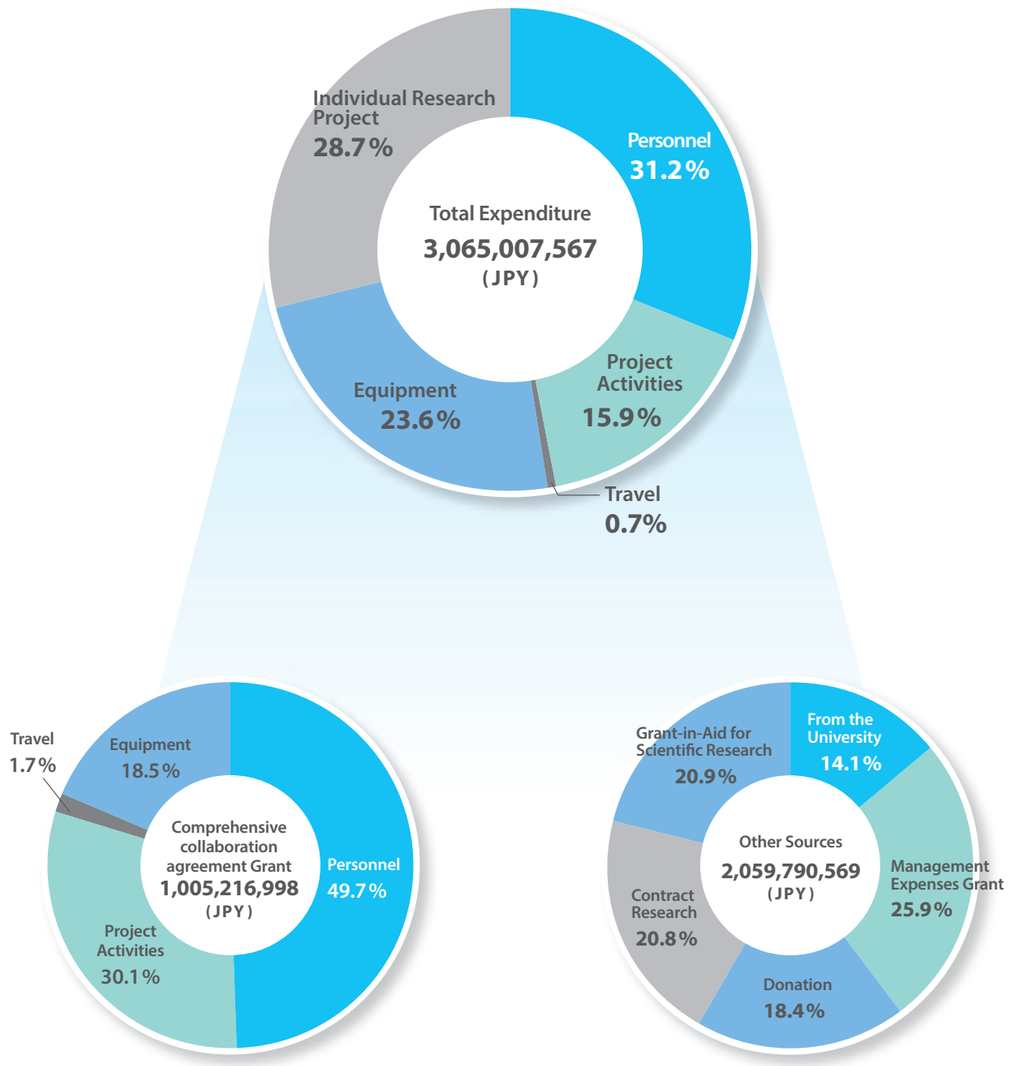



Number of Researchers




## Finance

Break down of total expenditure at IFReC in FY2018



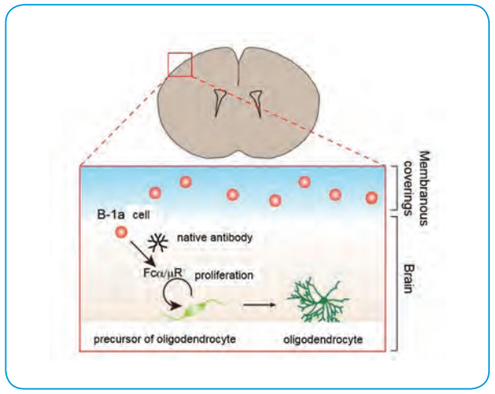
# Selected Articles

## B-1a lymphocytes promote oligodendrogenesis during brain development

***Nature Neuroscience*** 21:506–516 (2018).

Tanabe S and Yamashita T.

Toshiihide Yamashita (Molecular Neuroscience, IFReC) and his research group identified the subtypes of lymphocytes that are present in neonatal mouse brains and investigated their functions. They found that B-1a cells, a subtype of B cells, were abundant in the neonatal mouse brain and infiltrated into the brain in a CXCL13–CXCR5-dependent manner. B-1a cells promoted the proliferation of oligodendrocyte-precursor cells (OPCs) in vitro, and depletion of B-1a cells from developing brains resulted in a reduction of numbers of OPCs and mature oligodendrocytes. Furthermore, neutralizing Fcα/μR, the receptor for the Fc region of IgM secreted by B-1a cells, inhibited OPC proliferation and reduced the proportion of myelinated axons in neonatal mouse brains. These results demonstrate that B-1a cells infiltrate into the brain and contribute to oligodendrogenesis and myelination by promoting OPC proliferation via IgM–Fcα/μR signaling.



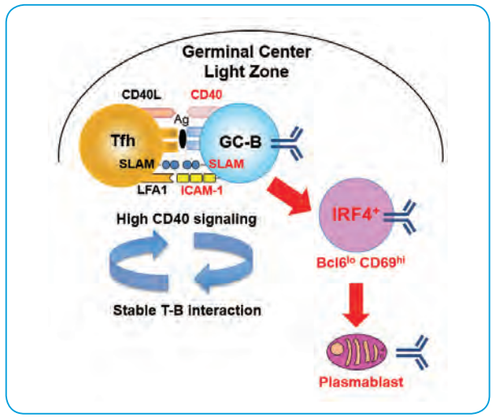

***Immunity.*** 48:702-715 (2018).

## T follicular helper cell-germinal center B cell interaction strength regulates entry into plasma cell or recycling GC cell fate

***Immunity*** 48:702-715 (2018).

Ise W, Fujii K, Shiroguchi K, et al.

Wataru Ise, Tomohiro Kurosaki (Lymphocyte Differentiation, IFReC) and the research group discovered how high affinity antibodies, which are essential for host protection from pathogens, are generated. The findings in this study are expected to contribute to the development of novel vaccine that targets efficient production of antibody against various virus. Using mouse model, the study clarified the cellular and molecular mechanism by which "high quality" antibodies, which have high affinity against pathogens such as influenza virus, are developed during immune response. Upon invasion of pathogens to our body, B cells are activated and differentiated to plasma cells which produce pathogen-specific antibodies. Importantly, some of activated B cells form germinal centers, microenvironments where B cells with high affinity antibodies are generated. Thus, germinal center B cells are sources of plasma cells producing high affinity antibodies. This study analyzed germinal center B cells carefully and identified plasma cell precursors among germinal center B cells. Furthermore, the study revealed what kind of signals or molecules are involved in the development of such plasma cell precursors in germinal center. Together, the efficient induction of plasma cell precursors in germinal center would be the one of the targets of new vaccine.

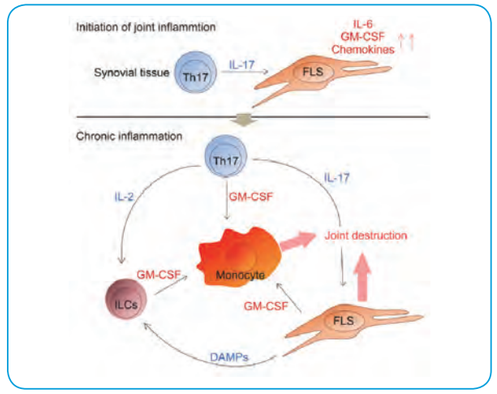



## Autoimmune Th17 cells induced synovial stromal and innate lymphoid cell secretion of GM-CSF to initiate and augment autoimmune arthritis

***Immunity*** 48:1220-1232.e5. (2018).

Hirota K, Hashimoto M, Yoshinaga I, et al.

Despite the importance of Th17 cells in autoimmune diseases, it remains unclear how they control other inflammatory cells in autoimmune tissue damage. Using a model of spontaneous autoimmune arthritis, Hirota and Sakaguchi's group (Experimental Immunology, IFReC) showed arthritogenic Th17 cells stimulated fibroblast-like synoviocytes via interleukin-17 (IL-17) to secrete the cytokine GM-CSF and also expanded synovial-resident innate lymphoid cells (ILCs) in inflamed joints. Activated synovial ILCs, which expressed CD25, IL33Ra, and TLR9, produced abundant GM-CSF upon stimulation by IL-2, IL-33, or CpG DNA. Loss of GM-CSF production by either ILCs or radio-resistant stroma cells prevented Th17 cell-mediated arthritis. GM-CSF production by Th17 cells augmented chronic inflammation but was dispensable for the initiation of arthritis. The authors showed GM-CSF-producing ILCs were present in inflamed joints of rheumatoid arthritis patients. Thus, a cellular cascade of autoimmune Th17 cells, ILCs, and stroma cells, via IL-17 and GM-CSF, mediates chronic joint inflammation and can be a target for therapeutic intervention.

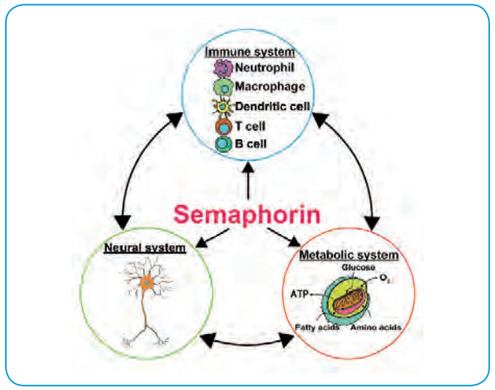



## Semaphorin 6D reverse signaling controls macrophage lipid metabolism and anti-inflammatory polarization

***Nature Immunology*** 19:561–570 (2018).

Kang S, Nakanishi Y, Kioi Y, et al.

Polarization of macrophages into pro-inflammatory or anti-inflammatory states has distinct metabolic requirements, with mechanistic target of rapamycin (mTOR) kinase signaling playing a critical role. However, it remains unclear how mTOR regulates metabolic status to promote polarization of these cells. Sujin Kang, Atsushi Kumanogoh (Immunopathology, IFReC) and the research group showed that an mTOR-Semaphorin 6D (Sema6D)-Peroxisome proliferator receptor γ (PPARγ) axis plays critical roles in macrophage polarization. Inhibition of mTOR or loss of Sema6D blocked anti-inflammatory macrophage polarization, concomitant with severe impairments in PPARγ expression, uptake of fatty acids, and lipid metabolic reprogramming. Macrophage expression of the receptor Plexin-A4 is responsible for Sema6D-mediated anti-inflammatory polarization. The group found that a tyrosine kinase, c-Abl, which associates with the cytoplasmic region of Sema6D, is required for PPARγ expression. Furthermore, Sema6D is important for generation of intestinal resident CX3CR1hi macrophages and prevents development of colitis. Collectively, these findings highlight crucial roles for Sema6D reverse signaling in macrophage polarization, coupling immunity, and metabolism via PPARγ.



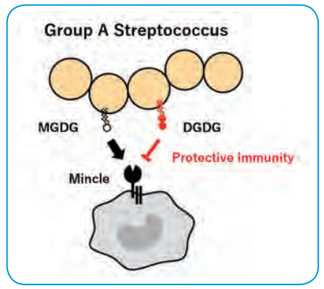
## Lipoteichoic acid anchor triggers Mincle to drive protective immunity against invasive group A Streptococcus infection

***Proc Natl Acad Sci USA*** 115:E10662-E10671 (2018).

Imai T, Matsumura T, Mayer-Lambertz S, et al.

Group A Streptococcus (GAS) causes invasive streptococcal infections in humans, resulting high mortality. Thus, GAS is also known as "killer bacteria" or "flesh-eating bacteria". However, the mechanisms by which the innate immune system recognizes GAS are not well understood.

Sho Yamasaki (Molecular Immunology, IFReC) and his research group reported that the C-type lectin receptor macrophage inducible C-type lectin (Mincle) recognizes GAS and initiates anti-bacterial immunity. Gene expression analysis of myeloid cells upon GAS stimulation revealed the contribution of the caspase recruitment domain-containing protein 9 (CARD9) pathway to the anti-bacterial responses. Among receptors signaling through CARD9, Mincle induced the production of inflammatory cytokines, inducible nitric oxide synthase (iNOS) and reactive oxygen species (ROS) upon recognition of the anchor of lipoteichoic acid (LTA), monoglucosyldiacylglycerol (MGDG), produced by GAS. Upon GAS infection, Mincle-deficient mice exhibited impaired production of pro-inflammatory cytokines, severe bacteremia and rapid lethality. GAS also possesses another Mincle ligand, diglucosyldiacylglycerol (DGDG); however, this glycolipid interfered with MGDG-induced activation. These results indicate that Mincle plays a central role in protective immunity against acute GAS infection.




## Humanized cereblon mice revealed two distinct therapeutic pathways of immunomodulatory drugs

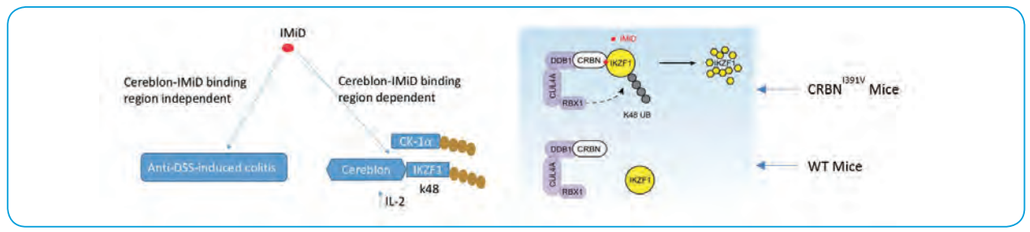
***Proc Natl Acad Sci USA*** 115:11802-11807 (2018).

Gemechu Y, Millrine D, Hashimoto S, et al.

After its appearance on the drug market, it was found out that thalidomide was highly teratogenic. Although thalidomide passed the safety check in pregnant mice, it was not safe among humans due to different actions of thalidomide among various species. Due to inactivity of immunomodulatory drugs (IMiDs) in mice, preclinical safety checks and clinical investigation of IMiDs is impossible in murine models. Further, murine cereblon (CRBN), the substrate receptor for IMiD action, is resistant to some of IMiDs therapeutic effects.

To overcome this difficulty, the research group of Tadamitsu Kishimoto (Immune Regulation, IFReC) generated humanized cereblon (CRBNI391V) mice thereby providing an animal model to unravel complex mechanisms of action in a murine physiological setup. This model may also permit investigation of the main safety concerns.

The group found the degradative effect of IMiDs on IKZF1 and CK-1α, as well as upregulation of IL-2, is dependent on the CRBN-IMiD binding region. Therefore, the anti-inflammatory bowel disease benefit of IMiD is mediated through a CRBN–IMiD binding region-independent pathway.



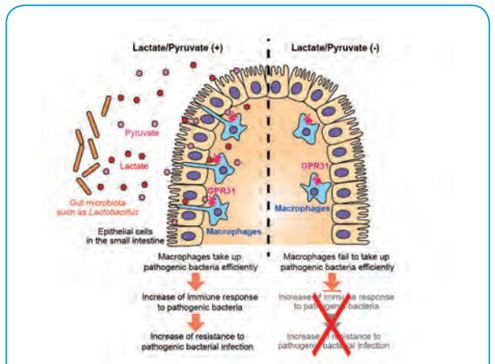

## GPR31-dependent dendrite protrusion of intestinal CX3CR1+ cells by bacterial metabolites

***Nature*** 566:110-114 (2019).

Morita N, Umemoto E, Fujita S, et al.

Eiji Umemoto, Naoki Morita, Kiyoshi Takeda (Mucosal Immunology, IFReC) and the research group showed common bacterial metabolites pyruvate and lactate enhance the intestinal immune response and guard against infection by important gut pathogens.

Gut microbiota such as lactobacillus produce lactate and pyruvate. These metabolites stimulate intestinal macrophages through the receptor GPR31, allowing macrophages to protrude trans-epithelial dendrites and take up pathogenic bacteria efficiently in the intestine. Accordingly, lactate and pyruvate cause enhanced immune responses to pathogenic bacteria and increased resistance to the infection.

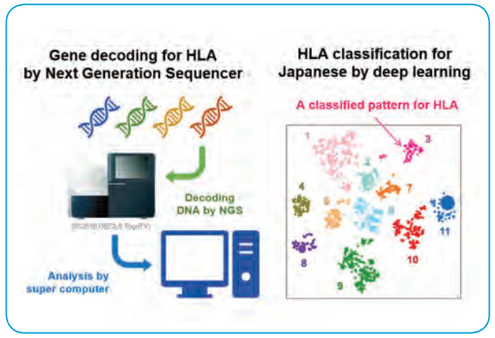



## Genetic and phenotypic landscape of the MHC region in the Japanese population

***Nature Genetics*** 51:470-480 (2019).

Hirata J, Hosomichi K, Sakaue S, et al.

Yukinori Okada (Statistical Immunology, IFReC) and the research group conducted NGS-based typing of the 33 human leukocyte antigen (HLA) genes of 1,120 Japanese, providing high resolution allele catalogue and linkage disequilibrium (LD) structure of both classical and non-classical HLA genes. Together with population-specific deep whole-genome sequencing (WGS) data (n = 1,276), they conducted NGS-based HLA, SNV, and indel imputation of large-scale genome-wide association (GWAS) data of 166,190 Japanese. A phenome-wide association study (PheWAS) assessing 106 clinical phenotypes identified abundant significant genotype-phenotype associations across 52 phenotypes. Fine-mapping highlighted multiple association patterns conferring independent risks from the classical HLA genes. Region-wide heritability estimates and genetic correlation network analysis elucidated polygenic architecture shared across the phenotypes.



# Publications


1 Adachi, T; Nakae, A; Maruo, T; Shi, K; Maeda, L; Saitoh, Y; Shibata, M; Sasaki, J. The Relationships Between Pain-Catastrophizing Subcomponents and Multiple Pain-Related Outcomes in Japanese Outpatients with Chronic Pain: A Cross-Sectional Study. Pain Practice 19, 27-36 (2019).

2 Akazawa, K; Sugihara, F; Minoshima, M; Mizukami, S; Kikuchi, K. Sensing caspase-1 activity using activatable F-19 MRI nanoprobes with improved turn-on kinetics. Chemical Communications 54, 11785-11788 (2018).

3 Akazawa, K; Sugihara, F; Nakamura, T; Matsushita, H; Mukai, H; Akimoto, R; Minoshima, M; Mizukami, S; Kikuchi, K. Perfluorocarbon-Based F-19 MRI Nanoprobes for In Vivo Multicolor Imaging. Angewandte Chemie-International Edition 57, 16742-16747 (2018).

4 Akazawa, K; Sugihara, F; Nakamura, T; Mizukami, S; Kikuchi, K. Highly Sensitive Detection of Caspase-3/7 Activity in Living Mice Using Enzyme-Responsive F-19 MRI Nanoprobes. Bioconjugate Chemistry 29, 1720-1728 (2018).

5 Baba, M; Endoh, M; Ma, WJ; Toyama, H; Hirayama, A; Nishikawa, K; Takubo, K; Hano, H; Hasumi, H; Umemoto, T; Hashimoto, M; Irie, N; Esumi, C; Kataoka, M; Nakagata, N; Soga, T; Yao, M; Kamba, T; Minami, T; Ishii, M; Suda, T. Folliculin Regulates Osteoclastogenesis Through Metabolic Regulation. Journal of Bone And Mineral Research 33, 1785-1798 (2018).

6 Balakrishnan, A; Karki, R; Berwin, B; Yamamoto, M; Kanneganti, TD. Guanylate binding proteins facilitate caspase-11-dependent pyroptosis in response to type 3 secretion system-negative Pseudomonas aeruginosa. Cell Death Discovery 4, (2018).

7 Bambouskova, M; Gorvel, L; Lampropoulou, V; Sergushichev, A; Loginicheva, E; Johnson, K; Korenfeld, D; Mathyer, ME; Kim, H; Huang, LH; Duncan, D; Bregman, H; Keskin, A; Santeford, A; Apte, RS; Sehgal, R; Johnson, B; Amarasinghe, GK; Soares, MP; Satoh, T; Akira, S; Hai, T; Strong, CD; Auclair, K; Roddy, TP; Biller, SA; Jovanovic, M; Klechevsky, E; Stewart, KM; Randolph, GJ; Artyomov, MN. Electrophilic properties of itaconate and derivatives regulate the I kappa B zeta-ATF3 inflammatory axis. Nature 556, 501 (2018).

8 Bando, H; Lee, Y; Sakaguchi, N; Pradipta, A; Ma, JS; Tanaka, S; Cai, YH; Liu, JF; Shen, JL; Nishikawa, Y; Sasai, M; Yamamoto, M. Inducible Nitric Oxide Synthase Is a Key Host Factor for Toxoplasma GRA15-Dependent Disruption of the Gamma Interferon-Induced Antiparasitic Human Response. mBIO 9, e01738-18 (2018).

9 Bando, H; Sakaguchi, N; Lee, Y; Pradipta, A; Ma, JS; Tanaka, S; Lai, DH; Liu, JF; Lun, ZR; Nishikawa, Y; Sasai, M; Yamamoto, M. Toxoplasma Effector TgIST Targets Host IDO1 to Antagonize the IFN-gamma-Induced Anti-parasitic Response in Human Cells. Frontiers In Immunology 9, 2073 (2018).

10 Beshr, R; Isohashi, K; Watabe, T; Naka, S; Horitsugi, G; Romanov, V; Kato, H; Miyatake, SI; Shimosegawa, E; Hatazawa, J. Preliminary feasibility study on differential diagnosis between radiation-induced cerebral necrosis and recurrent brain tumor by means of [F-18]fluoro-borono-phenylalanine PET/CT. Annals of Nuclear Medicine 32, 702-708 (2018).

11 Briard, B; Karki, R; Malireddi, RKS; Bhattacharya, A; Place, DE; Mavuluri, J; Peters, JL; Vogel, P; Yamamoto, M; Kanneganti, TD. Fungal ligands released by innate immune effectors promote inflammasome activation during Aspergillus fumigatus infection. Nature Microbiology 4, 316-327 (2019).

12 Chu, SW; Fujita, K; Kemper, B; Pavillon, N; Smith, NI. Trends in Label-Free Imaging. Optics Communications 422, 1-2 (2018).

13 Coban, C; Lee, MSJ; Ishii, KJ. Tissue-specific immunopathology during malaria infection. Nature Reviews Immunology 18, 266-278 (2018).

14 Deng, M; Gui, X; Kim, J; Xie, L; Chen, WN; Li, ZL; He, LC; Chen, YZ; Chen, HY; Luo, WG; Lu, ZG; Xie, JJ; Churchill, H; Xu, YX; Zhou, Z; Wu, GJ; Yu, CY; John, S; Hirayasu, K; Nguyen, N; Liu, XY; Huang, FF; Li, LK; Deng, H; Tang, HD; Sadek, AH; Zhang, LB; Huang, T; Zou, YZ; Chen, B; Zhu, H; Arase, H; Xia, NS; Jiang, YX; Collins, R; You, MJ; Homsi, J; Unni, N; Lewis, C; Chen, GQ; Fu, YX; Liao, XC; An, ZQ; Zheng, JK; Zhang, NY; Zhang, CC. LILRB4 signalling in leukaemia cells mediates T cell suppression and tumour infiltration. Nature 562, 605 (2018).

15 Diaz-Gallo, LM; Ramskold, D; Shchetynsky, K; Folkersen, L; Chemin, K; Brynedal, B; Uebe, S; Okada, Y; Alfredsson, L; Klareskog, L; Padyukov, L. Systematic approach demonstrates enrichment of multiple interactions between non-HLA risk variants and HLA-DRB1 risk alleles in rheumatoid arthritis. Annals of the Rheumatic Diseases 77, 1454-1462 (2018).

16 Edwards, CL; de Oca, MM; Rivera, FD; Kumar, R; Ng, SS; Wang, YL; Amante, FH; Kometani, K; Kurosaki, T; Sidwell, T; Kallies, A; Engwerda, CR. The Role of BACH2 in T Cells in Experimental Malaria Caused by Plasmodium chabaudi chabaudi AS. Frontiers In Immunology 9, -2578 (2018).

17 Ekemen, S; Uzay, A; Bassullu, N; Dikicioglu-Cetin, E; Matsuda, K; Ince, U; Coban, C. Does it take three to tango? An unsuspected multimorbidity of CD8(+) T cell lymphoproliferative disorder, malaria, and EBV infection. Malaria Journal 17, 349 (2018).

18 Flores-Langarica, A; Cook, C; Luda, KM; Persson, EK; Marshall, JL; Beristain-Covarrubias, N; Yam-Puc, JC; Dahlgren, M; Persson, JJ; Uematsu, S; Akira, S; Henderson, IR; Lindbom, BJ; Agace, W; Cunningham, AF. Intestinal CD103(+) CD11b(+) cDC2 Conventional Dendritic Cells Are Required for Primary CD4(+) T and B Cell Responses to Soluble Flagellin. Frontiers In Immunology 9, 2409 (2018).

19 Fujita, Y; Yamashita, T. Sirtuins in Neuroendocrine Regulation and Neurological Diseases. Frontiers In Neuroscience 12, 778 (2018).

20 Fujita, Y; Yamashita, T. Spatial organization of genome architecture in neuronal development and disease. Neurochemistry International 119, 49-56 (2018).

21 Fukata, T; Mizushima, T; Nishimura, J; Okuzaki, D; Wu, X; Hirose, H; Yokoyama, Y; Kubota, Y; Nagata, K; Tsujimura, N; Inoue, A; Miyoshi, N; Haraguchi, N; Takahashi, H; Hata, T; Matsuda, C; Kayama, H; Takeda, K; Doki, Y; Mori, M; Yamamoto, H. The Supercarbonate Apatite-MicroRNA Complex Inhibits Dextran Sodium Sulfate-Induced Colitis. Molecular Therapy-Nucleic Acids 12, 658-671 (2018).

22 Fukunaga, T; Fujita, Y; Kishima, H; Yamashita, T. Methylation dependent down-regulation of G0S2 leads to suppression of invasion and improved prognosis of IDH1-mutant glioma. Plos One 13, e0206552 (2018).

23 Gemechu, Y; Millrine, D; Hashimoto, S; Prakash, J; Sanchenkova, K; Metwally, H; Gyanu, P; Kang, SJ; Kishimoto, T. Humanized cereblon mice revealed two distinct therapeutic pathways of immunomodulatory drugs. Proceedings of the National Academy of Sciences of the United States of America 115, 11802-11807 (2018).

24 Gyori, D; Lim, EL; Grant, FM; Spensberger, D; Roychoudhuri, R; Shuttleworth, SJ; Okkenhaug, K; Stephens, LR; Hawkins, PT. Compensation between CSF1R(+) macrophages and Foxp3(+) Treg cells drives resistance to tumor immunotherapy. Jci Insight 3, e120631 (2018).

25 Ha, D; Tanaka, A; Kibayashi, T; Tanemura, A; Sugiyama, D; Wing, JB; Lim, EL; Teng, KWW; Adeegbe, D; Newell, EW; Katayama, I; Nishikawa, H; Sakaguchi, S. Differential control of human Treg and effector T cells in tumor immunity by Fc-engineered anti-CTLA-4 antibody. Proceedings of the National Academy of Sciences of the United States of America 116, 609-618 (2019).

26 Han, B; Akiyama, M; Kim, KK; Oh, H; Choi, H; Lee, CH; Jung, S; Lee, HS; Kim, EE; Cook, S; Haritunians, T; Yamazaki, K; Park, SH; Ye, BD; McGovern, DPB; Esaki, M; Kawaguchi, T; Khor, SS; Taylor, KD; Rotter, JI; Suzuki, Y; Matsui, T; Motoya, S; Bang, SY; Kim, TH; Momozawa, Y; Kamatani, Y; Tokunaga, K; Kubo, M; Okada, Y; Yang, SK; Song, K. Amino acid position 37 of HLA-DR beta 1 affects susceptibility to Crohn's disease in Asians. Human Molecular Genetics 27, 3901-3910 (2018).

27 Hanieh, H; Masuda, K; Metwally, H; Chalise, JP; Mohamed, M; Nyati, KK; Standley, DM; Li, SL; Higa, M; Zaman, MM; Kishimoto, T. Arid5a stabilizes OX40 mRNA in murine CD4(+) Tcells by recognizing a stem-loop structure in its 3 ' UTR. European Journal of Immunology 48, 593-604 (2018).

28 Hasegawa, T; Kikuta, J; Ishii, M. Imaging the Bone-Immune Cell Interaction in Bone Destruction. Frontiers in Immunology 10, 596 (2019).

29 Hashimoto, T; Takahashi, H; Sakaguchi, S. Regulatory T-cell deficiency and autoimmune skin disease: Beyond the scurfy mouse and immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome. Journal of Allergy and Clinical Immunology 142, 1754-1756 (2018).

30 Hayama, Y; Kimura, T; Takeda, Y; Nada, S; Koyama, S; Takamatsu, H; Kang, S; Ito, D; Maeda, Y; Nishide, M; Nojima, S; Sarashina-Kida, H; Hosokawa, T; Kinehara, Y; Kato, Y; Nakatani, T; Nakanishi, Y; Tsuda, T; Koba, T; Okada, M; Kumanogoh, A. Lysosomal Protein Lamtor1 Controls Innate Immune Responses via Nuclear Translocation of Transcription Factor EB. Journal of Immunology 200, 3790-3800 (2018).

31 Hayashi, T; Momota, M; Kuroda, E; Kusakabe, T; Kobari, S; Makisaka, K; Ohno, Y; Suzuki, Y; Nakagawa, F; Lee, MSJ; Coban, C; Onodera, R; Higashi, T; Motoyama, K; Ishii, KJ; Arima, H. DAMP-Inducing Adjuvant and PAMP Adjuvants Parallelly Enhance Protective Type-2 and Type-1 Immune Responses to Influenza Split Vaccination. Frontiers in Immunology 9, 2619 (2018).

32 Herndler-Brandstetter, D; Ishigame, H; Shinnakasu, R; Plajer, V; Stecher, C; Zhao, J; Lietzenmayer, M; Kroehling, L; Takumi, A; Kometani, K; Inoue, T; Kluger, Y; Kaech, SM; Kurosaki, T; Okada, T; Flavell, RA. KLRG1(+) Effector CD8(+) T Cells Lose KLRG1, Differentiate into All Memory T Cell Lineages, and Convey Enhanced Protective Immunity. Immunity 48, 716 (2018).

33 Hiradate, Y; Sasaki, E; Momose, H; Asanuma, H; Furuhata, K; Takai, M; Aoshi, T; Yamada, H; Ishii, KJ; Tanemura, K; Mizukami, T; Hamaguchi, I. Development of screening method for intranasal influenza vaccine and adjuvant safety in preclinical study. Biologicals 55, 43-52 (2018).

34 Hirata, J; Hosomichi, K; Sakaue, S; Kanai, M; Nakaoka, H; Ishigaki, K; Suzukil, K; Akiyama, M; Kishikawa, T; Ogawa, K; Masuda, T; Yamamoto, K; Hirata, M; Matsuda, K; Momozawa, Y; Inoue, I; Kubo, M; Kamatani, Y; Okada, Y. Genetic and phenotypic landscape of the major histocompatibilty complex region in the Japanese population. Nature Genetics 51, 470 (2019).

35 Hirota, K; Hashimoto, M; Ito, Y; Matsuura, M; Ito, H; Tanaka, M; Watanabe, H; Kondoh, G; Tanaka, A; Yasuda, K; Kopf, M; Potocnik, AJ; Stockinger, B; Sakaguchi, N; Sakaguchi, S. Autoimmune Th17 Cells Induced Synovial Stromal and Innate Lymphoid Cell Secretion of the Cytokine GM-CSF to Initiate and Augment Autoimmune Arthritis. Immunity 48, 1220 (2018).

36 Hobro, AJ; Smith, NI. Vibrational spectroscopic imaging of pathogens, microorganisms, and their interactions with host systems. Optics Communications 422, 75-84 (2018).

37 Huy, LV; Tanaka, C; Imai, T; Yamasaki, S; Miyamoto, T. Synthesis of 12-O-Mono- and Diglycosyl-oxystearates, a New Class of Agonists for the C-type Lectin Receptor Mincle. Acs Medicinal Chemistry Letters 10, 44-49 (2019).

38 Ichiyama, K; Dong, C. The role of miR-183 cluster in immunity. Cancer Letters 443, 108-114 (2019).

39 Ikeda, H; Hayashi, Y; Takahashi, N; Watabe, T; Kanai, Y; Shinohara, A; Kato, H; Watabe, H; Shimosegawa, E; Hatazawa, J. Application of astatine-210: Evaluation of astatine distribution and effect of pre-injected iodide in whole body of normal rats. Applied Radiation And Isotopes 139, 251-255 (2018).

40 Ikeno, Y; Seo, S; Iwaisako, K; Yoh, T; Nakamoto, Y; Fuji, H; Taura, K; Okajima, H; Kaido, T; Sakaguchi, S; Uemoto, S. Preoperative metabolic tumor volume of intrahepatic cholangiocarcinoma measured by F-18-FDG-PET is associated with the KRAS mutation status and prognosis. Journal of Translational Medicine 16, 95 (2018).

41 Imai, T; Matsumura, T; Mayer-Lambertz, S; Wells, CA; Ishikawa, E; Butcher, SK; Barnett, TC; Walker, MJ; Imamura, A; Ishida, H; Ikebe, T; Miyamoto, T; Ato, M; Ohga, S; Lepenies, B; van Sorge, NM; Yamasaki, S. Lipoteichoic acid anchor triggers Mincle to drive protective immunity against invasive group A Streptococcus infection. Proceedings of the National Academy of Sciences of the United States of America 115, E10662-E10671 (2018).

42 Imanishi, T; Unno, M; Kobayashi, W; Yoneda, N; Matsuda, S; Ikeda, K; Hoshii, T; Hirao, A; Miyake, K; Barber, GN; Arita, M; Ishii, KJ; Akira, S; Saito, T. Reciprocal regulation of STING and TCR signaling by mTORC1 for T-cell activation and function. Life Science Alliance 2, e201800282 (2019).

43 Inoue, T; Moran, I; Shinnakasu, R; Phan, TG; Kurosaki, T. Generation of memory B cells and their reactivation. Immunological Reviews 283, 138-149 (2018).

44 Ise, W; Fujii, K; Shiroguchi, K; Ito, A; Kometani, K; Takeda, K; Kawakami, E; Yamashita, K; Suzuki, K; Okada, T; Kurosaki, T. T Follicular Helper Cell-Germinal Center B Cell Interaction Strength Regulates Entry into Plasma Cell or Recycling Germinal Center Cell Fate. Immunity 48, 702 (2018).

45 Ise, W; Kurosaki, T. Plasma cell differentiation during the germinal center reaction. Immunological Reviews 288, 64-74 (2019).

46 Kanemaru, H; Yamane, F; Tanaka, H; Maeda, K; Satoh, T; Akira, S. BATF2 activates DUSP2 gene expression and up-regulates NF-kappa B activity via phospho-STAT3 dephosphorylation. International Immunology 30, 255-265 (2018).

47 Kang, SJ; Nakanishi, Y; Kioi, Y; Okuzaki, D; Kimura, T; Takamatsu, H; Koyama, S; Nojima, S; Nishide, M; Hayama, Y; Kinehara, Y; Kato, Y; Nakatani, T; Shimogori, T; Takagi, J; Toyofuku, T; Kumanogoh, A. Semaphorin 6D reverse signaling controls macrophage lipid metabolism and anti-inflammatory polarization. Nature Immunology 19, 561 (2018).

48 Katayama, Y; Tachibana, M; Kurisu, N; Oya, Y; Terasawa, Y; Goda, H; Kobiyama, K; Ishii, KJ; Akira, S; Mizuguchi, H; Sakurai, F. Oncolytic Reovirus Inhibits Immunosuppressive Activity of Myeloid-Derived Suppressor Cells in a TLR3-Dependent Manner. Journal of Immunology 200, 2987-2999 (2018).

49 Kato, Y; Park, J; Takamatsu, H; Konaka, H; Aoki, W; Aburaya, S; Ueda, M; Nishide, M; Koyama, S; Hayama, Y; Kinehara, Y; Hirano, T; Shima, Y; Narazaki, M; Kumanogoh, A. Apoptosis-derived membrane vesicles drive the cGAS-STING pathway and enhance type I IFN production in systemic lupus erythematosus. Annals of the Rheumatic Diseases 77, 1507-1515 (2018).

50 Kawano, M; Nagata, S. Efferocytosis and autoimmune disease. International Immunology 30, 551-558 (2018).

51 Kayama, H; Kohyama, M; Okuzaki, D; Motooka, D; Barman, S; Okumura, R; Muneta, M; Hoshino, K; Sasaki, I; Ise, W; Matsuno, H; Nishimura, J; Kurosaki, T; Nakamura, S; Arase, H; Kaisho, T; Takeda, K. Heme ameliorates dextran sodium sulfate-induced colitis through providing intestinal macrophages with noninflammatory profiles. Proceedings of the National Academy of Sciences of the United States of America 115, 8418-8423 (2018).

52 Kayama, H; Takeda, K. Recasting the Tissue-Resident Lymphocyte in Celiac Disease. Immunity 50, 549-551 (2019).

53 Kenai, M; Maeda, Y; Okada, Y. Grimon: graphical interface to visualize multi-omics networks. Bioinformatics 34, 3934-3936 (2018).

54 Kidoya, H; Muramatsu, F; Shimamura, T; Jia, WZ; Satoh, T; Hayashi, Y; Naito, H; Kunisaki, Y; Arai, F; Seki, M; Suzuki, Y; Osawa, T; Akira, S; Takakura, N. Regnase-1-mediated post-transcriptional regulation is essential for hematopoietic stem and progenitor cell homeostasis. Nature Communications 10, 1072 (2019).

55 Kinehara, Y; Nagatomo, I; Koyama, S; Ito, D; Nojima, S; Kurebayashi, R; Nakanishi, Y; Suga, Y; Nishijima-Futami, Y; Osa, A; Nakatani, T; Kato, Y; Nishide, M; Hayama, Y; Higashiguchi, M; Morimura, O; Miyake, K; Kang, SJ; Minami, T; Hirata, H; Iwahori, K; Takimoto, T; Takamatsu, H; Takeda, Y; Hosen, N; Hoshino, S; Shintani, Y; Okumura, M; Kumagai, T; Nishino, K; Imamura, F; Nakatsuka, S; Kijima, T; Kida, H; Kumanogoh, A. Semaphorin 7A promotes EGFR-TKI resistance in EGFR mutant lung adenocarcinoma cells. Jci Insight 3, e123093 (2018).

56 Kishikawa, T; Momozawa, Y; Ozeki, T; Mushiroda, T; Inohara, H; Kamatani, Y; Kubo, M; Okada, Y. Empirical evaluation of variant calling accuracy using ultra-deep whole-genome sequencing data. Scientific Reports 9, 1784 (2019).

57 Kitadate, Y; Jorg, DJ; Tokue, M; Maruyama, A; Ichikawa, R; Tsuchiya, S; Segi-Nishida, E; Nakagawa, T; Uchida, A; Kimura-Yoshida, C; Mizuno, S; Sugiyama, F; Azami, T; Ema, M; Noda, C; Kobayashi, S; Matsuo, I; Kanai, Y; Nagasawa, T; Sugimoto, Y; Takahashi, S; Simons, BD; Yoshida, S. Competition for Mitogens Regulates Spermatogenic Stem Cell Homeostasis in an Open Niche. Cell Stem Cell 24, 79 (2019).

58 Kiyozumi, D; Taniguchi, Y; Nakano, I; Toga, J; Yagi, E; Hasuwa, H; Ikawa, M; Sekiguchi, K. Laminin gamma 1 C-terminal Glu to Gln mutation induces early postimplantation lethality. Life Science Alliance 1, e201800064 (2018).

59 Koga, S; Hozumi, K; Hirano, K; Yazawa, M; Terooatea, T; Minoda, A; Nagasawa, T; Koyasu, S; Moro, K. Peripheral PDGFR alpha(+) gp38(+) mesenchymal cells support the differentiation of fetal liver-derived ILC2. Journal of Experimental Medicine 215, 1609-1626 (2018).

60 Komine, O; Yamashita, H; Fujimori-Tonou, N; Koike, M; Jin, SJ; Moriwaki, Y; Endo, F; Watanabe, S; Uematsu, S; Akira, S; Uchiyama, Y; Takahashi, R; Misawa, H; Yamanaka, K. Innate immune adaptor TRIF deficiency accelerates disease progression of ALS mice with accumulation of aberrantly activated astrocytes. Cell Death And Differentiation 25, 2130-2146 (2018).

61 Kong, MS; Hashimoto-Tane, A; Kawashima, Y; Sakuma, M; Yokosuka, T; Kometani, K; Onishi, R; Carpino, N; Ohara, O; Kurosaki, T; Phua, KK; Saito, T. Inhibition of T cell activation and function by the adaptor protein CIN85. Science Signaling 12, eaav4373 (2019).

62 Kuwabara, R; Hamaguchi, M; Fukuda, T; Sakaguchi, S; Iwata, H. Preparation of Immunotolerant Space Under the Skin and Transplantation of Islets in the Space. Tissue Engineering Part A 25, 183-192 (2019).

63 Li, G; Martinez-Bonet, M; Wu, D; Yang, Y; Cui, J; Nguyen, HN; Cunin, P; Levescot, A; Bai, M; Westra, HJ; Okada, Y; Brenner, MB; Raychaudhuri, S; Hendrickson, EA; Maas, RL; Nigrovic, PA. High-throughput identification of noncoding functional SNPs via type IIS enzyme restriction. Nature Genetics 50, 1180 (2018).

64 Liu, BYC; Sarhan, J; Panda, A; Muendlein, HI; Ilyukha, V; Coers, J; Yamamoto, M; Isberg, RR; Poltorak, A. Constitutive Interferon Maintains GBP Expression Required for Release of Bacterial Components Upstream of Pyroptosis and Anti-DNA Responses. Cell Reports 24, 155 (2018).

65 Lu, XY; Nagata, M; Yamasaki, S. Mincle: 20 years of a versatile sensor of insults. International Immunology 30, 233-239 (2018).

66 Malik, R; Chauhan, G; Traylor, M; Sargurupremraj, M; Okada, Y; Mishra, A; Rutten-Jacobs, L; Giese, AK; van der Laan, SW; Gretarsdottir, S; Anderson, CD; Chong, M; Adams, HHH; Ago, T; Almgren, P; Amouyel, P; Ay, H; Bartz, TM; Benavente, OR; Bevan, S; Boncoraglio, GB; Brown, RD; Butterworth, AS; Carrera, C; Carty, CL; Chasman, DI; Chen, WM; Cole, JW; Correa, A; Cotlarciuc, I; Cruchaga, C; Danesh, J; de Bakker, PIW; DeStefano, AL; den Hoed, M; Duan, Q; Engelter, ST; Falcone, GJ; Gottesman, RF; Grewal, RP; Gudnason, V; Gustafsson, S; Haessler, J; Harris, TB; Hassan, A; Havulinna, AS; Heckbert, SR; Holliday, EG; Howard, G; Hsu, FC; Hyacinth, HI; Ikram, MA; Ingelsson, E; Irvin, MR; Jian, XQ; Jimenez-Conde, J; Johnson, JA; Jukema, JW; Kanai, M; Keene, KL; Kissela, BM; Kleindorfer, DO; Kooperberg, C; Kubo, M; Lange, LA; Langefeld, CD; Langenberg, C; Launer, LJ; Lee, JM; Lemmens, R; Leys, D; Lewis, CM; Lin, WY; Lindgren, AG; Lorentzen, E; Magnusson, PK; Maguire, J; Manichaikul, A; McArdle, PF; Meschia, JF; Mitchell, BD; Mosley, TH; Nalls, MA; Ninomiya, T; O'Donnell, MJ; Psaty, BM; Pulit, SL; Rannikmae, K; Reiner, AP; Rexrode, KM; Rice, K; Rich, SS; Ridker, PM; Rost, NS; Rothwell, PM; Rotter, JI; Rundek, T; Sacco, RL; Sakaue, S; Sale, MM; Salomaa, V; Sapkota, BR; Schmidt, R; Schmidt, CO; Schminke, U; Sharma, P; Slowik, A; Sudlow, CLM; Tanislav, C; Tatlisumak, T; Taylor, KD; Thijs, VNS; Thorleifsson, G; Thorsteinsdottir, U; Tiedt, S; Trompet, S; Tzourio, C; van Duijn, CM; Walters, M; Wareham, NJ; Wassertheil-Smoller, S; Wilson, JG; Wiggins, KL; Yang, Q; Yusuf, S; Bis, JC; Pastinen, T; Ruusalepp, A; Schadt, EE; Koplev, S; Bjorkegren, JLM; Codoni, V; Civelek, M; Smith, NL; Tregouet, DA; Christophersen, IE; Roselli, C; Lubitz, SA; Ellinor, PT; Tai, ES; Kooner, JS; Kato, N; He, J; van der Harst, P; Elliott, P; Chambers, JC; Takeuchi, F; Johnson, AD; Sanghera, DK; Melander, O; Jern, C; Strbian, D; Fernandez-Cadenas, I; Longstreth, WT; Rolfs, A; Hata, J; Woo, D; Rosand, J; Pare, G; Hopewell, JC; Saleheen, D; Stefansson, K; Worrall, BB; Kittner, SJ; Seshadri, S; Fornage, M; Markus, HS; Howson, JMM; Kamatani, Y; Debette, S; Dichgans, M. Multiancestry genome-wide association study of 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes. Nature Genetics 50, 524 (2018).

67 Maruyama, K; Takayama, Y; Sugisawa, E; Yamanoi, Y; Yokawa, T; Kondo, T; Ishibashi, K; Sahoo, BR; Takemura, N; Mori, Y; Kanemaru, H; Kumagai, Y; Martino, MM; Yoshioka, Y; Nishijo, H; Tanaka, H; Sasaki, A; Ohno, N; Iwakura, Y; Moriyama, Y; Nomura, M; Akira, S; Tominaga, M. The ATP Transporter VNUT Mediates Induction of Dectin-1-Triggered Candida Nociception. Iscience 6, 306 (2018).

68 Matsumoto, R; Dainichi, T; Tsuchiya, S; Nomura, T; Kitoh, A; Hayden, MS; Ishii, KJ; Tanaka, M; Honda, T; Egawa, G; Otsuka, A; Nakajima, S; Sakurai, K; Nakano, Y; Kobayashi, T; Sugimoto, Y; Kabashima, K. Epithelial TRAF6 drives IL-17-mediated psoriatic inflammation. JCI Insight 3, e121175 (2018).

69 Matsuura, Y; Kikuta, J; Kishi, Y; Hasegawa, T; Okuzaki, D; Hirano, T; Minoshima, M; Kikuchi, K; Kumanogoh, A; Ishii, M. In vivo visualisation of different modes of action of biological DMARDs inhibiting osteoclastic bone resorption. Annals of the Rheumatic Diseases 77, 1220-1226 (2018).

70 Mazaki, Y; Higashi, T; Onodera, Y; Nam, JM; Hashimoto, A; Hashimoto, S; Horinouchi, T; Miwa, S. Endothelin type B receptor interacts with the 78-kDa glucose-regulated protein. Febs Letters 593, 644-651 (2019).

71 Mendoza, P; Martinez-Martin, N; Bovolenta, ER; Reyes-Garau, D; Hernansanz-Agustin, P; Delgado, P; Diaz-Munoz, MD; Oeste, CL; Fernandez-Pisonero, I; Castellano, E; Martinez-Ruiz, A; Alonso-Lopez, D; Santos, E; Bustelo, XR; Kurosaki, T; Alarcon, B. R-Ras2 is required for germinal center formation to aid B cells during energetically demanding processes. Science Signaling 11, eaal1506 (2018).

72 Mizuno, H; Kikuta, J; Ishii, M. In vivo live imaging of bone cells. Histochemistry and Cell Biology 149, 417-422 (2018).

73 Morita, N; Umemoto, E; Fujita, S; Hayashi, A; Kikuta, J; Kimura, I; Haneda, T; Imai, T; Inoue, A; Mimuro, H; Maeda, Y; Kayama, H; Okumura, R; Aoki, J; Okada, N; Kida, T; Ishii, M; Nabeshima, R; Takeda, K. GPR31-dependent dendrite protrusion of intestinal CX3CR1(+) cells by bacterial metabolites. Nature 566, 110 (2019).

74 Nagahama, Y; Shimoda, M; Mao, GL; Singh, SK; Kozakai, Y; Sun, X; Motooka, D; Nakamura, S; Tanaka, H; Satoh, T; Maeda, K; Akira, S. Regnase-1 controls colon epithelial regeneration via regulation of mTOR and purine metabolism. Proceedings of the National Academy of Sciences of the United States of America 115, 11036-11047 (2018).

75 Nagatake, T; Suzuki, H; Hirata, SI; Matsumoto, N; Wada, Y; Morimoto, S; Nasu, A; Shimojou, M; Kawano, M; Ogami, K; Tsujimura, Y; Kuroda, E; Iijima, N; Hosomi, K; Ishii, KJ; Nosaka, T; Yasutomi, Y; Kunisawa, J. Immunological association of inducible bronchus-associated lymphoid tissue organogenesis in Ag85B-rHPIV2 vaccine-induced anti-tuberculosis mucosal immune responses in mice. International Immunology 30, 471-481 (2018).

76 Naito, Y; Hamaoka, S; Kinoshita, M; Kainuma, A; Shimizu, M; Katoh, H; Moriyama, K; Ishii, KJ; Sawa, T. The protective effects of nasal PcrV-CpG oligonucleotide vaccination against Pseudomonas aeruginosa pneumonia. Microbiology And Immunology 62, 774-785 (2018).

77 Nakagawa, S; Serada, S; Kakubari, R; Hiramatsu, K; Sugase, T; Matsuzaki, S; Matsuzaki, S; Ueda, Y; Yoshino, K; Ohkawara, T; Fujimoto, M; Kishimoto, T; Kimura, T; Naka, T. Intratumoral Delivery of an Adenoviral Vector Carrying the SOCS-1 Gene Enhances T-Cell-Mediated Antitumor Immunity By Suppressing PD-L1. Molecular Cancer Therapeutics 17, 1941-1950 (2018).

78 Nakanishi, T; Fujita, Y; Yamashita, T. Neuropilin-1-mediated pruning of corticospinal tract fibers is required for motor recovery after spinal cord injury. Cell Death & Disease 10, 67 (2019).

79 Nakasone, A; Muramatsu, R; Kato, Y; Kawahara, Y; Yamashita, T. Myotube-derived factor promotes oligodendrocyte precursor cell proliferation. Biochemical And Biophysical Research Communications 500, 609-613 (2018).

80 Nakata, J; Nakae, Y; Kawakami, M; Morimoto, S; Motooka, D; Hosen, N; Fujiki, F; Nakajima, H; Hasegawa, K; Nishida, S; Tsuboi, A; Oji, Y; Oka, Y; Kumanogoh, A; Sugiyama, H. Wilms tumour 1 peptide vaccine as a cure-oriented post-chemotherapy strategy for patients with acute myeloid leukaemia at high risk of relapse. British Journal of Haematology 182, 287-290 (2018).

81 Narazaki, M; Kishimoto, T. The Two-Faced Cytokine IL-6 in Host Defense and Diseases. International Journal of Molecular Sciences 19, 3528 (2018).

82 Nawa, Y; Yonemaru, Y; Kasai, A; Oketani, R; Hashimoto, H; Smith, NI; Fujita, K. Saturated excitation microscopy using differential excitation for efficient detection of nonlinear fluorescence signals. Apl Photonics 3, 80805 (2018).

83 Nishibe, M; Katsuyama, Y; Yamashita, T. Developmental abnormality contributes to cortex-dependent motor impairments and higher intracortical current requirement in the reeler homozygous mutants. Brain Structure & Function 223, 2575-2587 (2018).

84 Nishizawa, N; Ito, Y; Eshima, K; Ohkubo, H; Kojo, K; Inoue, T; Raouf, J; Jakobsson, PJ; Uematsu, S; Akira, S; Narumiya, S; Watanabe, M; Majima, M. Inhibition of microsomal prostaglandin E synthase-1 facilitates liver repair after hepatic injury in mice. Journal of Hepatology 69, 110-120 (2018).

85 Noguchi, S; Honda, S; Saitoh, T; Matsumura, H; Nishimura, E; Akira, S; Shimizu, S. Beclin 1 regulates recycling endosome and is required for skin development in mice. Communications Biology 2, 37 (2019).

86 Nonaka, H; Nakanishi, Y; Kuno, S; Ota, T; Mochidome, K; Saito, Y; Sugihara, F; Takakusagif, Y; Aoki, I; Nagatoishi, S; Tsum, K; Sando, S. Design strategy for serine hydroxymethyltransferase probes based on retro-aldol-type reaction. Nature Communications 10, 876 (2019).

87 Okada, Y; Momozawa, Y; Sakaue, S; Kanai, M; Ishigaki, K; Akiyama, M; Kishikawa, T; Arai, Y; Sasaki, T; Kosaki, K; Suematsu, M; Matsuda, K; Yamamoto, K; Kubo, M; Hirose, N; Kamatani, Y. Deep whole-genome sequencing reveals recent selection signatures linked to evolution and disease risk of Japanese. Nature Communications 9, 1631 (2018).

88 Okamoto, K; Germond, A; Fujita, H; Furusawa, C; Okada, Y; Watanabe, TM. Single cell analysis reveals a biophysical aspect of collective cell-state transition in embryonic stem cell differentiation. Scientific Reports 8, 11965 (2018).

89 Okumura, R; Takeda, K. Maintenance of intestinal homeostasis by mucosal barriers. Inflammation and Regeneration 38, UNSP 5 (2018).

90 Okuyama, Y; Tanaka, Y; Jiang, JJ; Kamimura, D; Nakamura, A; Ota, M; Ohki, T; Higo, D; Ogura, H; Ishii, N; Atsumi, T; Murakami, M. Bmi1 Regulates I kappa B alpha Degradation via Association with the SCF Complex. Journal of Immunology 201, 2264-2272 (2018).

91 Ono, E; Murota, H; Mori, Y; Yoshioka, Y; Nomura, Y; Munetsugu, T; Yokozeki, H; Katayama, I. Sweat glucose and GLUT2 expression in atopic dermatitis: Implication for clinical manifestation and treatment. Plos One 13, e0195960 (2018).

92 Osa, A; Uenami, T; Koyama, S; Fujimoto, K; Okuzaki, D; Takimoto, T; Hirata, H; Yano, Y; Yokota, S; Kinehara, Y; Naito, Y; Otsuka, T; Kanazu, M; Kuroyama, M; Hamaguchi, M; Koba, T; Futami, Y; Ishijima, M; Suga, Y; Akazawa, Y; Machiyama, H; Iwahori, K; Takamatsu, H; Nagatomo, I; Takeda, Y; Kida, H; Akbay, EA; Hammerman, PS; Wong, KK; Dranoff, G; Mori, M; Kijima, T; Kumanogoh, A. Clinical implications of monitoring nivolumab immunokinetics in non-small cell lung cancer patients. Jci Insight 3, e59125 (2018).

93 Ota, F; Hirayama, T; Kizuka, Y; Yamaguchi, Y; Fujinawa, R; Nagata, M; Ismanto, HS; Lepenies, B; Aretz, J; Rademacher, C; Seeberger, PH; Angata, T; Kitazume, S; Yoshida, K; Betsuyaku, T; Kida, K; Yamasaki, S; Taniguchi, N. High affinity sugar ligands of C-type lectin receptor langerin. Biochimica et Biophysica Acta-General Subjects 1862, 1592-1601 (2018).

94 Pagnamenta, AT; Murakami, Y; Anzilotti, C; Titheradge, H; Oates, AJ; Morton, J; Kinoshita, T; Kini, U; Taylor, JC.. A homozygous variant disrupting the PIGH start-codon is associated with developmental delay, epilepsy, and microcephaly. Human Mutation 39, 822-826 (2018).

95 Raju, S; Kometani, K; Kurosaki, T; Shaw, AS; Egawa, T. The adaptor molecule CD2AP in CD4 T cells modulates differentiation of follicular helper T cells during chronic LCMV infection. Plos Pathogens 14, e1007053 (2018).

96 Saijou, E; Enomoto, Y; Matsuda, M; Kok, CYY; Akira, S; Tanaka, M; Miyajima, A. Neutrophils Alleviate Fibrosis in the CCl4-Induced Mouse Chronic Liver Injury Model. Hepatology Communications 2, 703-717 (2018).

97 Sakaguchi, Y; Nishikawa, K; Seno, S; Matsuda, H; Takayanagi, H; Ishii, M. Roles of Enhancer RNAs in RANKL-induced Osteoclast Differentiation Identified by Genome-wide Cap-analysis of Gene Expression using CRISPR/Cas9. Scientific Reports 8, 7504 (2018).

98 Sakaue, S; Hirata, J; Maeda, Y; Kawakami, E; Nii, T; Kishikawa, T; Ishigaki, K; Terao, C; Suzuki, K; Akiyama, M; Suita, N; Masuda, T; Ogawa, K; Yamamoto, K; Saeki, Y; Matsushita, M; Yoshimura, M; Matsuoka, H; Ikari, K; Taniguchi, A; Yamanaka, H; Kawaji, H; Lassmann, T; Itoh, M; Yoshitomi, H; Ito, H; Ohmura, K; Forrest, ARR; Hayashizaki, Y; Carninci, P; Kumanogoh, A; Kamatani, Y; de Hoon, M; Yamamoto, K; Okada, Y. Integration of genetics and miRNA-target gene network identified disease biology implicated in tissue specificity. Nucleic Acids Research 46, 11898-11909 (2018).

99 Sakuragi, T; Kosako, H; Nagata, S. Phosphorylation-mediated activation of mouse Xkr8 scramblase for phosphatidylserine exposure. Proceedings of the National Academy of Sciences of the United States of America 116, 2907-2912 (2019).

100 Segawa, K; Yanagihashi, Y; Yamada, K; Suzuki, C; Uchiyama, Y; Nagata, S. Phospholipid flippases enable precursor B cells to flee engulfment by macrophages. Proceedings of the National Academy of Sciences of the United States of America 115, 12212-12217 (2018).

101 Sekido, Y; Yasumizu, Y; Nishimura, J; Kayama, H; Matsuno, H; Ogino, T; Miyoshi, N; Takahashi, H; Haraguchi, N; Hata, T; Matsuda, C; Doki, Y; Mori, M; Takeda, K; Ohkura, N; Sakaguchi, S; Mizushima, T. Innate Myeloid Cell Subset-Specific Gene Expression Patterns in the Human Colon are Altered in Crohn's Disease Patients. Digestion 99, 194-204 (2019).

102 Shibata, N; Kunisawa, J; Hosomi, K; Fujimoto, Y; Mizote, K; Kitayama, N; Shimoyama, A; Mimuro, H; Sato, S; Kishishita, N; Ishii, KJ; Fukase, K; Kiyono, H. Lymphoid tissue-resident Alcaligenes LPS induces IgA production without excessive inflammatory responses via weak TLR4 agonist activity. Mucosal Immunology 11, 693-702 (2018).

103 Shigeta, N; Nakamura, H; Kumasawa, K; Imai, K; Saito, S; Sakaguchi, S; Kimura, T. Are naive T cells and class-switched memory (IgD(-) CD27(+)) B cells not essential for establishment and maintenance of pregnancy? Insights from a case of common variable immunodeficiency with pregnancy. Medical Hypotheses 121, 36-41 (2018).

104 Shiroyama, T; Nagatomo, I; Koyama, S; Hirata, H; Nishida, S; Miyake, K; Fukushima, K; Shirai, Y; Mitsui, Y; Takata, S; Masuhiro, K; Yaga, M; Iwahori, K; Takeda, Y; Kida, H; Kumanogoh, A. Impact of sarcopenia in patients with advanced non-small cell lung cancer treated with PD-1 inhibitors: A preliminary retrospective study. Scientific Reports 9, 2447 (2019).

105 Shishido, T; Kohyama, M; Nakai, W; Matsumoto, M; Miyata, H; Suenaga, T; Arase, H. Invariant chain p41 mediates production of soluble MHC class II molecules. Biochemical And Biophysical Research Communications 509, 216-221 (2019).

106 Stocker, BL; Kodar, K; Wahi, K; Foster, AJ; Harper, JL; Mori, D; Yamasaki, S; Timmer, MSM. The effects of trehalose glycolipid presentation on cytokine production by GM-CSF macrophages. Glycoconjugate Journal 36, 69-78 (2019).

107 Sueyoshi, T; Kawasaki, T; Kitai, Y; Ori, D; Akira, S; Kawai, T. Hu Antigen R Regulates Antiviral Innate Immune Responses through the Stabilization of mRNA for Polo-like Kinase 2. Journal of Immunology 200, 3814-3824 (2018).

108 Sugawa, M; Masaike, T; Mikami, N; Yamaguchi, S; Shibata, K; Saito, K; Fujii, F; Toyoshima, YY; Nishizaka, T; Yajima, J. Circular orientation fluorescence emitter imaging (COFEI) of rotational motion of motor proteins. Biochemical And Biophysical Research Communications 504, 709-714 (2018).

109 Sugiyama, T; Omatsu, Y; Nagasawa, T. Niches for hematopoietic stem cells and immune cell progenitors. International Immunology 31, 5-11 (2019).

110 Suzuki, K; Akiyama, M; Ishigaki, K; Kanai, M; Hosoe, J; Shojima, N; Hozawa, A; Kadota, A; Kuriki, K; Naito, M; Tanno, K; Ishigaki, Y; Hirata, M; Matsuda, K; Iwata, N; Ikeda, M; Sawada, N; Yamaji, T; Iwasaki, M; Ikegawa, S; Maeda, S; Murakami, Y; Wakai, K; Tsugane, S; Sasaki, M; Yamamoto, M; Okada, Y; Kubo, M; Kamatani, Y; Horikoshi, M; Yamauchi, T; Kadowaki, T. Identification of 28 new susceptibility loci for type 2 diabetes in the Japanese population. Nature Genetics 51, 379 (2019).

111 Suzuki, K; Nakai, A. Immune modulation by neuronal electric shock waves. Journal of Allergy and Clinical Immunology 141, 2022-2023 (2018).

112 Svensson, MND; Doody, KM; Schmiedel, BJ; Bhattacharyya, S; Panwar, B; Wiede, F; Yang, S; Santelli, E; Wu, DJ; Sacchetti, C; Gujar, R; Seumois, G; Kiosses, WB; Aubry, I; Kim, G; Mydel, P; Sakaguchi, S; Kronenberg, M; Tiganis, T; Tremblay, ML; Ay, F; Vijayanand, P; Bottini, N. Reduced expression of phosphatase PTPN2 promotes pathogenic conversion of Tregs in autoimmunity. Journal of Clinical Investigation 129, 1193-1210 (2019).

113 Takahashi, H; Misato, K; Aoshi, T; Yamamoto, Y; Kubota, Y; Wu, X; Kuroda, E; Ishii, KJ; Yamamoto, H; Yoshioka, Y. Carbonate Apatite Nanoparticles Act as Potent Vaccine Adjuvant Delivery Vehicles by Enhancing Cytokine Production Induced by Encapsulated Cytosine-Phosphate-Guanine Oligodeoxynucleotides. Frontiers in Immunology 9, 783 (2018).

114 Takaoka, Y; Uchinomiya, S; Kobayashi, D; Endo, M; Hayashi, T; Fukuyama, Y; Hayasaka, H; Miyasaka, M; Ueda, T; Shimada, I; Hamachi, I. Endogenous Membrane Receptor Labeling by Reactive Cytokines and Growth Factors to Chase Their Dynamics in Live Cells. Chem 4, 1451-1464 (2018).

115 Takeda, K; Sakakibara, S; Yamashita, K; Motooka, D; Nakamura, S; El Hussien, MA; Katayama, J; Maeda, Y; Nakata, M; Hamada, S; Standley, DM; Hayama, M; Shikina, T; Inohara, H; Kikutani, H. Allergic conversion of protective mucosal immunity against nasal bacteria in patients with chronic rhinosinusitis with nasal polyposis. Journal of Allergy and Clinical Immunology 143, 1163 (2019).

116 Takeuchi, A; Ozawa, M; Kanda, Y; Kozai, M; Ohigashi, I; Kurosawa, Y; Rahman, MA; Kawamura, T; Shichida, Y; Umemoto, E; Miyasaka, M; Ludewig, B; Takahama, Y; Nagasawa, T; Katakai, T. A Distinct Subset of Fibroblastic Stromal Cells Constitutes the Cortex-Medulla Boundary Subcompartment of the Lymph Node. Frontiers In Immunology 9, 2196 (2018).

117 Tanabe, S; Fujita, Y; Ikuma, K; Yamashita, T. Inhibiting repulsive guidance molecule-a suppresses secondary progression in mouse models of multiple sclerosis. Cell Death & Disease 9, 1061 (2018).

118 Tanabe, S; Yamashita, T. B lymphocytes: Crucial contributors to brain development and neurological diseases. Neuroscience Research 139, 37-41 (2019).

119 Tanabe, S; Yamashita, T. B-1a lymphocytes promote oligodendrogenesis during brain development. Nature Neuroscience 21, 506 (2018).

120 Tanabe, S; Yamashita, T. The role of immune cells in brain development and neurodevelopmental diseases. International Immunology 30, 437-444 (2018).

121 Tanaka, K; Martinez, GJ; Yan, XW; Long, WW; Ichiyama, K; Chi, XX; Kim, BS; Reynolds, JM; Chung, Y; Tanaka, S; Liao, L; Nakanishi, Y; Yoshimura, A; Zheng, P; Wang, XH; Tian, Q; Xu, JM; O'Malley, BW; Dong, C. Regulation of Pathogenic T Helper 17 Cell Differentiation by Steroid Receptor Coactivator-3. Cell Reports 23, 2318-2329 (2018).

122 Tanaka, T; Narazaki, M; Kishimoto, T. Interleukin (IL-6) Immunotherapy. Cold Spring Harbor Perspectives in Biology 10, a028456 (2018).

123 Temizoz, B; Kuroda, E; Ishii, KJ. Combination and inducible adjuvants targeting nucleic acid sensors. Current Opinion in Pharmacology 41, 104-113 (2018).

124 Teraguchi, S; Kumagai, Y. Estimation of diffusion constants from single molecular measurement without explicit tracking. Bmc Systems Biology 12, 15 (2018).

125 Terao, C; Yoshifuji, H; Matsumura, T; Naruse, TK; Ishii, T; Nakaoka, Y; Kirino, Y; Matsuo, K; Origuchi, T; Shimizu, M; Maejima, Y; Amiya, E; Tamura, N; Kawaguchi, T; Takahashi, M; Setoh, K; Ohmura, K; Watanabe, R; Horita, T; Atsumi, T; Matsukura, M; Miyata, T; Kochi, Y; Suda, T; Tanemoto, K; Meguro, A; Okada, Y; Ogimoto, A; Yamamoto, M; Takahashi, H; Nakayamada, S; Saito, K; Kuwana, M; Mizuki, N; Tabara, Y; Ueda, A; Komuro, I; Kimura, A; Isobe, M; Mimori, T; Matsuda, F. Genetic determinants and an epistasis of LILRA3 and HLA-B(star)52 in Takayasu arteritis. Proceedings of the National Academy of Sciences of the United States of America 115, 13045-13050 (2018).

126 Teumer, A; Chaker, L; Groeneweg, S; Li, Y; Di Munno, C; Barbieri, C; Schultheiss, UT; Traglia, M; Ahluwalia, TS; Akiyama, M; Appel, EVR; Arking, DE; Arnold, A; Astrup, A; Beekman, M; Beilby, JP; Bekaert, S; Boerwinkle, E; Brown, SJ; De Buyzere, M; Campbell, PJ; Ceresini, G; Cerqueira, C; Cucca, F; Deary, IJ; Deelen, J; Eckardt, KU; Ekici, AB; Eriksson, JG; Ferrrucci, L; Fiers, T; Fiorillo, E; Ford, I; Fox, CS; Fuchsberger, C; Galesloot, TE; Gieger, C; Gogele, M; De Grandi, A; Grarup, N; Greiser, KH; Haljas, K; Hansen, T; Harris, SE; van Heemst, D; den Heijer, M; Hicks, AA; den Hollander, W; Homuth, G; Hui, JN; Ikrarm, MA; Ittermann, T; Jensen, RA; Jing, J; Jukema, JW; Kajantie, E; Kamatani, Y; Kasbohm, E; Kaufman, JM; Kiemeney, LA; Kloppenburg, M; Kronenberg, F; Kubo, M; Lahti, J; Lapauw, B; Li, S; Liewald, DCM; Lim, EM; Linneberg, A; Marina, M; Mascalzoni, D; Matsuda, K; Medenwald, D; Meisinger, C; Meulenbelt, I; De Meyer, T; zu Schwabedissen, HEM; Mikolajczyk, R; Moed, M; Netea-Maier, RT; Nolte, IM; Okadah, Y; Pala, M; Pattaro, C; Pedersen, O; Petersmann, A; Porcu, E; Postmus, I; Pramstaller, PP; Psaty, BM; Ramos, YFM; Rawal, R; Redmond, P; Richards, JB; Rietzschel, ER; Rivadeneira, F; Roef, G; Rotter, JI; Sala, CF; Schlessinger, D; Selvin, E; Slagboom, PE; Soranzo, N; Sorensen, TIA; Spector, TD; Starr, JM; Stott, DJ; Taes, Y; Taliun, D; Tanaka, T; Thuesen, B; Tiller, D; Toniolo, D; Uitterlinden, AG; Visser, WE; Walsh, JP; Wilson, SG; Wolffenbuttel, BHR; Yang, Q; Zheng, HF; Cappola, A; Peeters, RP; Naitza, S; Volzke, H; Sanna, S; Kottgen, A; Visser, TJ; Medici, M. Genome-wide analyses identify a role for SLC17A4 and AADAT in thyroid hormone regulation. Nature Communications 9, 4455 (2018).

127 Tran, TT; Nguyen, NT; Pham, NB; Chu, HN; Nguyen, TD; Kishimoto, T; Chau, MV; Chu, HM. Hairy Root Cultures of Eurycoma longifolia and Production of Anti-inflammatory 9-Methoxycanthin-6-one. Natural Product Communications 13, 539-542 (2018).

128 Tsai, CY; Sakakibara, S; Yasui, T; Minamitani, T; Okuzaki, D; Kikutani, H. Bystander inhibition of humoral immune responses by Epstein-Barr virus LMP1. International Immunology 30, 579-590 (2018).

129 Tsuboi, A; Hashimoto, N; Fujiki, F; Morimoto, S; Kagawa, N; Nakajima, H; Hosen, N; Nishida, S; Nakata, J; Morita, S; Sakamoto, J; Oji, Y; Oka, Y; Sugiyama, H. A phase I clinical study of a cocktail vaccine of Wilms' tumor 1 (WT1) HLA class I and II peptides for recurrent malignant glioma. Cancer Immunology Immunotherapy 68, 331-340 (2019).

130 Uenaka, T; Satake, W; Cha, PC; Hayakawa, H; Baba, K; Jiang, SY; Kobayashi, K; Kanagawa, M; Okada, Y; Mochizuki, H; Toda, T. In silico drug screening by using genome-wide association study data repurposed dabrafenib, an anti-melanoma drug, for Parkinson's disease. Human Molecular Genetics 27, 3974-3985 (2018).

131 Vandenbon, A; Kumagai, Y; Lin, MJ; Suzuki, Y; Nakai, K. Waves of chromatin modifications in mouse dendritic cells in response to LPS stimulation. Genome Biology 19, 138 (2018).

132 Wang, YC; Hirata, T; Maeda, Y; Murakami, Y; Fujita, M; Kinoshita, T. Free, unlinked glycosylphosphatidylinositols on mammalian cell surfaces revisited. Journal of Biological Chemistry 294, 5038-5049 (2019).

133 Wing, JB; Tanaka, A; Sakaguchi, S. Human FOXP3(+) Regulatory T Cell Heterogeneity and Function in Autoimmunity and Cancer. Immunity 50, 302-316 (2019).

134 Wing, JB; Tekguc, M; Sakaguchi, S. Control of Germinal Center Responses by T-Follicular Regulatory Cells. Frontiers in Immunology 9, 1910 (2018).

135 Yamamoto, T; Masuta, Y; Momota, M; Kanekiyo, M; Kanuma, T; Takahama, S; Moriishi, E; Yasutomi, Y; Saito, T; Graham, BS; Takahashi, Y; Ishii, KJ. A unique nanoparticulate TLR9 agonist enables a HA split vaccine to confer Fc gamma R-mediated protection against heterologous lethal influenza virus infection. International Immunology 31, 81-90 (2019).

136 Yasuda, K; Kitagawa, Y; Kawakami, R; Isaka, Y; Watanabe, H; Kondoh, G; Kohwi-Shigematsu, T; Sakaguchi, S; Hirota, K. Satb1 regulates the effector program of encephalitogenic tissue Th17 cells in chronic inflammation. Nature Communications 10, 549 (2019).

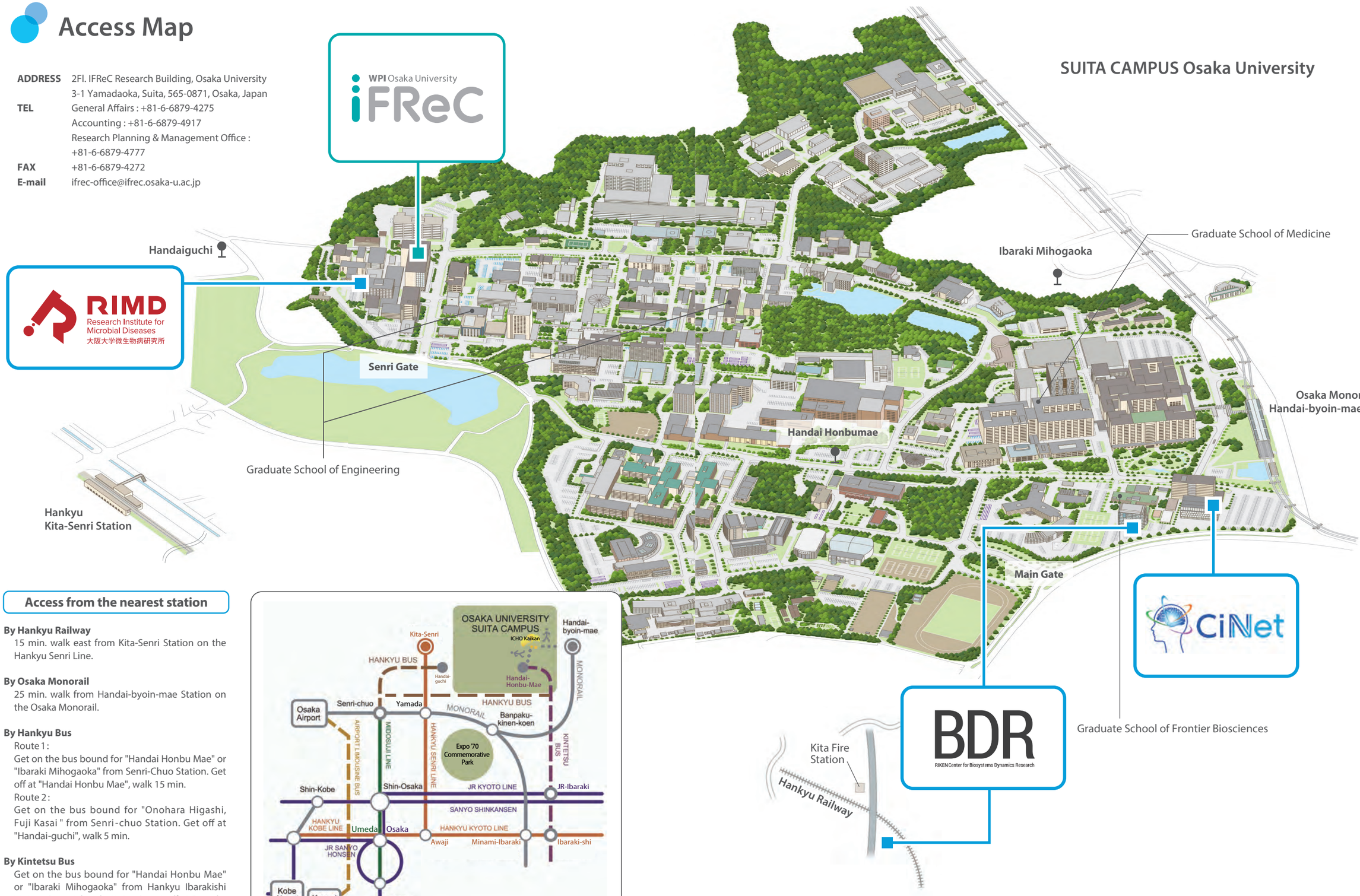
137 Yoshino, T; Miyazaki, J; Kojima, T; Kandori, S; Shiga, M; Kawahara, T; Kimura, T; Naka, T; Kiyohara, H; Watanabe, M; Yamasaki, S; Akaza, H; Yano, I; Nishiyama, H. Cationized liposomal keto-mycolic acids isolated from Mycobacterium bovis bacillus Calmette-Guerin induce antitumor immunity in a syngeneic murine bladder cancer model. Plos One 14, e0209196 (2019).

138 Zhang, SX; Fujita, Y; Matsuzaki, R; Yamashita, T. Class I histone deacetylase (HDAC) inhibitor CI-994 promotes functional recovery following spinal cord injury. Cell Death & Disease 9, 460 (2018).


The articles were published between April, 2018 and March, 2019. The data were acquired using Web of Science Core Collection™ on May 22, 2019, and sorted by alphabetical order of the first authors.

# Access Map

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




## Access from the nearest station

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

**By Osaka Monorail**
25 min. walk from Handai-byoin-mae Station on the Osaka Monorail.

**By Hankyu Bus**
Route 1 :
Get on the bus bound for "Handai Honbu Mae" or "Ibaraki Mihogaoka" from Senri-Chuo Station. Get off at "Handai Honbu Mae", walk 15 min.
Route 2 :
Get on the bus bound for "Onohara Higashi, Fuji Kasai " from Senri-chuo Station. Get off at "Handai-guchi", walk 5 min.

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