WPI Immunology Frontier Research Center 2019-2020

World Premier
International
Research Center

Osaka University

Immunology Frontier Research Center

Annual Report
of IFReC
2019-2020

Osaka University

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World Premier International
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WPI Osaka University
iFReC

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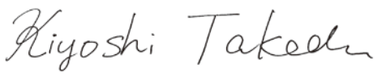
Message from the Director



Kiyoshi TAKEDA, MD/PhD
Director
WPI Immunology Frontier Research Center

As the Director of the Immunology Frontier Research Center (WPI-IFReC) at Osaka University, I am very pleased to present the IFReC annual report for the year 2019-2020.

Since its inception in 2007, IFReC has established itself as a high-profile international research center of immunology with the broad support of many people. Furthermore, since the establishment of the WPI Academy in 2017, IFReC has strived to create a new mark in its history through a novel academic-industry partnership agreement.

In 2019, I assumed the directorship of IFReC from Dr. Shizuo Akira. Fortunately, in 2019-2020, we were able to make great strides in our research resulting in many academic achievements. In addition, we embarked on a couple of new initiatives. The first is the emphasis on human immunology research. The IFReC Human Immunology Laboratory was launched in 2019 and two new PIs have joined. In this laboratory, the human immune system will be analyzed through the collaboration of the many research colleagues in IFReC. The second is the promotion of international cooperation. We started mutual visits and joint symposiums with overseas partner institutions, such as the University of Bonn (Germany) and the University College of London (UK), and we also promoted cooperation with institutions in Asia. The NIF Winter School, which began as an international educational program, will celebrate its 10th anniversary next year. We are proud of our graduates who have taken up prominent positions at world-famous institutions. We hope that our Winter School contributes to the worldwide circulation of talented young researchers, which is one of the aims of the WPI.

Starting from the latter part of last year, coronavirus disease 2019 (COVID-19) caused by a coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread throughout the world and turned into a pandemic. As various countries deal with this ongoing crisis, we continue our efforts in basic immunology research and look for ways in which we can contribute to the society in the fight against COVID-19.

We are totally committed to continuing our contributions to scientific advances through research and education and our evolvement into a world-class immunology research center.

Organization

Organization Chart

As of June, 2020

Osaka University
International Advanced Research Institute (IARI)

MEXT
WPI Initiative Program Committee
JSPS

WPI Osaka University iFReC

International Scientific Advisory Board

Director

Research Groups
- **Immunology Group**
- **Imaging Group**
- **Bioinformatics Group**
- **Combined Research Fields**

Deputy Directors

Management Committee — **Board of Representatives**

Administrative Director — **Administrative Department**
- General Affairs Section
- Accounting Section

Research Planning and Management Office

Joint Research Chairs with the Pharmaceutical Companies
- Innovative Drug Discovery in Immunology (Chugai Pharmaceutical)
- Immune-therapeutic Drug Discovery (Otsuka Pharmaceutical)
- Innate Immunity
- Innovative Drug Discovery in Host Defense

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

Cooperative Institutions
- Institute for Frontier Life and Medical Sciences, Kyoto University, Japan
- RIKEN Center for Integrative Medical Sciences, Japan
- National Institute of Biomedical Innovation, Health and Nutrition, Japan
- ImmunoSensation, Clusters of Excellence, the Rheinische Friedrich-Wilhelms-University of Bonn, Germany

Functions of Administrative Office

General Affairs Section
- Employment, acceptance, and management of work hours for researchers and staff
- Social insurance (for part-time staff), employment insurance
- Various research supports (MTA, patents, animal experiments, safety etc.)
- Support for international researchers and students
- IFReC Kishimoto Foundation Fellowship Program

Accounting Section
- Budget drafting / implementation / management
- Acceptance and implementation of third-party funding
- Purchasing and payment of payroll, travel expense and honorarium
- Health insurance procedures
- Management of facilities

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.)

Committees & Advisory Board for IFReC

World Premier International Research Center Initiative (WPI)

Program Director

As of Mar. 2020

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 University Professor, University of Maryland, USA
Richard Dasher	Director, US-Asia Technology Management Center, Stanford University, USA
Victor Joseph Dzau	President, National Academy of Medicine, USA
Michinari Hamaguchi	President, Japan Science and Technology Agency (JST), 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 Nobel Laureate in Physics (1985)
Makoto Kobayashi	Honorary Professor Emeritus, High Energy Accelerator Research Organization, Japan Nobel Laureate in Physics (2008)
Kiyoshi Kurokawa	Professor Emeritus, National Graduate Institute for Policy Studies, Japan
Chuan Poh Lim	Chairman, Singapore Food Agency, Singapore
Hiroshi Matsumoto	President, RIKEN, Japan
Ryozo Nagai	President, Jichi Medical University, Japan
Michiharu Nakamura	Counselor to the President, JST, Japan
〈Chairperson〉 Ryoji Noyori	Director-General, Center for Research and Development Strategy, JST, Japan Nobel Laureate in Chemistry (2001)
Norihiko Suzuki	Chair of the Board/President, Akita International University, Japan
Harriet Wallberg	Professor, 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.

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. 2020

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. 2020

Jeffrey Ravetch	The Rockefeller University, USA	Immunology
Christopher Goodnow	The Garvan Institute of Medical Research, 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 E. Goldman	University of Pennsylvania, USA	Imaging

Laboratories

Laboratories of IFReC

Host Defense

Shizuo Akira

#InnateImmunity
#PathogenRecognition
#macrophage

Immunoglycobiology

Taroh Kinoshita
Yoshiko Murakami

#GPI-anchor
#ParoxysmalNocturnalHemoglobinuria

Immunopathology

Atsushi Kumanogoh

#ImmuneSemaphorin
#AutoimmuneDiseases
#T-cellActivation

Immunochemistry

Hisashi Arase

#MHC #neo-self
#MisfoldedProtein
#malaria

Immune Regulation

Tadamitsu Kishimoto

#rheumatism #IL-6
#Th17Differentiation

Immune Regulation

Hitoshi Kikutani

#SLE
#Anti-nuclearAntibody(ANA)

Mucosal Immunology

Kiyoshi Takeda

#GutImmunity
#InflammatoryBowelDisease(IBD)
#microbiota

Experimental Immunology

Shimon Sakaguchi

#Treg #ImmuneTolerance
#CancerImmunology

Cell Signaling

Takashi Saito

#T-cellActivation
#TCRSignal

Lymphocyte Differentiation

Tomohiro Kurosaki

#MemoryB-cell
#AntibodyProduction

Malaria Immunology

Cevayir Coban

#MalariaParasite
#vaccine

Vaccine Science

Ken J. Ishii

#vaccine
#adjuvant

Immunoparasitology

Masahiro Yamamoto

#parasite #toxoplasma
#ImmuneEvasion

Biochemistry and Immunology

Shigekazu Nagata

#macrophage
#CellDeathSignal
#apoptosis

Molecular Neuroscience

Toshihide Yamashita

#CentralNervousSystem
#encephalomyelitis

Stem Cell Biology and Developmental Immunology

Takashi Nagasawa

#CARCell #StemCell
#niche

Molecular Immunology

Sho Yamasaki

#lectin
#NovelImmuneReceptor

Aging Biology

Eiji Hara

#aging #SASP
#cancer

Oncogene Research

Masato Okada

#mTOR #SRC
#cancer

Signal Transduction

Nobuyuki Takakura

#BloodVessels #StemCell
#cancer

Cutaneous Immunology

Manabu Fujimoto

#IntractableSkinDiseases
#allergy

Innate Immune Systems

Kazuyo Moro

#ILC2
#AutoimmuneDiseases

Human Immunology

James B. Wing
Single Cell Immunology

Daisuke Okuzaki
Single Cell Genomics

#HumanDiseases
#SingleCell #genomics

Single Molecule Imaging

Toshio Yanagida
Ben Seymour

#SingleMoleculeImaging
#MembraneProtein

Immunology and Cell Biology

Masaru Ishii

#osteoclast #LiveImaging
#CancerMetastasis

Nuclear Medicine

Jun Hatazawa

#PET/MRI #FBPA
#CancerTherapy

Biophotonics

Nicholas Isaac Smith

#LabelFree #RamanScattering
#IntraCellImaging

Chemical Imaging Techniques

Kazuya Kikuchi

#ChemicalBiology
#FluorescentProbe

Immune Response Dynamics

Kazuhiro Suzuki

#AdrenergicRceptor
#LymphocyteTrafficking

Systems Immunology

Daron M. Standley

#ImmuneRepertoire
#ReceptorModeling

Statistical Immunology

Yukinori Okada

#StatisticalGenetics #BigData
#DiseaseRiskGenes

Host Defense

Shizuo Akira, MD/PhD

Professor	Shizuo Akira
Associate Professor	Kazuhiko Maeda Takashi Satoh
Assistant Professor	Hiroki Tanaka Kanako Kuniyoshi
Postdoctoral Fellow	2
Research Assistant	5
Visiting Scientist	7
Support Staff	5

We are focused on the innate immune system, which is an evolutionarily conserved host defense mechanism against various pathogens. Innate immune responses are initiated by pattern recognition receptors, 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.

Role of SatM, an atypical monocyte and committed progenitor involved in fibrosis

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. We identified a new macrophage subset that Ceacam1+Msr1+Ly6C-F4/80-Mac1+ monocytes, which we termed SatM (segregated-nucleus-containing atypical monocytes), share granulocyte characteristics, are regulated by C/EBPβ (CCAAT/enhancer binding protein beta), and are critical for fibrosis. To investigate the physiological role of SatM and related subsets, we recently identified an RNA-binding protein RBM7 that is a component of the NEXT (nuclear exosome targeting) complex. We found that the expression of Rbm7 is increased in the fibrotic phase. *Rbm7*-deletion in nonhematopoietic cells suppresses fibrosis. Dysregulated expression Rbm7 triggers apoptosis via nuclear degradation of noncoding RNA *Neat1*. Rbm7 in epithelial cells plays a critical role in the development of fibrosis by regulating ncRNA decay, thereby producing chemokines that recruit SatMs. Inhibition of RBM7 would provide an effective treatment of fibrosis in patients.

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 protein 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, yet the precise role of phosphorylation largely remains unknown. 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. 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. We showed that Regnase-1 controls colon epithelial regeneration by regulating protein kinase mTOR (mechanistic target of rapamycin kinase) and purine metabolism. During dextran sulfate sodium-induced intestinal epithelial injury and acute colitis, *Regnase-1*-deficiency in IECs (*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.

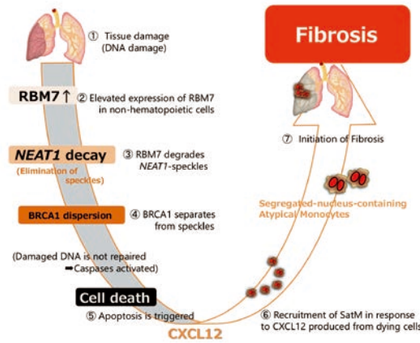


Figure 1. Dysregulated expression of the nuclear exosome targeting complex component Rbm7 in non-hematopoietic cells licenses the development of fibrosis.

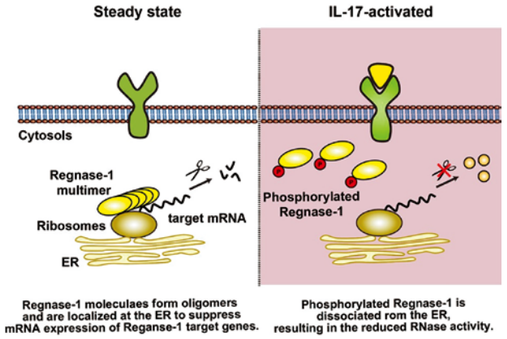


Figure 2. IL-17 induces Regnase-1 phosphorylation in an Act-1/TBK1/IKKi –dependent manner. Phosphorylation of Regnase-1 is released from the endoplasmic reticulum into the cytosols, resulting in loss of the mRNA degradation function.

Recent Publications

- Fukushima K, Satoh T., et al. Dysregulated expression of the nuclear exosome targeting complex component Rbm7 in non-hematopoietic cells licenses the development of fibrosis. Immunity 52, 542-556 (2020).
- Tanaka H., et al. Phosphorylation-dependent Regnase-1 release from endoplasmic reticulum is critical in IL-17 response. J. Exp. Med. 216, 1431-1449 (2019).
- Maeda K., et al. Innate immunity in allergy. Allergy 74, 1660-1674 (2019).
- 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).
- Satoh T., et al. Identification of an atypical monocyte and committed progenitor involved in fibrosis. Nature 541, 96-101 (2017).

Immunoglycobiology

Taroh Kinoshita

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

Professor	Taroh Kinoshita Yoshiko Murakami
Research Assistant	1
Visiting Scientist	3
Support Staff	4

We have been studying biosynthesis, functions and deficiencies of glycosylphosphatidylinositol (GPI) and GPI-anchored proteins (GPI-APs). In 2019, we made the following advances in studies on GPI deficiencies and biosynthesis.

Studies on paroxysmal nocturnal hemoglobinuria caused by *PIGT* mutations (PIGT-PNH)

In collaborations with Dr. Krawitz's and Dr. Schrezenmeier's groups in Germany and Dr. Kohara's and Dr. Kanakura's groups in Kobe and Osaka, respectively, we reported genetic, biochemical, immunological and clinical characteristics of patients with PNH caused by *PIGT* mutations, and compared them with those of classical PNH caused by mutations in *PIGA* (Hochsmann, Murakami, Osato et al. J. Clin. Invest. 2019). PNH, characterized by complement-mediated hemolysis and thrombosis, is caused by generation and clonal expansion of GPI-AP deficient hematopoietic stem cells. Single somatic mutation in X-linked *PIGA* causes GPI-AP-deficiency in classical PNH, whereas two mutations are required for *PIGT* on chromosome 20q to cause GPI-AP-deficiency. Patients with PIGT-PNH had a germline mutation in the maternal *PIGT* and in their PNH cells, the paternal *PIGT* was lost by a somatic, Mb-size deletion including the entire *PIGT* gene. We found that the Mb-size deletion always included a region called "myeloid common deleted region (CDR)" implicated in 20q- myeloproliferative neoplasm (Figure 1). The deletion results in loss of tumor-suppressive genes in the myeloid CDR because those in the maternal myeloid CDR are imprinted, suggesting causality to clonal expansion in PIGT-PNH. PIGA protein is essential for the initial step in GPI biosynthesis, so a PIGA defect does not cause accumulation of any GPI intermediate. In contrast, PIGT is essential for the transfer of preformed GPI to proteins. We showed accumulation of un-protein-linked free GPI in PIGT-defective cells (Figure 1). Clinically, patients with PIGT-PNH had recurrent autoinflammatory symptoms such as urticaria, arthralgia and aseptic meningitis, as well as typical PNH symptoms. Moreover, the patients had the autoinflammation for many years before the onset of PNH. Eculizumab, an anti-complement C5 antibody drug, was effective in preventing intravascular hemolysis, thrombosis, and inflammatory symptoms, suggesting that the autoinflammation was complement-dependent. Being consistent with the autoinflammation, elevation of serum IL-18 was documented. To clarify the mechanistic basis of autoinflammation, we used macrophages derived from PIGT-KO and PIGA-KO THP-1 cells. The PIGT-KO cells activated the lectin pathway of complement and bound more C4- and C3-fragments and C5b-9 membrane attack complexes than those on the PIGA-KO cells. The elevated activation of complement on the PIGT-KO cells was associated with higher levels of C5b-9 dependent IL-1β secretion by the PIGT-KO cells than the PIGA-KO cells. These results suggest that overactivation of complement and inflammasome in PIGT-defective cells is the basis of autoinflammation seen in PIGT-PNH and PIGT-PNH is a distinct form of PNH (Figure 1).

Studies on inherited GPI deficiencies (IGD)

In collaborations with Dr. Campeau's group in Canada and many clinicians and medical geneticists in several other countries, we reported the first group of individuals who suffer from IGD caused by bi-allelic hypomorphic mutations in the *PIGB* gene (Murakami et al. Am. J. Hum. Genet. 2019). We also contributed to a study on the first group of individuals with IGD caused by *PIGU* mutations (Knaus et al. Am. J. Hum. Genet. 2019).

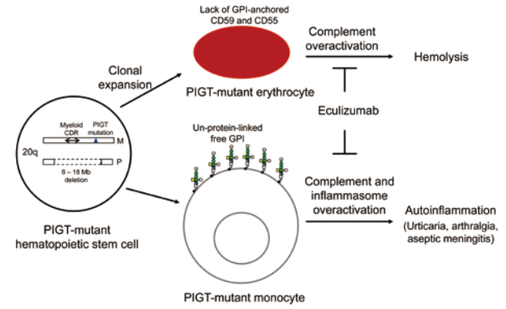


Figure 1. Mechanistic basis of PIGT-PNH. Patients' maternal chromosomes 20q have a germline loss-of-function PIGT mutation and imprinted myeloid common deleted region (CDR). Mutant hematopoietic stem cells in those patients lost paternal normal PIGT and active myeloid CDR because of a megabase-size deletion. The mutant stem cell expands similar to those of 20q- myeloproliferative neoplasm and generates PNH-type erythrocytes and monocytes. Intravascular hemolysis and recurrent autoinflammation occur because of uncontrolled activation of complement and inflammasomes. Anti-C5 antibody eculizumab is effective for both hemolysis and inflammatory symptoms. (Adopted from Hochsmann, B., Murakami, Y., Osato, M., et al., J. Clin. Invest., 2019, 129:5123-5136)

Studies on GPI biosynthesis

Some GPI-APs have an N-acetylgalactosamine (GalNAc)-side chain (Figure 2). The GalNAc side-chain can be elongated with β1-3-linked galactose (Gal). The Gal transferase (GPI-GalT) that mediates this elongation has been unknown. Through a CRISPR genome-wide knockout screen we identified B3GALT4, which is known as ganglioside GM1 synthase, as GPI-GalT (Figure 2). We also found that lactosylceramide is required for efficient GPI galactosylation. These results revealed close interactions of the GPI-AP biosynthetic pathway with the glycosphingolipid biosynthetic pathway in the Golgi apparatus (Wang Y et al. Nat. Commun. 2020).

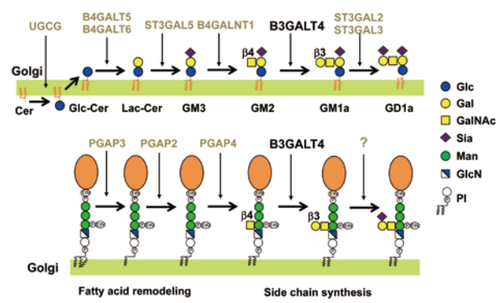


Figure 2. Identification of B3GALT4, previously known as ganglioside GM1 synthase, to be a galactosyltransferase that modifies N-acetylgalactosamine side chain of GPI anchors.

Recent Publications

- Wang Y, Maeda Y, Liu Y-S, Takada Y, Ninomiya A, Hirata T, Fujita M, Murakami M and Kinoshita T. Cross-talks of glycosylphosphatidylinositol biosynthesis with glycosphingolipid biosynthesis and ER-associated degradation. Nat. Commun.11, 860 (2020).
- Hochsmann B, Murakami Y, Osato M, Knaus A, Kawamoto M, Inoue N, Hirata T, Murata S, Anliker M, Eggerman T, Jaeger M, Floettmann R, Hoellein A, Murase S, Ueda Y, Nishimura J, Kanakura Y, Kohara N, Schrezenmeier H, Krawitz PM and Kinoshita T. Complement and inflammasome overactivation mediates paroxysmal nocturnal hemoglobinuria with autoinflammation. J. Clin. Invest.129, 5123-5136 (2019).
- 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 and 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 and Murakami Y. Phenotype-genotype correlations of PIGO deficiency with variable phenotypes from infantile lethality to mild learning difficulties. Hum. Mutat.38, 805-815 (2017).

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 Sema4D in Eosinophilic chronic rhinosinusitis (ECRS). ECRS is characterized by intractable nasal polyps with eosinophilic infiltration based on Th2 inflammation. Conservative treatments for ECRS are endoscopic sinus surgery (ESS) and systemic administration of glucocorticoids. However, ECRS frequently recurs after ESS, and glucocorticoid therapy occasionally causes multiple side effects. Therefore, it is necessary to identify not only the disease pathogenesis, but also potentially novel therapeutic targets and/or predictive biomarkers for ECRS.

We found serum soluble SEMA4D levels were significantly higher in ECRS patients than in patients with other paranasal diseases and serum SEMA4D levels were positively correlated with disease features. The expression of membrane SEMA4D on eosinophils was downregulated in ECRS patients, whereas downregulation of SEMA4D was not detected in other leukocytes, suggesting that increasing of serum SEMA4D in ECRS patients is due to proteolytic cleavage of membrane-bound SEMA4D from eosinophils. Furthermore, we showed that MMP-9 levels were elevated in ECRS patients and MMP-9 proteolytically cleaved SEMA4D from the eosinophil surface, yielding its soluble form. Soluble SEMA4D promotes transendothelial migration of eosinophils, causing them to infiltrate nasal polyps, in addition, SEMA4D can increase nasal epithelium permeability, thereby facilitating allergen infiltration. Investigations using animal models demonstrated that loss of SEMA4D made ECRS milder. Furthermore, treatment with anti-SEMA4D antibody significantly reduced the inflammation. Collectively, these data show that the SEMA4D plays pivotal roles in ECRS pathogenesis. Moreover, our results reveal the potential utility of SEMA4D not only as a predictive biomarker, but also as a potentially novel therapeutic target in ECRS.

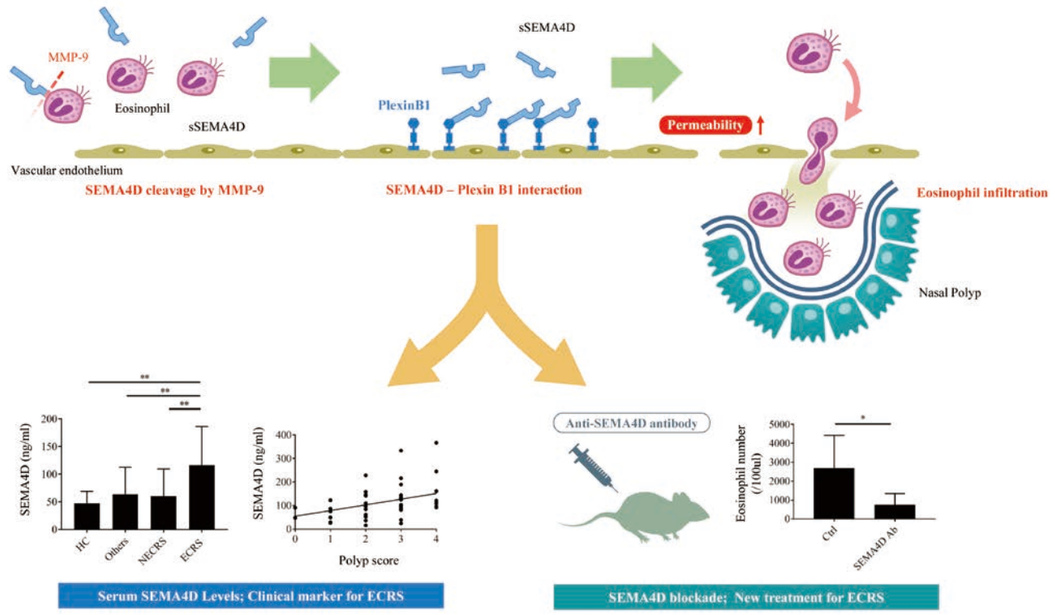


Figure. Serum soluble SEMA4D levels were elevated in patients with ECRS and positively correlated with disease severity. Cell surface expression of SEMA4D on eosinophils from ECRS patients was reduced, which was due to MMP-9-mediated cleavage of membrane SEMA4D. Soluble SEMA4D induced eosinophil transendothelial migration. Treatment with anti-SEMA4D antibody ameliorated eosinophilic infiltration in sinus tissues and nasal lavage fluid in the ECRS animal model.

Recent Publications

- Tsuda T, Nishide M, Maeda Y, Hayama Y, Koyama S, Nojima S, Takamatsu H, Okuzaki D, Morita T, Nakatani T, Kato Y, Nakanishi Y, Futami Y, Suga Y, Naito Y, Konaka H, Satoh S, Naito M, Izumi M, Obata S, Nakatani A, Shikina T, Takeda K, Hayama M, Inohara H, and Kumanogoh. Pathological and therapeutic implications of eosinophil-derived semaphorin 4D in eosinophilic chronic rhinosinusitis. J. Allergy Clin. Immunol. 145(3), 843-854 (2020).
- 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, Takagi J, Toyofuku T and Kumanogoh A. Semaphorin 6D reverse signaling controls macrophage lipid metabolism and anti-inflammatory polarization. Nature 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 *β*. Nat. Med. 23,1436-1443 (2017).

Immunochemistry

Hisashi Arase, MD/PhD

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We have been working on the interactions between pathogens and various paired receptors. In addition, we have found that MHC class II molecules function as molecular chaperones 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 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 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 β2GPI/HLA class II complex in the patients with refractory cutaneous ulcers (Arase et al. Br. J. Dermatol. 2017). Similarly, we also found that 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).

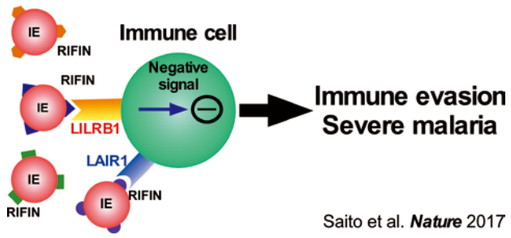


Figure 1. Immune evasion of *Plasmodium falciparum* by RIFIN via inhibitory receptors.
Plasmodium falciparum induces the expression of RIFINs on the surface of infected erythrocytes. Individual RIFINs may have evolved to target host inhibitory receptors, thus facilitating escape from host immune systems,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

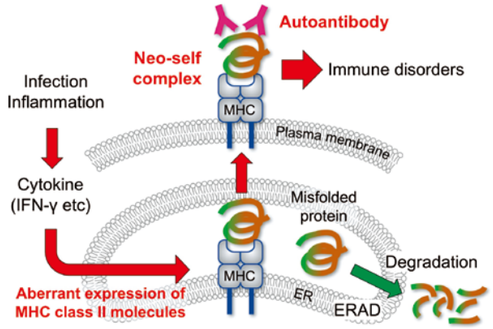
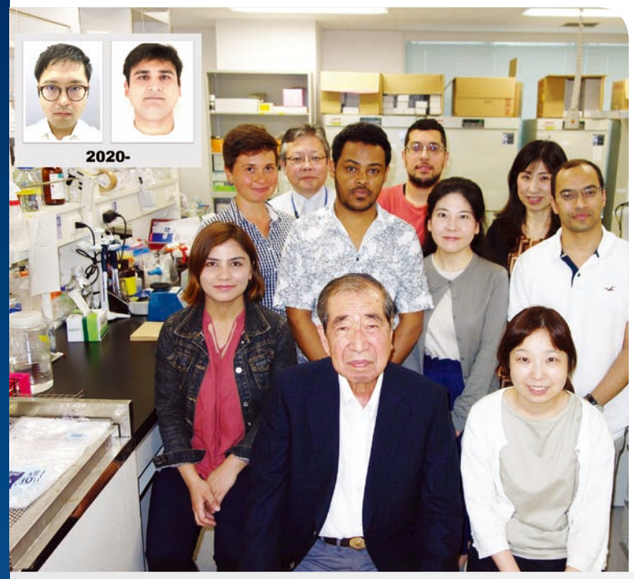


Figure 2. Misfolded proteins transported to the cell surface by MHC class II molecules are targets for autoantibodies.
Cellular misfolded proteins are generally degraded in the cells and are not transported to outside the cells. Therefore, misfolded proteins transported to the cell surface by MHC class II molecules may be recognized as 'neo-self' antigens by immune system, which might initiate aberrant immune response to self-antigens (Jiang et al. Int. Immunol. 2013; Jin et al. Proc. Natl. Acad. Sci. USA. 2014; Tanimura et al. Blood 2015, Arase Adv. Immunol. 2016; 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. 31, 303-314 (2019).
- 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. Nature Microbiology 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, 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

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IL-6 induced vascular endothelial cell activation plays a key role in cytokine storms

The cytokine storm is a systemic inflammatory response, caused by CAR-T cell therapy, infections including sepsis or COVID-19. Cytokine storms are characterized by excessive proinflammatory cytokines production. These proinflammatory cytokines including interleukin-6 (IL-6) elicit an increase in vascular permeability and the influx of fluid, resulting in respiratory failure. However, the precise mechanism as to how IL-6 signaling induces the dysregulation of endothelial cells remains to be elucidated. We found that vascular endothelial cell-driven IL-6 promptly activates IL-6 receptor (IL-6R) in a trans-signaling pathway, and this process accelerated the formation of the proinflammatory cytokine network, including IL-6, IL-8, and MCP-1, plasminogen activator inhibitor-1, which reflects endothelial injury. Furthermore, treatment with tocilizumab which is an IL-6R antagonist, substantially abolished the formation of this cytokine network in LPS-stimulated vascular endothelial cells, suggesting that endothelial cells activated by IL-6 signaling form a positive loop during a cytokine storm. Taken together, these results indicate that blockade of IL-6 signaling is a potential biological therapy for cytokine storm.

STAT1 phosphorylation confers distinct DNA-binding and gene-regulatory properties

The LPS–induced endocytosis of TLR4 is essential in the production of IFN-β, which activates the transcription of antiviral response genes by STAT1 phosphorylated at Tyr^{701}. In human macrophages, TLR4 endocytosis activated the noncanonical phosphorylation of STAT1 at Thr^{749}, which subsequently promoted the production of IL-6 and IL-12p40 through distinct mechanisms. Noncanonical phosphorylation of STAT1 activated the expression of gene encoding AT-rich interaction domain-containing protein 5A (ARID5A), which stabilizes *IL6* mRNA. Moreover, noncanonical phosphorylation of STAT1 enhanced the transcription of gene encoding IL-12p40 (*IL12B*). Phosphorylation of Thr^{749} facilitated the binding of STAT1 to a noncanonical DNA motif in the promoter regions of *ARID5A* and *IL12B*. In LPS-stimulated macrophages, endogenous STAT1 bound to the *ARID5A* and *IL12B* promoters, respectively. Endocytosis of TLR4 induced the formation of a complex between the kinases TBK1 and IKKβ through noncanonical phosphorylation of STAT1. Collectively, our study indicates that phosphorylation of STAT1 at different sites confers distinct DNA binding and gene regulation activities, in which canonical phosphorylation at Tyr^{701} and Ser^{727} facilitates STAT1 anti-viral gene transcription. On the other hand, noncanonical phosphorylation at Thr^{749} confers the proinflammatory functions of STAT1.

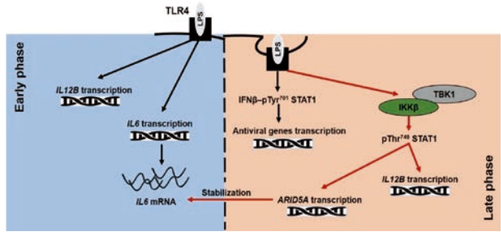

Figure. Scheme of proinflammatory cytokines production through Thr^{749} STAT1 phosphorylation downstream of TLR4 endocytosis.
LPS–TLR4 ligation on cell surface induces MYD88-dependent signaling cascades resulting in early phase transcriptional induction of proinflammatory cytokines. LPS–induced TLR4 endocytosis induces the transcription of antiviral genes through IFNβ–Tyr^{701} STAT1 phosphorylation. Moreover, LPS–induced TLR4 endocytosis promotes Thr^{749} STAT1 phosphorylation through TBK1/IKKβ kinase complex at the late phase of LPS stimulation. In turn, phosphorylated STAT1 at Thr^{749} induces *ARID5A* and *IL12B* transcription resulting in augmented production of proinflammatory cytokines. Red arrows indicate the noncanonical signaling pathway.

Arid5a orchestrates the immunosuppressive microenvironment of pancreatic cancer

Arid5a was identified as an RNA-binding protein that plays crucial roles in the development of inflammatory diseases via the augmentation of IL-6 signaling. However, the roles of Arid5a in cancer remain elusive. We found that although KPC cells derived from pancreatic ductal adenocarcinoma (PDAC) mouse model lacking *Arid5a* (Arid5a-KO), had similar growth rates as wild-type KPC cells when subcutaneously inoculated into immunodeficient mice, Arid5a-KO KPC cells had substantially reduced growth in syngeneic mice. Using mass cytometry, we observed that in tumors derived from Arid5a-KO mice, the infiltration of granulocytic myeloid-derived suppressor cells (gMDSCs) and regulatory T cells (Tregs) is suppressed, whereas the recruitment and activation of CD8$^+$T lymphocytes is induced. Interestingly, Arid5a-KO KPC cells showed significantly lower expression of indoleamine 2,3-dioxygenase 1 (Ido1), which augments the production of kynurenine, an immunoregulatory metabolite of tryptophan catabolism, and augments the production of some chemokines leading to recruitment of Tregs and gMDSCs. Moreover, Arid5a stabilized *Ido1* and chemokine mRNAs.

Taken together, our results indicate that Arid5a acts as a dual regulator in PDAC to generate immunosuppressive tumor microenvironments; first, Arid5a-Ido1 axis controls Treg differentiation/activation; and second, the increase in chemokine levels induced by Arid5a recruits Treg and gMDSCs, in the tumor microenvironment. Thus, our findings provide insights into the molecular basis of the immune evasion of PDAC via Arid5a, and indicate that Arid5a is a promising druggable target for tumor immunotherapy.

Assessment of the physiological action of immunomodulatory drugs in humanized cereblon mice

Thalidomide and its derivatives are immunomodulatory drugs (IMiDs) that have therapeutic actions on hematological malignancies and inflammatory diseases. However, the molecular basis of these pleiotropic activities of IMiDs is still unknown.

IMiDs bind directly to cereblon (CRBN), which is a substrate receptor of the CRL4-CRBN E3 ligase complex 6, which results in the ubiquitination and degradation of specific protein targets. Using mice expressing humanized CRBN, which mimics the action of IMiDs in human cells, we found that T-lymphocytes from humanized CRBN mice treated with a novel IMiD result in the increased production of IL-22, IL-2, and IL-9 via the degradation of Aiolos and interferon regulatory factor 4, whereas WT mice did not show such an increase. Moreover, immunodeficient mice, which were engrafted with the myelodysplastic syndrome (MDS) patient-derived cell line MDS-L, and treated with IMiDs showed a longer survival rate and less infiltration of MDS cells into their bone marrow than WT mice. Consistently, IMiDs showed significant suppressive effects on the growth of MDS-L cells *in vitro*. Assessment of protein expression in humanized CRBN mice treated with IMiDs is currently under investigation.

Recent Publications

- Kang S, Narazaki M, Metwally H, Kishimoto T. Historical overview of the interleukin-6 family cytokine. J. Exp. Med. 217, pii: e20190347 (2020).
- Choy E, Benedetti F, Takeuchi T, Hashizume M, Markus RJ, Kishimoto T. Translating IL-6 biology into effective treatments. Nat. Rev. Rheumatol 23, 1-11 (2020).
- Metwally H, Tanaka T, Li S, Parajuli G, Kang S, Hanieh H, Hashimoto S, Chalise JP, Gemechu Y, Standley DM, Kishimoto T. Noncanonical STAT1 phosphorylation expands its transcriptional activity into promoting LPS-induced IL-6 and IL-12p40 production. Sci Signal. 13, pii: eaay0574 (2020).
- Nyati KK, Zaman MM, Sharma P, Kishimoto T. Arid5a, an RNA-binding protein in immune regulation: RNA stability, inflammation, and autoimmunity. Trends Immunol. 41, 255-268 (2020).
- Nyati KK, Agarwal RG, Sharma P, Kishimoto T. Arid5a regulation and the roles of Arid5a in the inflammatory response and disease. Front. Immunol. 10, 2790 (2019).
- Chalise JP, Hashimoto S, Parajuli G, Kang S, Singh SK, Gemechu Y, Metwally H, Nyati KK, Dubey PK, Zaman MM, Nagahama Y, Hamza H, Masuda K, Kishimoto T. Feedback regulation of Arid5a and Ppar-γ2 maintains adipose tissue homeostasis. Proc. Natl. Acad. Sci. USA. 116, 15128-15133 (2019).
- Kang S, Tanaka T, Narazaki M, Kishimoto T. Targeting interleukin-6 signaling in clinic. Immunity, 50(4), 1007-1023 (2019).

Immune Regulation

Hitoshi Kikutani, MD/PhD

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Self-reactive and polyreactive B cells are generated and selected in the germinal center during γ-herpesvirus infection

Murine γ-herpesvirus 68 (MHV68) is closely related to Epstein–Barr virus (EBV) and induces splenomegaly and hypergammaglobulinemia at 2–3 weeks after infection in mouse inbred strains. Although a humoral immune response is initiated in the acute phase, production of neutralizing antibodies is delayed and a high titer of IgG autoantibody is produced instead. Nevertheless, it has remained unclear how self-reactive B cells are selectively generated. To determine how self-reactive B cells are generated during MHV68 infection, we analyzed the reactivity of recombinant antibodies obtained from single, isolated germinal center (GC) B cells, transient short-lived plasmablasts, and long-lived plasma cells (PCs). We revealed that about a quarter of IgG^+ GC B cells emerge as polyreactive to multiple self-antigens and/or virion antigens. The self-reactivity of most polyreactive clones was dependent on somatic hypermutation (SHM), but not due to intrinsic alteration of virally infected B cells. Our findings suggest that MHV68 infection generates polyreactive B cells through the GC reaction.

Furthermore, both virus-mono-specific and polyreactive clones were selected to differentiate into $B220^{lo}$ $CD138^+$ plasma cells (PCs). However, the representation of GC-derived polyreactive clones was reduced and that of virus-mono-specific clones was markedly increased in terminally differentiated PCs as compared to transient plasmablasts. Collectively, our findings demonstrate that, during acute MHV68 infection, self-reactive B cells are generated through SHM and selected for further differentiation into short-lived plasmablasts but not terminally differentiated PCs (Sakaibara S et al. Int. Immunol. 2020).

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

Epstein-Barr virus (EBV) infects over 95% of adults and persists throughout their lifespan. EBV establishes latent infection in B cells with expression of limited viral genes. Latent membrane protein1 (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 1). 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. Int. Immunol. 2018).

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

From our previous study on monoclonal anti-dsDNA antibodies isolated from acute SLE patients (Sakakibara S et al., 2017), we demonstrated that low-affinity anti-ssDNA B cells can acquire high-affinity to both ss- and dsDNA by only one or two mutations. This raises the questions of whether low-affinity anti-ssDNA precursor B cells are subjected to immunological tolerance 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 SLE anti-DNA antibody clones (Figure 2). The G9gl KI mice had reduced number of splenic B cells, in which only one third of them expressed the KI BCR and the rest of B cells expressed endogenous BCR due to receptor editing. As expected, the KI BCR-expressing B cells reacted to ssDNA, but not dsDNA. These low-affinity anti-ssDNA precursor B cells exhibited anergic phenotype such as lowered BCR expression and shorter half-life *in vitro*. Furthermore, they were excluded from GC reaction induced in several experimental settings including ssDNA-conjugated mBSA immunization and Treg depletion. Therefore, multiple tolerance checkpoints regulate low-affinity anti-DNA precursor B cells not only at the early development stage but also GC to prevent pathogenic autoantibody production.

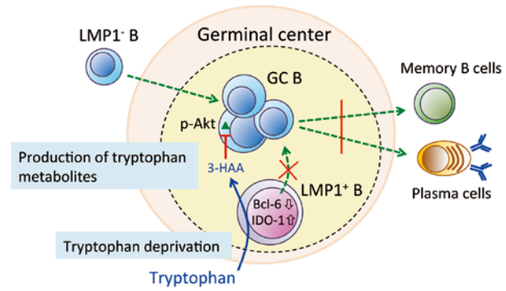

Figure 1.

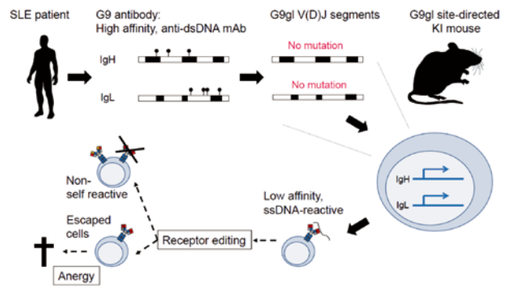

Figure 2.

Recent Publications

- Sakakibara S., et al. Self-reactive and polyreactive B cells are generated and selected in the germinal center during γ-herpesvirus infection. Int. Immunol. 32, 27-38 (2020).
- 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 antinuclear antibodies in acute systemic lupus erythematosus. Scientific Reports 7, 16428 (2017).

Mucosal Immunology

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Mucosal barriers including mucins and antimicrobial peptides serve as a segregator of commensal microbes and intestinal epithelial cells (IECs) to maintain gut homeostasis. We previously reported that Ly6/Plaur domain containing 8 (Lypd8), a highly glycosylated GPI-anchored protein expressed on enterocytes of the colon, promotes the segregation of commensal bacteria and colonic epithelia by inhibiting bacterial invasion of the colonic epithelia. We analyzed the role of Lypd8 in the host defense against enteric bacterial pathogens such as *Citrobacter rodentium*, which induces attachment and effacement (A/E) lesions on colonic epithelia.

Lypd8-deficient mice are sensitive to *C. rodentium* infection

To determine the role of Lypd8 in protection against enteric pathogens, we first analyzed the ability of orally infected *Lypd8*$^{-/-}$ mice to eliminate *C. rodentium*. We evaluated the numbers of *C. rodentium* in fecal samples from *Lypd8*$^{-/-}$ mice over the course of infection. *C. rodentium* was more abundant in the feces of *Lypd8*$^{-/-}$ mice compared with wild-type (WT) mice at the early time points, suggesting that *C. rodentium* more rapidly colonized and proliferated in the colons of *Lypd8*$^{-/-}$ mice. Intimin-mediated bacterial attachment to IECs is necessary for colonization of A/E bacteria. Thus, we next analyzed the attachment of *C. rodentium* to colonic epithelia of *Lypd8*$^{-/-}$ mice. The total number of adherent *C. rodentium* was markedly increased in *Lypd8*$^{-/-}$ mice compared with WT mice from day 3 to day 12 post-infection. These findings indicate that Lypd8 inhibits the colonization of *C. rodentium* in the colon by suppressing bacterial attachment to colonic epithelia. In accordance with the increased number of *C. rodentium* adhering to colonic epithelia, *Lypd8*$^{-/-}$ mice showed more severe colitis, characterized by a thicker mucosa and increased cellular infiltration to the lamina propria. These results suggest that Lypd8 deficiency impairs host defenses against *C. rodentium*.

Lypd8 suppresses the attachment of A/E bacteria to colonic epithelia by binding to bacterial cells

To assess the mechanism by which Lypd8 inhibits the attachment of *C. rodentium* to the colonic epithelia, we next analyzed whether Lypd8 binds to *C. rodentium* in the colon. We used flow cytometry with anti-Lypd8 antibody and measured Lypd8 binding to GFP-expressing *C. rodentium* in the feces of WT mice following infection. Lypd8 bound to GFP-expressing *C. rodentium* in the colon. *C. rodentium* attachment is strengthened by the generation of A/E lesions involving the interaction of intimin expressed on the bacterial surface and translocated intimin receptor (Tir) on the host cell membrane after being injected via the type III secretion system (T3SS). To test whether Lypd8 binds to these virulence factors, we generated eae-deficient (Δ*eae*; equivalent to intimin-deficient) and escN-deficient (Δ*escN*; equivalent to T3SS-deficient) *C. rodentium* strains, and then examined Lypd8 binding to these mutant strains by flow cytometry. While no decrease in Lypd8 binding to the T3SS-deficient strain compared with the WT was observed, Lypd8 binding to the intimin-deficient strain was substantially reduced compared with the WT strain, suggesting that intimin on the surface of *C. rodentium* is targeted by Lypd8 in the colon.

Lypd8 interferes with the interaction between intimin and Tir of A/E bacteria by binding to intimin

To further define Lypd8 binding to intimin expressed by *C. rodentium*, we analyzed whether FLAG-tagged Lypd8 recombinant proteins bind to GST-tagged intimin recombinant proteins by an enzyme-linked immunosorbent assay (ELISA) with anti-FLAG antibody. The Ig-like and C-type lectin (CTL) domains of intimin are pivotal for intimin binding to Tir of A/E bacteria. Thus, we examined the binding of Lypd8 to each intimin protein domain. Predictably, highly glycosylated Lypd8 proteins bound to the CTL domain of intimin. In addition, Lypd8 bound to the Ig-like domain. The interaction between intimin and Tir is indispensable for the generation of A/E lesions. We next examined whether Lypd8 inhibits the interaction between intimin and Tir. High absorbance readings indicative of intimin-Tir binding gradually decreased following the addition of increasing concentrations of Lypd8. These data clearly indicate that Lypd8 inhibits A/E bacteria attachment to enterocytes by suppressing the association between intimin and Tir (Figure).

Taken together, our studies demonstrate that enterocytes, previously regarded as passive contributors to the defense against invading pathogens compared with other intestinal epithelial cells such as goblet cells and Paneth cells, actively contribute to the fight against enteric pathogens by expressing Lypd8.

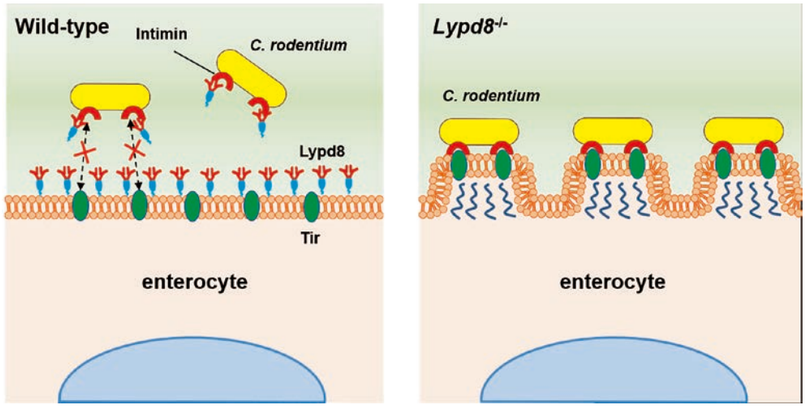

Figure. Lypd8, a highly glycosylated GPI-anchored protein on enterocytes, inhibits the attachment of *C. rodentium* to enterocytes by suppressing the interaction of Intimin and Tir, both of which are critical virulence factors for A/E lesion.

Recent Publications

- Kayama H, Okumura R, Takeda K. Interaction between the microbiota, epithelia, and immune cells in the intestine. Ann. Rev. Immunol. 38, 23-48 (2020).
- Okumura R, Kodama T, Hsu CC, Sahlgren BH, Hamano S, Kurakawa T, Iida T, Takeda K. Lypd8 inhibits attachment of pathogenic bacteria to colonic epithelia. Mucosal Immunol. 13, 75-85 (2020).
- 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 CX_3CR1^+ cells by bacterial metabolites. 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 non-inflammatory profiles. Proc. Natl. Acad. Sci. USA 115, 8418-8423 (2018).
- 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 and Takeda K. Lypd8 promotes the segregation of flagellated microbiota and colonic epithelia. Nature 532, 117-121 (2016).

Experimental Immunology

Shimon Sakaguchi, MD/PhD

Professor	Shimon Sakaguchi
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Assistant Professor	Kenji Ichiyama
Postdoctoral Fellow	4
Research Assistant	3
Visiting Scientist	13
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 mechanisms of immunologic self-tolerance; and (iii) the causes and pathogenetic mechanisms 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. Depletion of Treg cells or attenuation of their suppressive activity can enhance immune responses such as tumor immunity, while expansion of Treg cells or augmentation of their suppressive activity can induce immunological tolerance and treat autoimmune and other immunological diseases. This year, we have explored chemical reagents which are able to induce or deplete Treg cells and thereby suppress or enhance immune responses, respectively.

By screening libraries of tyrosine or serine/threonine kinase inhibitors for *in vitro* Foxp3 inducing activity in conventional T cells, we found that chemical inhibition of cyclin-dependent kinases (CDK) 8/19, a serine/threonine kinase, was able to induce Foxp3 in antigen-stimulated effector/memory as well as naïve $CD4^+$ and $CD8^+$ T cells. Knockdown/ knockout of the CDK8 or CDK19 gene also similarly induced Foxp3. The induction was associated with STAT5 activation, independent of TGF-β action, and not affected by inflammatory cytokines. Furthermore, *in vivo* administration of a newly developed CDK8/19 inhibitor along with antigen immunization generated functionally stable antigen-specific $Foxp3^+$ Treg cells, which effectively suppressed skin contact hypersensitivity and autoimmune disease in animal models. The results indicate that CDK8/19 is physiologically repressing Foxp3 expression in activated conventional T cells and that its pharmacological inhibition enables conversion of antigen-specific effector/memory T cells into $Foxp3^+$ Treg cells for the treatment of various immunological diseases (Figure 1).

In contrast to such a Treg-inducing serine/threonine kinase inhibitor, we found that a certain tyrosine inhibitor can deplete Treg cells. Imatinib, a tyrosine kinase inhibitor of oncogenic BCR-ABL protein expressed by chronic myelogenous leukemia (CML) cells, possesses off-targets including LCK expressed in T cells. We showed in humans that imatinib-treated CML patients in complete molecular remission (CMR) exhibited selective depletion of effector Treg (eTreg) cells and significant increase in effector/memory $CD8^+$ T cells while non-CMR patients did not. Imatinib at CML-therapeutic concentrations indeed induced apoptosis specifically in eTreg cells and expanded tumor-antigen-specific $CD8^+$ T cells *in vitro* in healthy individuals and melanoma patients, and suppressed colon tumor growth *in vivo* in mice. Mechanistically, because of FoxP3-dependent much lower expression of LCK and ZAP-70 in Treg cells compared with other T cells, imatinib inhibition of LCK further reduced their TCR signal intensity, rendering them selectively susceptible to signal-deprived apoptotis. Taken together, eTreg cell depletion by imatinib is instrumental in evoking effective immune responses to various cancers (Figure 2).

Taken together, it is expected that small molecules capable of reducing or expanding Treg cells can be clinically useful for treatment of a variety of immunological diseases and control of physiological and pathological immune responses.

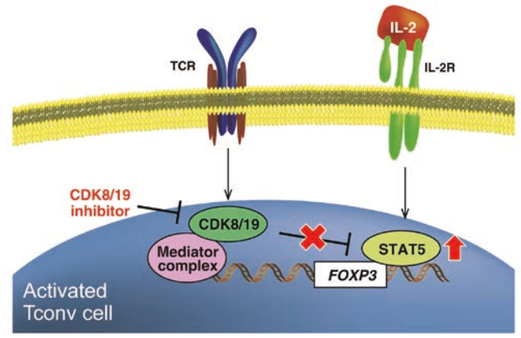


Figure 1. Induction of Foxp3 in activated conventional T cells by inhition of CDK8/19.
CDK8/19 is physiologically repressing Foxp3 expression in activated conventional T cells. Its pharmacological inhibition enables conversion of antigen-specific effector/memory T cells into $Foxp3^+$ Treg cells by mainly upregulating STAT5. Adapted from Akamatsu, Mikami, et al. *Sci. Immunol.* 2019.

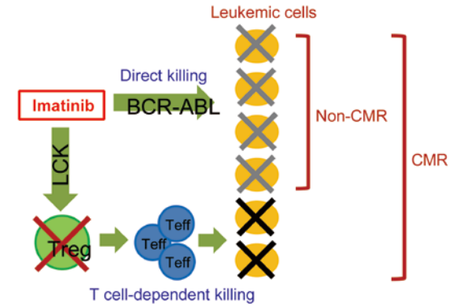
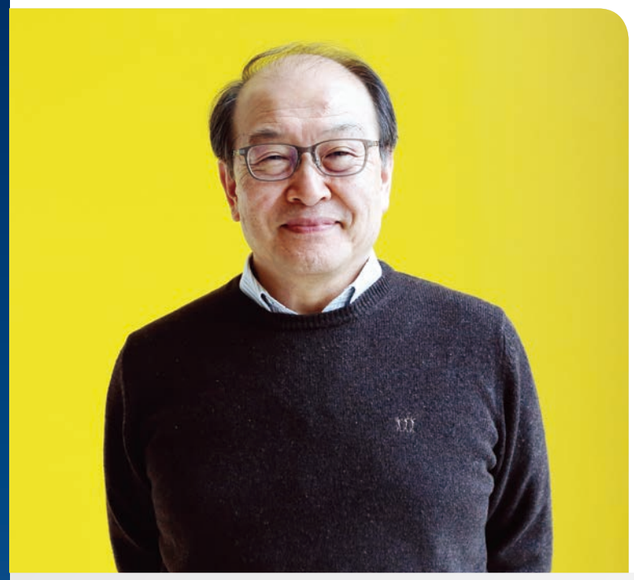


Figure 2. Direct or T cell-dependent killing of CML cells by imatinib.
Imatinib is able to not only kill leukemic cells directly but also deplete Treg cells, thereby inducing immunological killing of leukemic cells. Imatinib is therefore instrumental in immunological treatment of various other cancers as well. Adapted from Tanaka, et al. *J. Exp. Med.* 2020.

Recent Publications

- Sakaguchi S, Mikami N, Wing JB, Tanaka A, Ichiyama K, and Ohkura N. Regulatory T cells and human disease. Ann. Rev. Immunol. 38, 541-566 (2020).
- Ohkura N, Yasumizu Y, Kitagawa Y, Tanaka A, Nakamura Y, Motooka D, Nakamura S, Okada Y, Sakaguchi S. Treg-specific epigenomic region variants as a key determinant of susceptibility to common autoimmune diseases. Immunity (2020) in press.
- Mikami N, Kawakami R, Chen KY, Sugimoto A, Ohkura N, Sakaguchi S. Epigenetic conversion of conventional T cells into regulatory T cells by CD28 signal deprivation. Proc. Natl. Acad. Sci. USA, in press.
- Tanaka A, Nishikawa H, Noguchi S, Sugiyama D, Morikawa H, Takeuchi Y, Ha D, Shigeta N, Kitawaki T, Maeda Y, Saito T, Shinohara Y, Kameoka Y, Iwaisako K, Monma F, Ohishi K, Karbach J, Jäger E, Sawada K, Katayama N, Takahashi N, Sakaguchi S. Tyrosine kinase inhibitor imatinib augments tumor immunity by depleting effector regulatory T cells. J. Exp. Med. pii: jem.20191009. doi: 10.1084/jem.20191009 (2020).
- Akamatsu M, Mikami N, Ohkura N, Kawakami R, Kitagawa Y, Sugimoto A, Hirota K, Nakamura N, Ujihara S, Kurosaki T, Hamaguchi H, Harada H, Xia G, Morita Y, Aramori I, Narumiya S, Sakaguchi S. Conversion of antigen-specific effector/memory T cells into Foxp3-expressing Treg cells by inhibition of CDK8/19. Sci. Immunol. pii: eaaw2707. doi: 10.1126/sciimmunol.aaw2707 (2019).

Cell Signaling



Takashi Saito, PhD

Professor	Takashi Saito
Support Staff	1

The objective of our team is to determine the molecular mechanisms of T cell activation, differentiation and function, and ultimately aim to elucidate the onset of and to modulate T cell function/activation to prevent immune diseases such as autoimmunity and allergic inflammation. Toward this goal, we have analyzed regulation of T cell function from a signaling perspective.

Our finding that TCR-microclusters (MC) initiate T cell activation led us to analyze the dynamics of signal molecules at the immune synapse. We have also found that such microcluster formation also regulates the negative signaling particularly through inhibitory receptors such as CTLA4 and PD-1. Following these analyses, we then analyzed the dynamics of negative regulation of T cell activation through phosphatases, particularly PTPN22 whose SNPs are strongly related to various autoimmune diseases. The deficiency of PTPN22 exhibited enhanced activation of T cells and an increase in effector/memory T cells. Imaging analysis revealed that that PTPN22 was recruited to TCR-MC upon activation. MS analysis of the PTPN22-associated proteins showed several inhibitory molecules and following imaging analysis suggested that PTPN22 exhibited a “inhibitory complex” with other inhibitory molecules to inhibit T cell activation. The PTPN22 mutant, susceptible to the development of autoimmune diseases, was defective of TCR-MC recruitment, provided the inhibitory mechanisms and a cause of the autoimmune susceptibility by the mutation.

We have also analyzed the modulation of T cell activation and function by innate signaling. We have already shown that T cell stimulation by some TLR ligands induced co-stimulation of T cells to induce cell proliferation and cytokine production. Particularly TLR2 activates Th1 cells but not naïve T cells without TCR stimulation. We found that naïve T cells failed to respond to TLR2 stimulation due to the defective expression of TIRAP. The function of the innate-sensor STING in T cells was also analyzed because T cells express similar level of STING as innate immune cells. STING activation in T cells by STING ligands such as cGAMP induced growth inhibition on one hand, and type I-IFN production on the other hand. Both functions were mediated through mTORC1 activation, and we found the reciprocal regulation between STING and mTORC1 signals for T cell function (Figure). Furthermore, we showed that STING activation in T cells contributes to anti-tumor immunity probably through the great amount of type I IFN production by tumor-specific T cells.

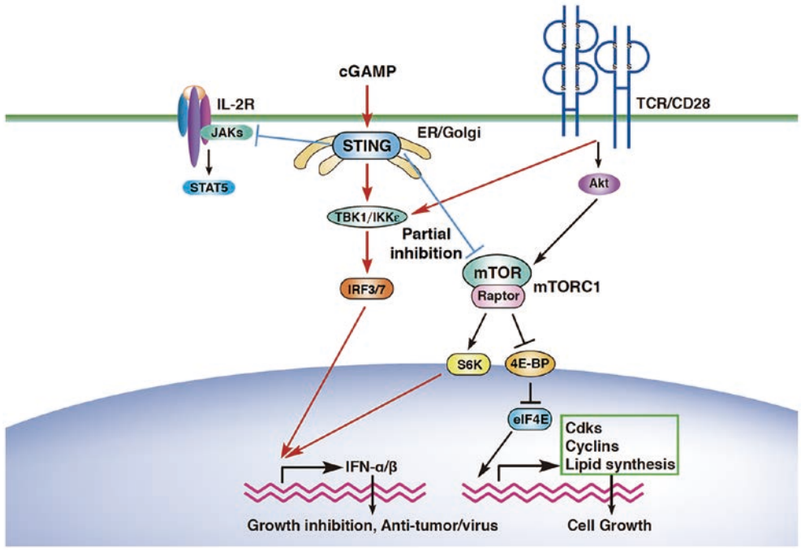
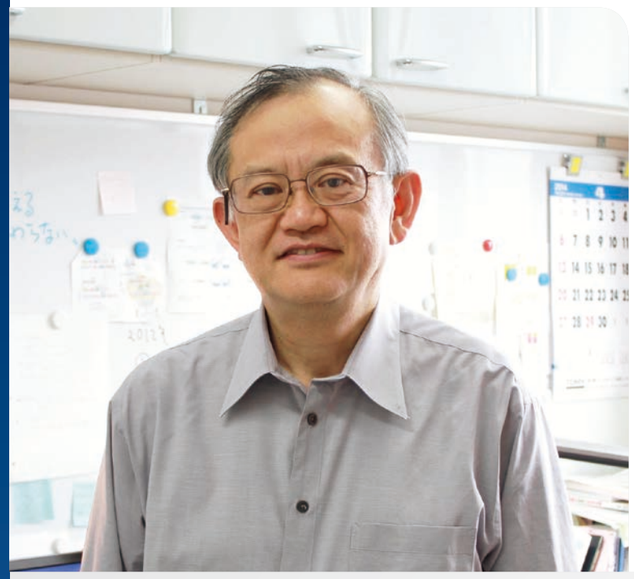


Figure. Molecular mechanism of reciprocal regulation of STING and TCR signaling.
Co-stimulation of T cells by STING and TCR activation can induce growth arrest by partially inhibiting mTORC1 activation, and simultaneously induces IFN-I production though sustained activation of IRF3 and the partial activation of mTORC1. The STING-mediated inhibition of mTORC1 signals is partly dependent on IRF3/IRF7 but not on TBK1/IKKε. cGAMP-induced STING activation also leads to the inhibition of IL-2 signaling pathways.

Recent Publications

- Imanishi T and Saito T. T cell co-stimulation and functional modulation by innate signals. Trends Immunol. 41, 200-212 (2020).
- Sasaki T, Yajima T, Shimaoka T, Ogawa S, Saito T, Yamaoka K, Takeuchi T and Kubo M. Synergistic effect of IgG4 antibody and CTLs causes tissue inflammation in IgG4-related disease. Int. Immunol. (IF:4.168) 32, 163-174 (2020).
- Kong MS, Hashimoto-Tane A, Kawashima Y, Sakuma M, Yokosuka T, Kometani K, Onishi R, Carpino N, Ohara O, Kurosaki T, Phua KK and 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).
- Saito T. Molecular dynamics of co-signal molecules in T cell activation. Adv. Exp. Med. Biol. 1189, 135-152 (2019).

Lymphocyte Differentiation



Tomohiro Kurosaki, MD/PhD

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Research Assistant	1
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Generation mechanism of memory B cells during germinal center (GC) reactions

Memory B cells and long-lived plasma cells (PCs) are responsible for effective long-term immunity against pathogens. The majority of these cells responding to T cell-dependent antigens are generated from the germinal center (GC) reaction. Indeed, memory B cells emerge from the GC as recirculating cells and, upon a secondary antigen challenge, they are primed to elicit rapid antibody responses.

In contrast to these insights into the GC precursor cells for recycling and plasma cell fates, studies of the memory fate decision have been hampered by the lack of a known master transcription factor for differentiation of memory B cells. Hence, surrogate markers such as an S1PR2 reporter, CCR6 expression, or a cell cycle reporter has been recently employed for identification of memory precursor cells. Although informative, these studies have not identified key features for development of the GC precursor cells committed to the long-lived memory B cell fate, or what signals regulate these key features.

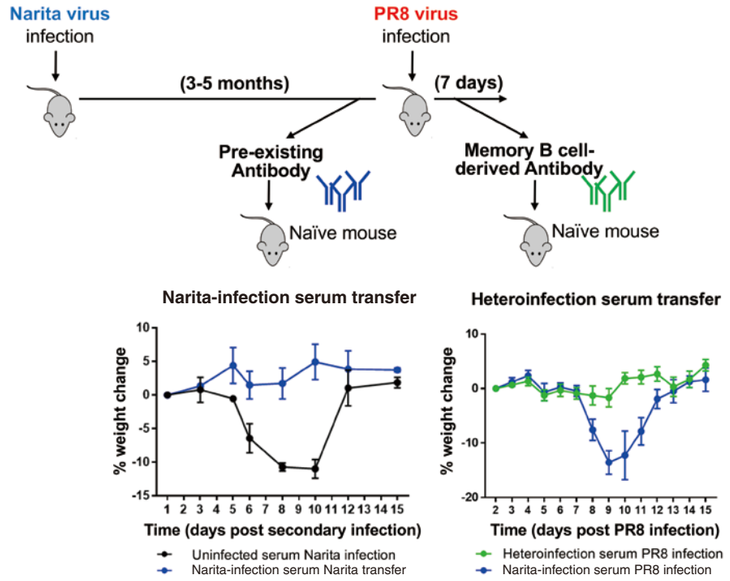
After identifying a memory-prone GC population ($CD38^{int}Bcl6^{hi/int}Efnb1^{+}$), we found that this small population exhibited lower mTORC1 activity than the recycling-prone population. Constitutive high mTORC1 activity led to defective development of $CD38^{int}Bcl6^{hi/int}Efnb1^{+}$ cells, whereas decreasing mTORC1 activity resulted in relative enrichment in this memory-prone cell population versus the recycling-prone one. Moreover, the $CD38^{int}Bcl6^{hi/int}Efnb1^{+}$ cells had higher levels of Bcl2 and surface BCR, thereby contributing to their survival. Given the positive correlation between the strength of T cell help and mTORC1 activity, our data support a model in which weak help from T cells together with provision of an increased survival signal is key for GC cells to assume the memory B cell fate.

Importance of memory B cell compartment for viral re-infection

Two humoral memory compartments, long-lived plasma cells (LLPCs) and memory B cells, co-exist in our body. LLPCs constitutively produce an antibody (Ab) and neutralize invading pathogens immediately upon re-infection, whereas memory B cells require re-stimulation by cognate antigen for their differentiation into Ab-secreting plasma cells. Since serum Ab titers are known to be correlated with vaccine efficacy, the importance of LLPCs is well-appreciated. In contrast, the importance of memory B cells in conferring protection from re-infection has been controversial.

The majority of, though not all, memory cells and LLPCs are thought to arise from germinal center (GC) reactions. Immunization with NP hapten leads to the introduction of the high-affinity-conferring, W33L VH mutation in a large proportion of LLPC Abs. Thus, the LLPC pool is thought to be primarily composed of specificities possessing the highest affinity for the primary antigen. On the other hand, we and others have recently demonstrated that GC B cells with relatively low affinities are preferentially recruited into the memory B cell compartment. This observation led us to propose that such mechanisms may prevent the memory B cell population from becoming overly committed to the primary antigen, and instead, allow them to potentially acquire a more diverse repertoire. Therefore, memory B cells might be intrinsically well-suited for recognition of and protection from secondary infection by related but antigenically variant pathogens.

To rigorously test this proposal, we used a mouse model of drifted viral infection with pandemic H1N1 A/Narita/2009 (Narita) virus first and then re-infection with the H1N1 A/Puerto Rico/8/1934 (PR8) virus. We demonstrate that GC-experienced LLPCs generated during primary infection with Narita virus are not effective in neutralizing the re-infecting PR8 virus. Instead, pre-existing anti-HA stem-specific memory B cells are activated upon PR8 virus re-infection, thereby contributing to host protection (Figure). Furthermore, our results suggest the requirement for maturation of anti-HA stem memory B cells in the GC during primary influenza infection, whereas GC-mediated maturation is not required for protection after secondary re-infection.



Leach S, Shinnakasu R, et al. ***Int Immunol*** 31, 771-779 (2019)

Figure. *De novo* generation of HA stem-specific antibodies from memory B cells is required for protection from heterologous infection.
Pre-existing antibodies generated in mice previously infected with Narita virus (3-5 months post infection) provided protection from weight loss in naïve recipient mice infected with Narita virus (Lower left; blue line). By contrast, transfer of serum from Narita-infected mice failed to protect naïve recipient mice infected with the PR8 virus (Lower right; blue line). On the other hand, antibodies generated from memory B cells, which were initially induced after the primary Narita infection, after PR8 heterologous re-infection at day 7 were able to provide protection to naïve recipient mice infected with the PR8 virus (Lower right; green line).

Recent Publications

- Fukuyama H, Shinnakasu R and Kurosaki T. Influenza vaccination strategies targeting the hemagglutinin stem region. Immunol. Rev. (2020) in press.
- Tanaka S, Ise W, Inoue T, Ito A, Ono C, Shima Y, Sakakibara S, Nakayama M, Fujii K, Miura I, Sharif J, Koseki H, Pandelakis K, Raman I, Li Q, Kubo M, Fujiki K, Nakato R, Shirahige K, Araki H, Miura F, Ito T, Kawakami E, Baba Y and Kurosaki T. Tet2 and Tet3 in B cells are required to repress CD86 and prevent autoimmunity. Nat. Immunol. (2020) in press.
- Mesin L, Schiepers A, Ersching J, Barbulescu A, Cavazzoni CB, Angelini A, Okada T, Kurosaki T and Victora GD. Restricted clonality and limited germinal center reentry characterize memory B cell reactivation by boosting. Cell 180, 92-106 (2020).
- Leach S, Shinnakasu R, Adachi Y, Momota M, Makino-Okamura C, Yamamoto T, Ishii KJ, Fukuyama H, Takahashi Y and Kurosaki T. Requirement for memory B cell activation in protection from heterologous influenza virus reinfection. Int. Immunol. 31, 771-779 (2019).
- Ise W and Kurosaki T. Plasma cell differentiation during the germinal center reaction. Immunol. Rev. 288, 64-74 (2019).

Malaria Immunology

Cevayir Coban, MD

Professor	Cevayir Coban
Assistant Professor	Michelle Sue Jann Lee
Visiting Scientist	1
Support Staff	2

Malaria is a severe disease affecting millions of lives every year. To solve the malaria problem, our approach is to 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 the conclusion that *Plasmodium* parasite presence in different organs should be considered in a tissue-specific context (Nature Reviews Immunology 2018). From this understanding, our final aim is to create successful vaccines for the elimination of malaria.

Recently we developed an automated analysis method to rapidly acquire and characterize neutrophil extracellular traps (NETs) by using in human PBMC as well as in whole blood during infection of mice with the malaria parasite (Cytometry A 2019). In subsequent studies using imaging flow cytometry, we have demonstrated an unexpected finding that heparin, a common anticoagulant, has the ability to induce NETs. NETs by heparin occurred with cell lysis and death, but live neutrophils releasing extracellular DNA strands, known as vital NETs, also occurred abundantly. Formation of NETs was time and dose dependent, and required reactive oxygen species and neutrophil elastase. Other compounds related to heparin such as low molecular weight heparin, fondaparinux and heparan sulfate either failed to induce NETs, or did so to a much lesser extent (International Immunology 2020). Heparin is used extensively, however, its biological effects are not limited to coagulation and remain incompletely understood. Our findings suggest the ability of heparin to directly induce NET formation and that should be considered in the context of heparin treatment and when heparin-related side effects occur.

In an effort to create vaccines against malaria and other infectious diseases, we developed a new adjuvant called synthetic hemozoin, a synthetic analog of *Plasmodium*-produced hemozoin, and completed its preliminary GLP non-clinical safety and toxicology studies in several animals and infection models (Vaccine 2016). Adjuvants improve the potency of vaccines, but the modes of action of most adjuvants are not unknown. In 2019, we demonstrated by using the T-dependent antigen NIPOVA and a synthetic hemozoin that MyD88 is required for early GC formation and enhanced antibody class-switch recombination in mice. Using cell-type-specific MyD88 KO mice, we found that IgG2c class switching, but not IgG1 class switching, was controlled by B cell-intrinsic MyD88 signaling. Notably, IFN-γ produced by various cells including T cells, NK cells, and dendritic cells was the primary cytokine for IgG2c CSR and B-cell intrinsic MyD88 is required for IFN-γ production. Moreover, IFN-γ receptor (IFNγR) deficiency abolished sHZ-induced IgG2c production, while recombinant IFN-γ administration successfully rescued IgG2c CSR impairment in mice lacking B-cell intrinsic MyD88. These findings suggest that B cell-intrinsic MyD88 signaling is involved in the mechanism of synthetic hemozoin adjuvants and this may enhance our specific understanding of how adjuvants and vaccines work. (European J. Immunology 2019).

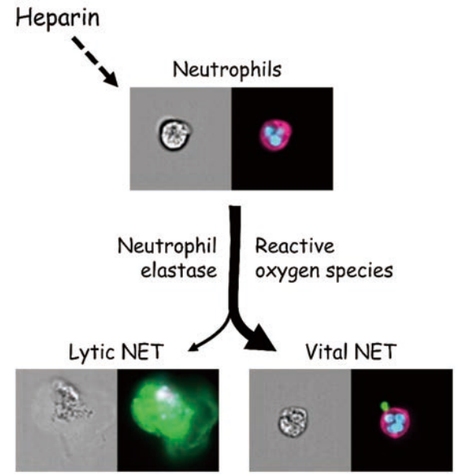


Figure 1. Heparin directly induces both lytic and vital NETs. This NET formation requires reactive oxygen species and neutrophil elastase but not NADPH oxidase or peptidyl-arginine deiminase 4.

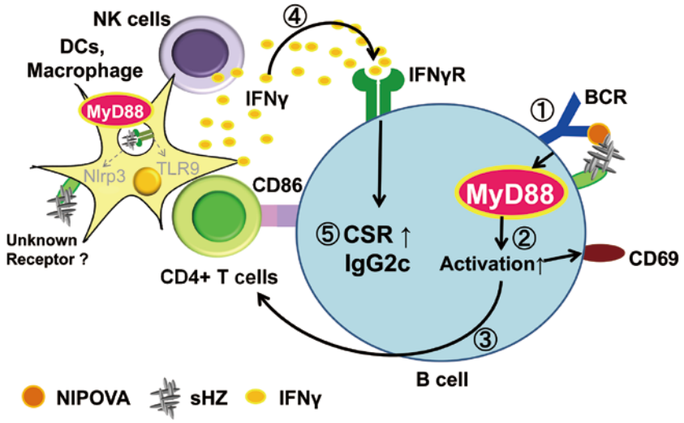


Figure 2. Cell-intrinsic MyD88 plays differential roles in the modes of action of vaccine adjuvants. Using the synthetic hemozoin particulate adjuvant, we found that B cell intrinsic MyD88 is required for IFNγ induction in T cells, NK cells and dendritic cells to induce IgG2c class switching in a TLR/IL1-independent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

Recent Publications

- Lelliott PM, Momota M, Shibahara T, Lee MSJ, Smith NI, Ishii KJ, Coban C. Heparin induces neutrophil elastase dependent vital and lytic NET formation. International Immunology, pii: dxz084. doi: 10.1093/intimm/dxz084 (2020).
- 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. European J. Immunology, doi: 10.1002/eji.201848084 (2019).
- 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. Nature Reviews Immunology, 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. Science Immunology, June 2, 2 (12), pii: eaam8093 (2017).

Vaccine Science

Ken J. Ishii, MD/PhD

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	Burcu Temizoz
Postdoctoral Fellow	1
Research Assistant	1
Support Staff	2

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 the 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. Three recent works published 2019-2020 are listed below;

1) ZBP1 governs the inflammasome-independent IL-1α and neutrophil inflammation that play a dual role in anti-influenza virus immunity.

Influenza A virus (IAV) triggers the infected lung to produce IL-1 and recruit neutrophil. Unlike IL-1β, however, little is known about IL-1α in terms of its mechanism of induction, action and physiological relevance to the host immunity against IAV infection. In particular, whether Z-DNA binding protein 1 (ZBP1), a key molecule for IAV-induced cell death, is involved in the IL-1α induction, neutrophil infiltration, and the physiological outcome have not been elucidated. Here we show in a murine model that the IAV-induced IL-1α is mediated solely by ZBP1, in an NLRP3-inflammasome-independent manner, and is required for the optimal IL-1β production followed by the formation of neutrophil extracellular traps. During IAV infection, ZBP1 displays a dual role in anti-IAV immune responses mediated by neutrophil, resulting in either a protective or pathological outcome *in vivo*. Thus, ZBP1-mediated IL-1α production is the key initial step of IAV-infected NETs, owing the duality of the consequent lung inflammation.

2) Cyclic GMP-AMP Triggers Asthma in an IL-33-Dependent Manner That Is Blocked by Amlexanox, a TBK1 Inhibitor.

Extracellular host-derived DNA, as one type of damage associated molecular patterns (DAMPs), is associated with allergic type 2 immune responses. Immune recognition of such DNA generates a second messenger cyclic GMP-AMP (cGAMP) and induces type-2 immune responses; however, its role in allergic diseases, such as asthma, has not been fully elucidated. This study aimed to determine whether cGAMP could induce asthma when used as an adjuvant. We intranasally sensitized mice with cGAMP together with house dust mite antigen (HDM), followed by airway challenge with HDM. We then assessed the levels of eosinophils in the broncho-alveolar lavage fluid (BALF) and serum HDM-specific antibodies. cGAMP promoted HDM specific allergic asthma, characterized by significantly increased HDM specific IgG1 and total IgE in the serum and infiltration of eosinophils in the BALF. cGAMP stimulated lung fibroblast cells to produce IL-33 *in vitro*, and mice deficient in IL-33 or IL-33 receptor (ST2) failed to develop asthma enhancement by cGAMP. Not only Il-33 -/- mice, but also Sting -/-, Tbk1 -/-, and Irf3 -/- Irf7 -/- mice which lack the cGAMP-mediated innate immune activation failed to increase eosinophils in the BALF than that from wild type mice. Consistently, intranasal and oral administration of amlexanox, a TBK1 inhibitor, decreased cGAMP-induced lung allergic inflammation. Thus, cGAMP functions as a type 2 adjuvant in the lung and can promote allergic asthma in manners that are dependent on the intracellular STING/TBK1/IRF3/7 signaling pathway and the resultant intercellular signaling pathway via IL-33 and ST2 might be a novel therapeutic target for allergic asthma.

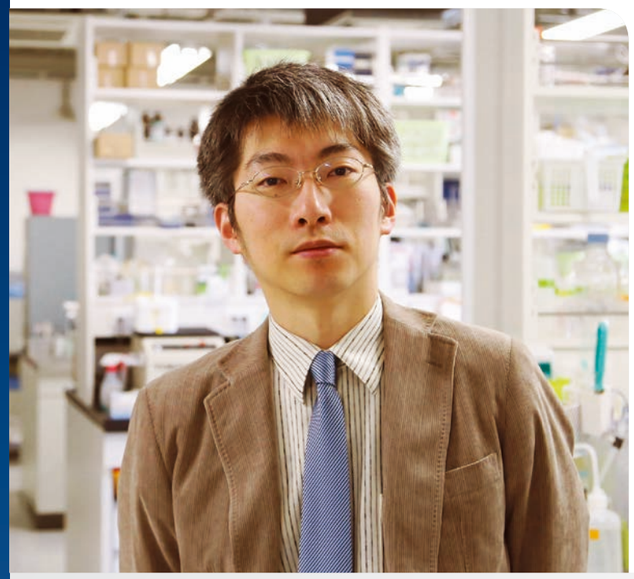
3) IL-33 Is Essential for Adjuvant Effect of Hydroxypropyl-β-Cyclodexrin on the Protective Intranasal Influenza Vaccination.

Vaccine adjuvants are traditionally used to augment and modulate the immunogenicity of vaccines, although in many cases it is unclear which specific molecules contribute to their stimulatory activity. We previously reported that both subcutaneous and intranasal administration of hydroxypropyl-β-cyclodextrin (HP-β-CD), a pharmaceutical excipient widely used to improve solubility, can act as an effective adjuvant for an influenza vaccine. However, the mechanisms by which mucosal immune pathway is critical for the intranasal adjuvant activity of HP-β-CD have not been fully delineated. Here, we show that intranasally administered HP-β-CD elicits a temporary release of IL-33 from alveolar epithelial type 2 cells in the lung; notably, IL-33 expression in these cells is not stimulated following the use of other vaccine adjuvants. The experiments using gene deficient mice suggested that IL-33/ST2 signaling is solely responsible for the adjuvant effect of HP-β-CD when it is administered intranasally. In contrast, the subcutaneous injection of HP-β-CD and the intranasal administration of alum, as a damage-associated molecular patterns (DAMPs)-inducing adjuvant, or cholera toxin, as a mucosal adjuvant, enhanced humoral immunity in an IL-33-independent manner, suggesting that the IL-33/ST2 pathway is unique to the adjuvanticity of intranasally administered HP-β-CD. Furthermore, the release of IL-33 was involved in the protective immunity against influenza virus infection which is induced by the intranasal administration of HP-β-CD-adjuvanted influenza split vaccine. In conclusion, our results suggest that an understanding of administration route- and tissue-specific immune responses is crucial for the design of unique vaccine adjuvants.

Recent Publications

- Toriyama M, Rizaldy D, Nakamura M, Fujita F, Okada F, Morita A, Ishii KJ. Immunological role of primary cilia of dendritic cells in human skin disease. bioRxiv, doi: https://doi.org/10.1101/2020.02.04.933333 (2020).
- Kobari S, Kusakabe T, Momota M, Shibahara T, Hayashi T, Ozasa K, Morita H, Matsumoto K, Saito H, Ito S, Kuroda E, Ishii KJ. IL-33 Is essential for adjuvant effect of hydroxypropyl-β-cyclodexrin on the protective intranasal influenza vaccination. Front Immunol. 11, 360 (2020).
- Momota M, Lelliott P, Kubo A, Kusakabe T, Kobiyama K, Kuroda E, Imai Y, Akira S, Coban C, Ishii KJ. ZBP1 governs the inflammasome-independent IL-1α and neutrophil inflammation that play a dual role in anti-influenza virus immunity. Int Immunol. 32(3), 203-212 (2020).
- Ozasa K, Temizoz B, Kusakabe T, Kobari S, Momota M, Coban C, Ito S, Kobiyama K, Kuroda E, Ishii KJ. Cyclic GMP-AMP triggers asthma in an IL-33-dependent manner that is blocked by amlexanox, a TBK1 inhibitor. Front Immunol. 10, 2212 (2019).

Immunoparasitology



Masahiro Yamamoto, PhD

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Associate Professor	Miwa Sasai
Assistant Professor	Ji Su Ma Youngae Lee
Postdoctoral Fellow	1
Research Assistant	1
Visiting Scientist	1
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The 2017 World Health Organization report estimates that nearly 440,000 deaths were caused by infection with the obligatory protozoan human malaria parasite, *Plasmodium*. Malaria, one of the most important vector-borne diseases, occurs when *Plasmodium* parasites are transmitted via *Anopheles* mosquitoes to mammalian hosts such as humans. The life cycle of *Plasmodium* parasites is divided into three stages, the vector stage in the mosquito, and the liver and blood stages in the host. During the vector stage, *Plasmodium* forms oocysts in the mosquito midgut from where it moves in the next life stage (as sporozoites) to salivary glands and acquires infectivity to mammalian hosts. Infection starts with the delivery of sporozoites to the host by the bite of a *Plasmodium* infected mosquito. Sporozoites first enter the blood circulation and then the liver, where they invade hepatocytes. After this, they develop further as exoerythrocytic forms (EEFs). Each EEF generates the release of thousands of merozoites into the blood stream where they invade erythrocytes, initiating the blood stages of infection and causing clinical malarial disease. Thus, the development of *Plasmodium* parasites in the liver is an obligatory stage for the successful infection of their mammalian hosts.

After their arrival at the liver, sporozoites traverse the host cells such as Kupffer cells and hepatocytes and form parasitophorous vacuoles (PVs) in the finally-invaded hepatocytes. Although the sporozoite traverse of dermis and Kupffer cells is indispensable for the liver stage development into EEFs *in vivo*, the cell traversal itself is dispensable for liver stage parasite development *in vitro*, since traverse-deficient parasites form normal liver stage development in hepatocytes, suggesting that sporozoite infection into hepatocytes rather than the cell traverse is important for the liver stage development. Sporozoites from the rodent malaria parasite *Plasmodium berghei* invade rodent hepatocytes causing hepatocyte growth factor (HGF) secretion and activating the MET receptor, resulting in suppressed hepatocyte apoptosis and facilitating sporozoite development into EEFs. Although the Leiriao et al. study strongly suggested that the development of *P. berghei* in the liver involves host factors, activation of the HGF/MET signaling pathway is not essential for liver stage *P. yoelii* rodent malaria parasites or for the *P. falciparum* human malaria parasite. Thus, little is known about the common regulatory circuit that is active during liver-stage development in *Plasmodium* species.

In the mosquito vector, *Plasmodium* sporozoites are rod-shaped. After invading hepatocytes, the rod-shaped sporozoites undergo bulbous expansion and transform into spherical EEFs. The morphological transformation of sporozoites into early EEFs can be induced outside of hepatocytes and is known to require serum and an optimal temperature of 37°C. Ca^{2+} is known to regulate temperature-dependent sporozoite development into EEFs under cell-free conditions. However, it is also known that ectopic morphological transformation of sporozoites outside hepatocytes is detrimental to the life cycle of *Plasmodium* parasites, as *P. berghei* lacking PUF2, an RNA binding protein, displays spherical EEF-like parasites in the mosquito salivary glands, resulting in failure of parasite invasion into the hepatocytes. Thus, it seems likely that morphological transformation into EEFs may be tightly controlled so that it only occurs in hepatocytes during the parasite's life cycle; however, the host factors that critically regulate the morphological transformation of sporozoites as well as their differentiation into EEFs in the infected hepatocytes are not well understood.

Here, we show that sporozoite differentiation into EEFs in the liver involves protein kinase C ζ-mediated NF-κB activation, which robustly induces the expression of C-X-C chemokine receptor type 4 (CXCR4) in hepatocytes and subsequently elevates intracellular Ca^{2+} levels, thereby triggering sporozoite transformation into EEFs. Blocking CXCR4 expression by genetic or pharmacological intervention profoundly inhibited the liver stage development of the *P. berghei* rodent malaria parasite and the human *P. falciparum* parasite also. Collectively, our experiments show that CXCR4 is a key host factor for *Plasmodium* development in the liver, and CXCR4 warrants further investigation for malaria prophylaxis.

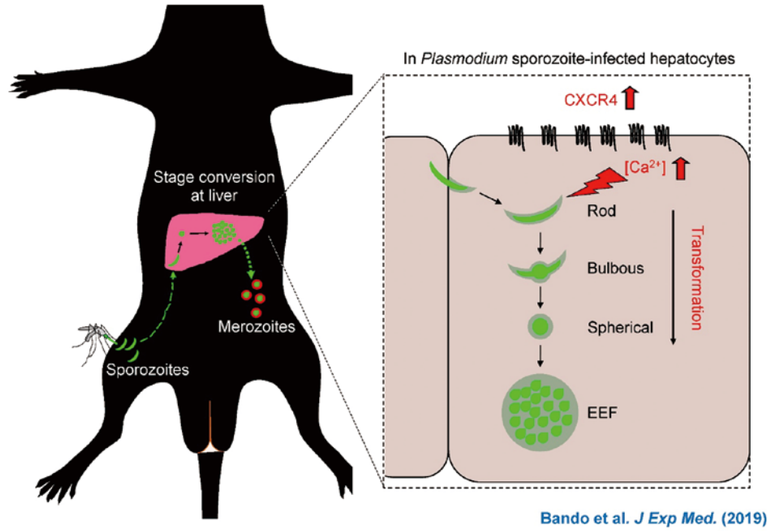
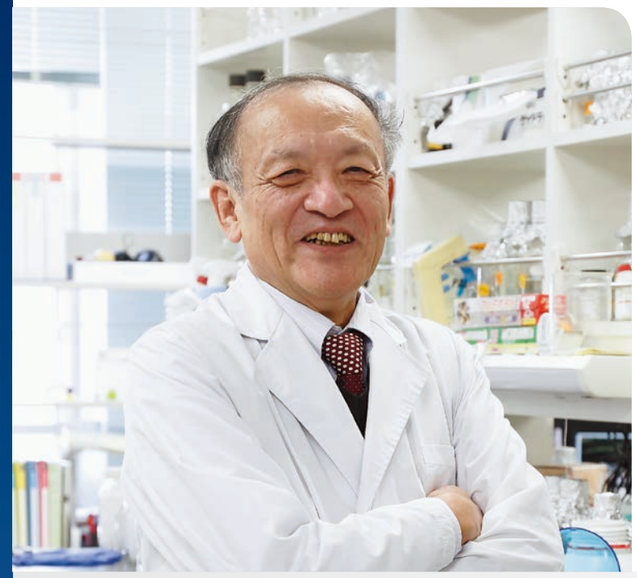


Figure. CXCR4 regulates the liver-stage *Plasmodium* development by Ca^{2+} increment in hepatocytes.

Recent Publications

- Bando H, Pradipta A, Iwanaga S, Okamoto T, Okuzaki D, Tanaka S, Vega-Rodríguez J, Lee Y, Ma JS, Sakaguchi N, Soga A, Fukumoto S, Sasai M, Matsuura Y, Yuda M, Jacobs-Lorena M, Yamamoto M. CXCR4 regulates *Plasmodium* development in mouse and human hepatocytes. J. Exp. Med. 216:1733-1748 (2019) doi: 10.1084/jem.20182227.
- 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) doi: 10.1128/mBio.01738-18.
- 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. Nature Immunology. (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

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Phospholipids are asymmetrically distributed between inner and outer leaflets of plasma membranes. Phosphatidylserine (PtdSer) 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 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 found that B cell progenitors express ATP11C but not ATP11A. Thus, B cells in ATP11C$^{-/-}$ mice have no flippase at plasma membranes, causing sustained PtdSer-exposure, and are engulfed alive by macrophages.

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. Using a microarray system with lipid bilayers in which phospholipids are asymmetrically distributed, we showed that a single dimeric molecule of TMEM16F can scramble phospholipids. TMEM16F is ubiquitously expressed, 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 of phospholipids during apoptosis. Xkr8 is expressed ubiquitously, while Xkr4 and Xkr9 are expressed in a tissue-specific manner, the brain or intestine, respectively. These Xkr members contain a caspase-recognition site in the C-terminal region, and are cleaved by caspase to function as a scramblase. Thus, in apoptotic cells, caspase cleaves and irreversibly inactivates ATP11A and 11C 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, 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 neutrophils and lymphocytes 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 SLE-type autoimmune disease. Similarly, a large number of male germ cells undergo apoptosis, and are cleared by Sertoli cells. We found that apoptotic germ cells expose PtdSer in an Xkr8-dependent manner, and many dead *Xkr8*$^{-/-}$ germ cells are left unengulfed in the testis, causing male infertility.

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 in which biological process it is activated. We are also interested in the molecular mechanism of how flippases flip phospholipids, and how scramblases scramble phospholipids at plasma membranes.

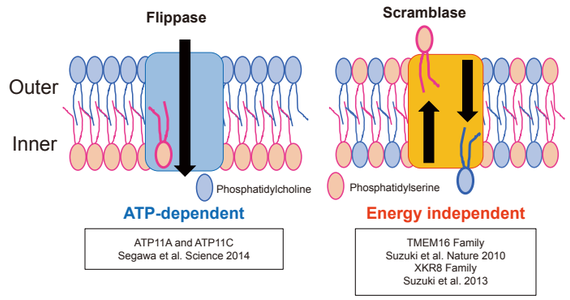

Figure 1. Flippase and Scramblase.
Plasma membranes in eukaryotic cells are comprised of outer and inner leaflets, in which phosphatidylserine (PtdSer) and phosphatidylcholine are located exclusively at inner or outer leaflets, respectively. The asymmetrical distribution of PtdSer is maintained by an 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.

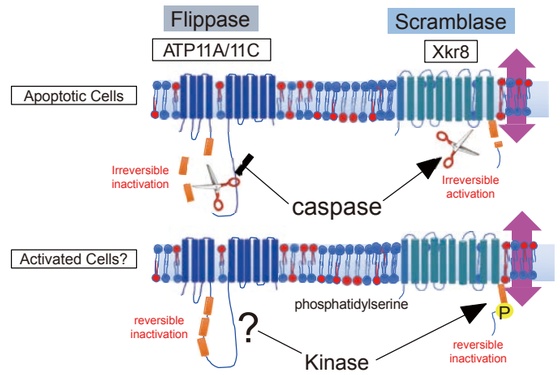

Figure 2. Activation of Xkr8’s scramblase and inactivation of flippases by two independent mechanisms.
In apoptosis, caspase cleave Xkr8 to activate scramblase while it cleaves flippases (ATP11A and 11C) to inactivate, causing irreversible exposure of PtdSer. Under activated conditions, unidentified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

Recent Publications

- Yamashita Y, Suzuki C, Uchiyama Y and Nagata S. Infertility caused by inefficient apoptotic germ cell clearance in Xkr8-deficient male mice. Mol. Cell Biol. 40, e00402-e00419 (2020).
- Ryoden Y, Fujii T, Segawa K and Nagata S. Functional expression of the P2X7 ATP receptor requires Eros. J. Immunol. 204, 559-568 (2020).
- 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 and Tanaka M. Programmed cell death and the immune system. Nat. Rev. Immunol. 17, 333-340 (2017).

Molecular Neuroscience

Toshihide Yamashita, MD/PhD

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Postdoctoral Fellow	3
Visiting Scientist	6
Support Staff	2

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.

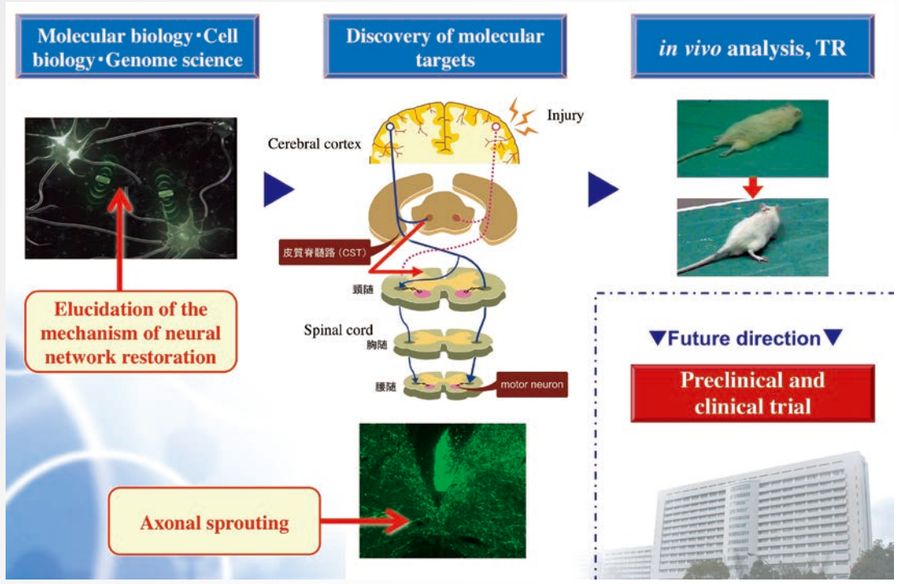

Figure 1. The mechanism of spontaneous functional recovery.

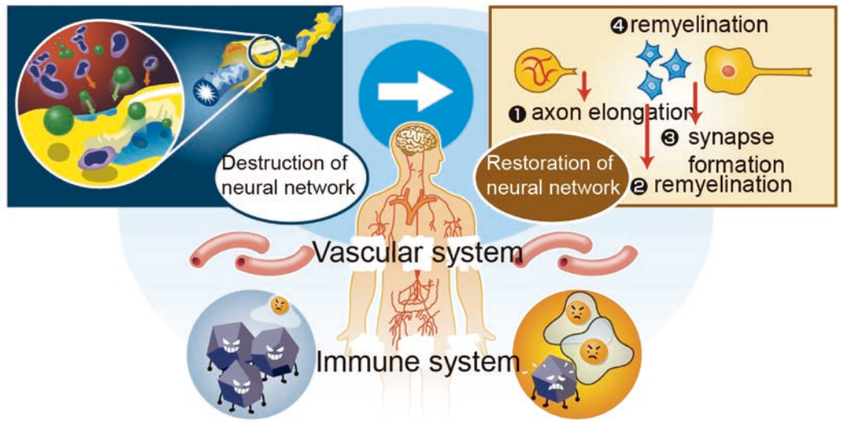

Figure 2. Biological systems that regulate rewiring of neural network after CNS injury.

Recent Publications

- Hamaguchi M, Muramatsu R, Fujimura H, Mochizuki H, Kataoka H and Yamashita T. Circulating transforming growth factor-β1 facilitates remyelination in the adult central nervous system. eLife 8, e41869 (2019).
- Tanabe S and 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 and 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 and 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 and Yamashita T. A chromosome 16p13.11 microduplication causes hyperactivity through dysregulation of miR-484/protocadherin-19 signaling. Mol. Psychiatry 22, 364-374 (2017).

Stem Cell Biology and Developmental Immunology

Takashi Nagasawa, MD/PhD

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Assistant Professor	Yoshiki Omatsu
Support Staff	1

Special microenvironments known as niches are essential for the maintenance of hematopoietic stem cells (HSCs), which give rise to blood cells, and lymphohematopoiesis within bone marrow cavities. We isolated a chemokine, CXCL12 (SDF-1/PBSF) as a molecule that stimulates the growth of B cell precursors (Nagasawa et al. PNAS 1994) and found that CXCL12 and its receptor CXCR4 are essential for colonization of bone marrow by HSCs during embryogenesis (Nagasawa et al. Nature 1996; Ara et al. Immunity 2003), maintenance of a pool of HSCs (Sugiyama et al. Immunity 2006), and development of immune cells, including B cells, plasmacytoid dendritic cells (pDCs) and NK cells in bone marrow (Nagasawa. Nat. Rev. Immunol. 2006) as well as vascular formation and cardiogenesis (Tachibana et al. Nature 1998). Subsequently, we identified a population of reticular cells expressing CXCL12 at high levels, termed CXCL12-abundant reticular (CAR) cells, within bone marrow (Tokoyoda et al. Immunity 2004; Sugiyama et al. Immunity 2006) and indicated that CAR cells are mesenchymal stem cells, which give rise to adipocytes and osteoblasts (Seike et al. Genes Dev. 2018), the major producer of CXCL12 and SCF (Omatsu at al. Immunity 2010), and the major cellular components of niches for HSCs and hematopoietic cells, including B cells (Omatsu at al. Immunity 2010; Shimoto et al. Blood 2017).

CAR cell-specific transcription factors, Foxc1 and Ebf1/Ebf3 are essential for formation and maintenance of niches for HSCs and immune cells

In 2014, 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 developing and maintaining niches for HSCs and immune cells (Omatsu et al. Nature 2014).

Bone marrow is the tissue filling the space between bone surfaces. CAR cells have potential to differentiate into osteoblasts in bone marrow. However, it had remained unclear how osteogenesis was prevented in most CAR cells to maintain HSC niches and marrow cavities. We found that the transcription factor Ebf3 is preferentially expressed in CAR cells in adult bone marrow, using lineage-tracing. When Ebf3 was deleted in CAR cells, HSC-niche function was severely impaired and bone marrow was osteosclerotic with increased bone in aged mice. In mice lacking both Ebf1 and Ebf3, CAR cells exhibiting a normal morphology were abundantly present but their niche function was markedly impaired with depleted HSCs at 1 week of age. Subsequently, the mutants become progressively more osteosclerotic, leading to the complete occlusion of marrow cavities in early adulthood. Expressions of osteogenic genes were increased in CAR cells with reduced HSC-niche factor expression in the absence of Ebf1/Ebf3. Thus, HSC cellular niches express Ebf3 that is required to create HSC niches, to inhibit their osteoblast differentiation, and to maintain spaces for HSCs (Seike et al. Genes Dev. 2018).

We are studying the molecular regulation of CAR cell properties and generation and the roles of CXCL12-CXCR4 signaling and CAR cells in the spatiotemporal regulation of lymphohematopoiesis during homeostasis and diseases.

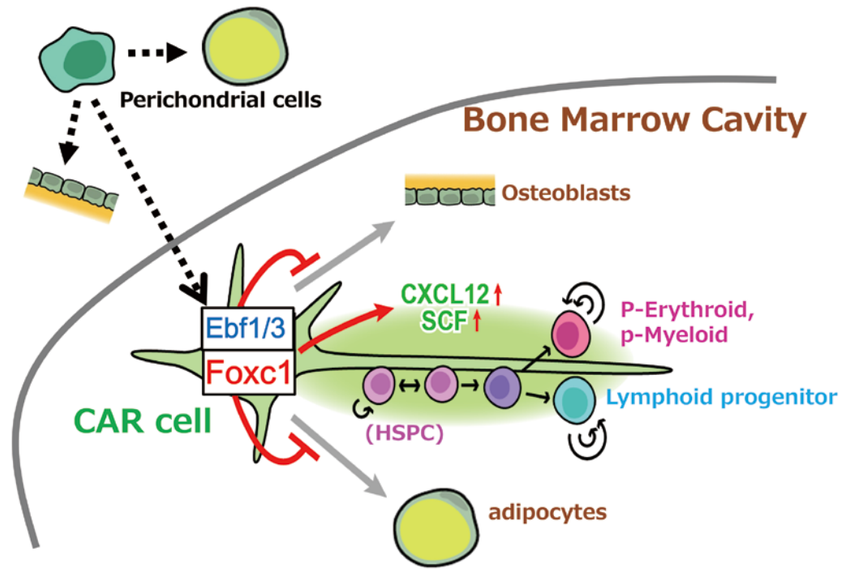

Figure. The development and functions of CAR cells.
The transcription factors, Foxc1 and Ebf1/Ebf3, and HSC niche factors, CXCL12 and SCF, are preferentially and abundantly expressed in CAR cells and critical for the formation and maintenance of niches for HSCs and hematopoietic cells, including B cells, within the bone marrow.

Recent Publications

- Matsushita Y, Nagata M, Kozloff KM, Welch JD, Mizuhashi K, Tokavanich N, Hallett SA, Link DC, Nagasawa T, Ono W and Ono N. A Wnt-mediated transformation of the bone marrow stromal cell identity orchestrates skeletal regeneration. Nat Commun. 11(1), 332(2020).
- 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. 24(5), 769-784(2019).
- Seike M, Omatsu Y, Watanabe H, Kondoh G, Nagasawa T. Stem cell nich-specific Ebf3 maintains the bone marrow cavity. Genes Dev. 32(5-6), 359-372(2018).
- Shimoto M, Sugiyama T and Nagasawa T. Numerous niches for hematopoietic stem cells remain empty during homeostasis. Blood 129(15), 2124-2131(2017).
- Omatsu Y, Seike M, Sugiyama T, Kume T, and Nagasawa, T. Foxc1 is a critical regulator of haematopoietic stem/progenitor cell niche formation. Nature 508, 536-540(2014).

Molecular Immunology

Sho Yamasaki, PhD

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Research Assistant	5
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Structural basis for the recognition of pathogen-derived phosphoglycolipids by C-type lectin receptor DCAR

The C-type lectin receptors (CLRs) form a family of pattern recognition receptors (PRRs) that recognize numerous pathogens, such as bacteria and fungi, and trigger innate immune responses. The extracellular carbohydrate recognition domain (CRD) of CLRs forms a globular structure that can coordinate a Ca^{2+} ion, allowing receptor interactions with sugar-containing ligands. Although well conserved, the CRD fold can also display differences that directly affect the specificity of the receptors for their ligands. Here, we report crystal structures at 1.8-2.3 Å resolutions of the CRD of murine dendritic cell–immunoactivating receptor (DCAR/*Clec4b1*), the only CLR that binds phosphoglycolipids such as acylated phosphatidyl-*myo*-inositol mannosides (AcPIMs) of mycobacteria. Using mutagenesis analysis, we identified critical residues, Ala136 and Gln198, on the surface surrounding the ligand-binding site of DCAR, as well as an atypical Ca^{2+}-binding motif (Glu-Pro-Ser/$EPS_{168–170}$). By chemically synthesizing a water-soluble ligand analog, inositol-monophosphate di-mannose (IPM2), we confirmed the direct interaction of DCAR with the polar moiety of AcPIMs by biolayer interferometry and co-crystallization approaches. We also observed a hydrophobic groove extending from the ligand-binding site that is in a suitable position to interact with the lipid portion of whole AcPIMs. These results suggest that the hydroxyl group-binding ability and hydrophobic groove of DCAR mediate its specific binding to pathogen-derived phosphoglycolipids such as mycobacterial AcPIMs.

Pathogenic contribution of Protein Kinase D in liver fibrosis

Liver fibrosis is a central pathological process that occurs in most types of chronic liver diseases. Advanced liver fibrosis causes cirrhosis, hepatocellular carcinoma, and liver failure. However, the exact molecular mechanisms underlying the initiation and progression of liver fibrosis remain largely unknown. This study was designed to investigate the role of protein kinase D3 (PKD3, gene symbol *Prkd3*) in the regulation of liver homeostasis. We generated global PKD3-deficient (*Prkd3*$^{-/-}$) mice and myeloid cell-specific PKD3-deficient (*Prkd3*$^{\Delta LysM}$) mice, and found that both *Prkd3*$^{-/-}$ mice and *Prkd3*$^{\Delta LysM}$ mice displayed spontaneous liver fibrosis. PKD3 deficiency also aggravated carbon tetrachloride-induced liver fibrosis. PKD3 is highly expressed in hepatic macrophages, and PKD3 deficiency skewed macrophage polarization toward a profibrotic phenotype. The activated profibrotic macrophages produced TGF-β that in turn activates hepatic stellate cells (HSCs) to become matrix-producing myofibroblasts. Moreover, PKD3 deficiency decreased the phosphatase activity of SHP1, a known PKD3 substrate, resulting in sustained STAT6 activation in macrophages. In addition, we observed that PKD3 expression in hepatic macrophages was downregulated in cirrhotic human liver tissues. Thus, PKD3 plays a protective role in the development of liver fibrosis.

Novel MR1 ligands that modulate Mucosal-Associated Invariant T (MAIT) Cells

Mucosal-associated invariant T (MAIT) cells are a subset of recently identified innate-like T lymphocytes that play an important role in many pathologies ranging from viral and bacterial infection, to autoimmune disorders and cancer. MAIT cells are activated via the presentation of ligands by MR1 on antigen presenting cells to the MAIT T cell receptor (TCR), however few studies have explored the effects of systematic changes to the ligand structure on MR1 binding and MAIT cell activation. We report on the first study into the effects of changes to the sugar motif in the known MAIT cell agonists 7-hydroxy-6-methyl-8-d-ribityllumazine (RL-6-Me-7-OH) and 5-(2-oxopropylideneamino)-6-d-ribitylaminouracil (5-OP-RU). Tetramer staining of MAIT cells revealed that the absence of the 2'-hydroxy group on the sugar backbone of lumazines improved MR1-MAIT TCR binding, which could be rationalised using computational docking studies. Although none of the lumazines activated MAIT cells, all 5-OP-RU analogues showed significant MAIT cell activation, with several analogues exhibiting comparable activity to 5-OP-RU. Docking studies with the 5-OP-RU analogues revealed different interactions between the sugar backbone and MR1 and the MAIT TCR compared to those observed for the lumazines and confirmed the importance of the 2'-hydroxy group for ligand binding and activity. Taken together, this information will assist in the development of future potent agonists and antagonists of MAIT cells.

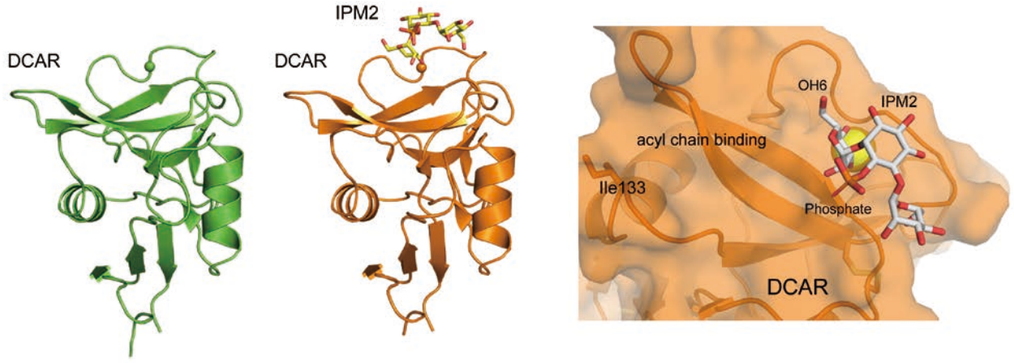

Figure. Crystal structure of the DCAR CRD in complex with IPM2.
Overall structure of the ligand-free DCAR CRD (ligand-free, PDB code: 6LKR)(left), the IPM2 complex (middle). Close-up view of the ligand binding site bound of DCAR to IPM2 (PDB code: 6LFJ). Hydrophobic groove extending from the ligand-binding site is in a suitable position to interact with the lipid portion of whole AcPIMs. Mannose and inositol residues are shown by Man and Ins respectively (right).

Recent Publications

- Omahdi Z, Horikawa Y, Nagae M, Toyonaga K, Imamura A, Takato K, Teramoto T, Ishida H, Kakuta Y, Yamasaki S. Structural insight into the recognition of pathogen-derived phosphoglycolipids by C-type lectin receptor DCAR. J. Biol. Chem. 295, 5807-5817 (2020).
- 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).
- 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).
- 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).

Aging Biology

Eiji Hara, PhD

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Assistant Professor	Shimpei Kawamoto
Postdoctoral Fellow	3
Research Assistant	3
Visiting Scientist	1
Support Staff	2

Oncogenic proliferative signals are coupled to a variety of growth inhibitory processes, such as the induction of apoptotic cell death and stable cell-cycle arrest, in phenomena termed cellular senescence. Both apoptosis and cellular senescence are considered to serve as important safeguards against neoplasia. However, unlike apoptotic cells, senescent cells are viable for long periods of time and thereby accumulate with age in various organs and tissues. Moreover, it has recently become apparent that senescent cells are not merely non-dividing, but eventually develop a secretory profile composed of pro-inflammatory cytokines, chemokines, and extracellular matrix-degrading proteases, a typical signature termed the senescence-associated secretory phenotype (SASP). Although SASP reportedly plays some beneficial roles, it also exhibits deleterious side effects such as chronic-inflammation and/or tumorigenesis, depending on the biological context. Thus, although cellular senescence primarily acts as a tumor suppression mechanism, the accumulation of senescent cells in aged tissues may eventually promote the age-related decline of organ function and/or associated diseases, such as cancer. Indeed, the clearance of $p16^{INK4a}$-positive senescent cells from aged transgenic mice reportedly delays in the onset of various age-related dysfunctions, such as sarcopenia, cataracts, atherosclerosis, loss of adipose tissue, and tumorigenesis, thus extending the healthy lifespan. Along similar lines, the elimination of therapy-induced senescent cells reduced several side-effects of chemotherapy and even cancer recurrence in mice. Thus, it is anticipated that the removal of senescent cells could prevent the toxicity of anticancer treatments and enhance their therapeutic benefits.

Senolytic drugs, which specifically induce cell death in senescent cells, are likely to represent a new therapeutic avenue, and several candidate drugs were identified using a bioinformatics approach. However, the senolytic drugs identified to date were not discovered by a truly unbiased high-throughput screening (HTS) method, and thus appear to have limitations in clinical applications. For example, in a phase II study of ABT263 (a specific inhibitor of the anti-apoptotic proteins BCL2 and BCL-xL) for the treatment of advanced and recurrent small-cell lung carcinoma patients, transient thrombocytopenia and neutropenia were reported as side-effects. Furthermore, the combination of dasatinib and quercetin (D+Q), another previously reported senolytic drug, apparently exhibited remarkable cell-type specificity, although its mechanisms of action remain obscure. Therefore, the identification of more effective senolytic drugs and the elucidation of their mechanisms of action are required towards a better strategy for the removal of senescent cells *in vivo*.

Here, we have identified bromodomain and extra-terminal domain (BET) family protein inhibitor (BETi) as a promising new class of senolytic drugs. The blockade of BRD4, a BET family protein, by chemical inhibitors or RNA interference robustly provokes senolysis. This is due, at least in part, to the combined effects of the attenuation of non-homologous end joining (NHEJ) repair and the activation of autophagic pathway in senescent cells. These results reveal the cellular vulnerability of senescent cells, and provide valuable novel insights into the resistance of senescent cells to death and open up new possibilities for its control.

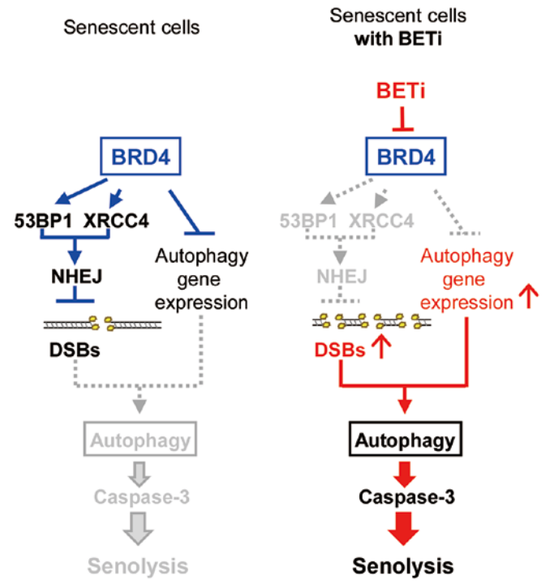

Figure. The model distinguishing mechanisms that may operate in the absence (left) or presence (right) of BETi in senescent cells.

Recent Publications

- Wakita M., et al. A BET family protein degrader provokes senolysis by targeting NHEJ and autophagy in senescent cells. Nat. Commun. 11, 1935 (2020).
- Gorgoulis V., et al. Cellular senescence: defining a path forward. Cell 179, 813-827 (2019).

Oncogene Research

Masato Okada, PhD

Professor	Masato Okada
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Assistant Professor	Kentaro Kajiwara
	(S.A.) Tetsuya Kimura
Postdoctoral Fellow	1
Research Assistant	2
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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 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 coupled with the AP1 transcription factors, 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 activated Src is ubiquitinated and promotes its secretion via exosomes to suppress its oncogenic potential. In addition, we identified CDCP1 as a Src-activating membrane glycoprotein in lipid rafts. Upregulation of CDCP1 induces prominent activation of Src and the STAT3 pathway, which promotes the invasive activity of epithelial cells. We also found that ablation of CDCP1 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 CDCP1 is a co-receptor of MET (Figure 1). Furthermore, ablation of CDCP1 suppresses the compensatory renal hypertrophy, indicating that CDCP1 is required for the HGF-MET signaling even *in vivo*. CDCP1 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

We previously 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, 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*. Recent studies in the intestinal tissues revealed that the p18-mediated mTORC1 signaling promotes the anabolic metabolism required for robust production of mucin in goblet cells. These findings underscore the critical role of p18 in the regulation of metabolic homeostasis in various tissues and cells. To further analyze the regulation of the p18 complex at the molecular level, we 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.

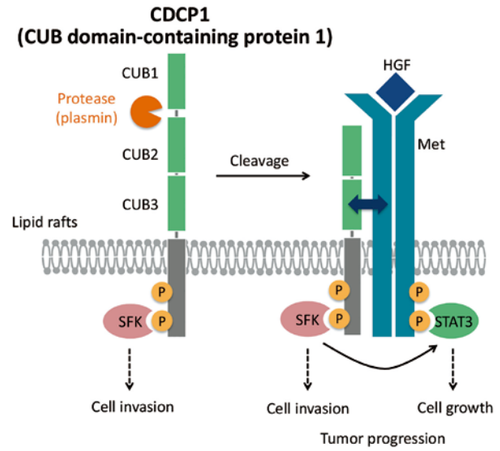


Figure 1. Role of CDCP1 in the Src-mediated growth factor signaling.

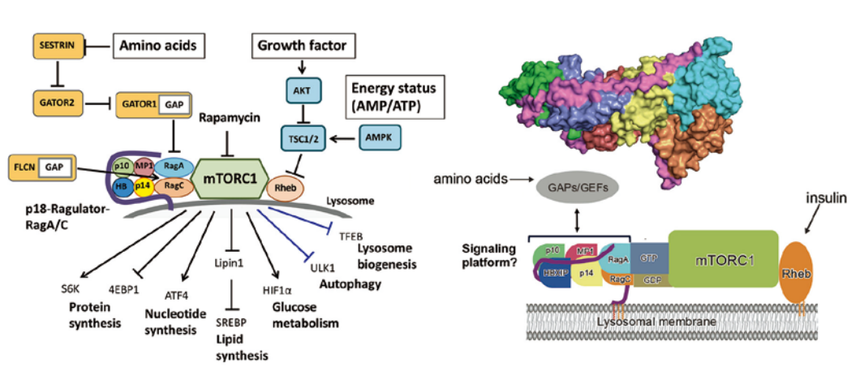
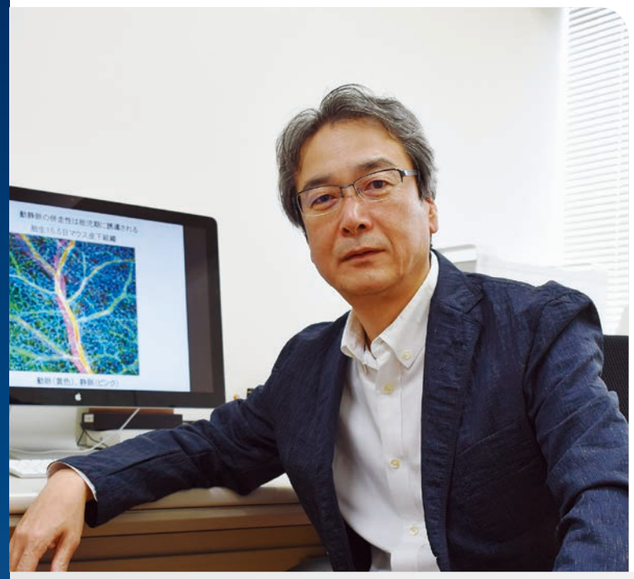


Figure 2. Molecular basis for the regulation of mTORC1 on lysosomes.

Recent Publications

- Tanaka K, Ito Y, Kajiwara K, Nada S, Okada M. Ubiquitination of Src promotes its secretion via small extracellular vesicles. Biochem. Biophys. Res. Commun. 525, 184-191 (2020).
- 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).
- 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, MD/PhD

Professor	Nobuyuki Takakura
Associate Professor	Hisamichi Naito Hiroyasu Kidoya
Postdoctoral Fellow	3
Research Assistant	2
Support Staff	1

Our research team is involved in studies in terms of vascular biology and stem cell biology. Among the results of our research in 2019, two of the interesting ones are shown here.

1) How integrity of blood vessels is induced after birth, is there stem cell population in endothelial cells?

So far, the existence of tissue specific stem cells has been elucidated in many organs, such as hematopoietic, neural, gut, epidermal (including hair) stem cells and so on. In the cardiovascular system, although the cardiac stem cell population was identified by using cell surface markers, vascular endothelial stem cells (VESCs) have not been identified by their cell surface markers. We previously reported that endothelial cells (ECs) with highly proliferative potential were fractioned in side population (SP) cells which express ABC transporter abundantly. EC-SP cells indeed differentiate into main population ECs and contribute to ECs for long term in the hindlimb ischemia model (Naito et al, EMBO J. 2012); however, we have not shown the hierarchy of ECs from stem cell population into terminally differentiated ECs through progenitor population. Recently, we identified specific cell surface markers (CD157 and CD200) to identify VESCs and showed abilities of single cell transplantation and clonal expansion of VESCs in the liver injury model (Wakabayashi et al. Cell Stem Cell 2018). This year, we reported the details of methods of how CD157 positive VESCs can be isolated from the liver with some modifications (Figure, Naito et al. Nature Protoc. 2020).

2) How human blood vessel formation can be induced and visualized in mice?

The cellular and molecular mechanisms of blood vessel formation in mice and humans is thought to be basically similar. Although gene modification is available to observe such mechanism in mice, it is not easy to assess *in vivo* function of cells or molecules for blood vessel formation in humans. So far, merely *in vitro* proliferation, migration, and tube formation analyses have been utilized to evaluate blood vessel formation by using human ECs from several organ origins. An important issue that we need to be concern about is that many different types of cells affect ECs to promote *in vivo* blood vessel formation and we need to evaluate the 3D structure of blood vessels with time course. We have recently succeeded in visualizing human blood vessels spatiotemporally using a patient derived xenograft (PDX) model generated in immunodeficient mice (Tsukada et al. paper in preparation). Using this PDX model, we will assess the mechanism of human vascular formation.

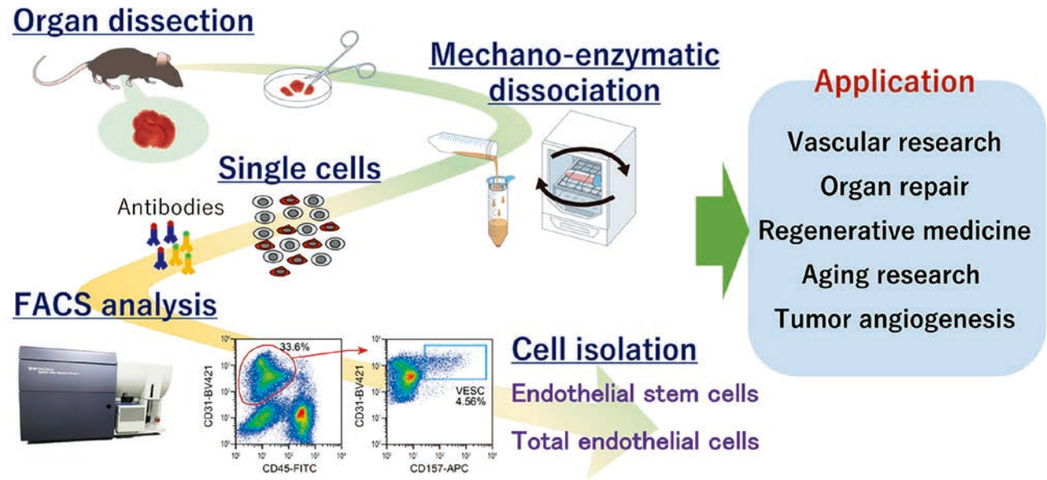


Figure. New mechanistic insight of blood vessel formation by endothelial stem cells.

Recent Publications

- Naito H, Wakabayashi T, Ishida M, Gil CH, Iba T, Rahmawati FN, Shimizu S, Yoder MC, Takakura N. Isolation of tissue-resident vascular endothelial stem cells from mouse liver. Nat. Protoc. 15, 1066-1081 (2020).
- 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).

Cutaneous Immunology

Manabu Fujimoto, MD/PhD

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Postdoctoral Fellow	1
Research Assistant	1

Trim21/IgG heavy chain/HLA-DR complex is expressed on the cell surface, and becomes a target for autoantibodies (ongoing)

We have found that some misfolded proteins generated in the ER can be transported to the cell surface by HLA class II alpha and beta heterodimers. Furthermore, these misfolded protein/HLA class II complexes are revealed as targets for autoantibodies. Immunoglobulin heavy chain (IgG HC) is regarded as one of the misfolded proteins that can be complexed with HLA class II molecules. This time, we have identified that cytosolic SS-A Ro52 protein: Trim21 associates with IgG HC/HLA-DR complex, and becomes a target for autoantibodies in several systemic autoimmune diseases, such as Sjogren's syndrome, or some dermatomyositis subtypes. We discovered that a cytoplasmic/nuclear protein complexes with HLA class II and becomes a target for autoantibodies.

Aging alteration of skin T cells is different from that of blood T cells (ongoing)

Immune function is known to decrease with age, resulting in increased numbers of tumors and infections. We have assessed T cell density, diversity and function in individuals of various ages to study the immunologic effects of aging on human skin. No decline in the density of T cells was noted in whole skin or dermis with advanced age. Of note, the frequency of epidermal T cells, particularly CD49a$^+$ CD8 resident memory T cells, was higher in elderly individuals. T cell diversity and antibacterial responses were maintained in the skin of elderly individuals but declined in the blood. Our findings suggest that skin T cells maintain diversity and protective cytokine production in elderly individuals despite reduced T-cell diversity and function in blood.

Dysregulation of circular and resident T cells targeting melanocyte in autoimmune vitiligo

Vitiligo is an autoimmune disorder related to melanocyte loss; however, the exact interplay between antigen-specific autoimmunity and local oxidative stress remains unclear. We found a reduced suppressive function of activated Tregs on Melan-A-specific CD8$^+$ T cells in the circulating cells of vitiligo patients compared with healthy controls. This result suggests that T cell anergy with Tregs dysfunction in circulation may participate in the immune response to melanocytes in vitiligo patients. In addition, skin resident memory CD8$^+$ T cells preferentially isolated from the vitiligo skin are now processed for single cell transcriptome analysis to unveil the specific character towards stressed melanocytes.

Elucidation of common mechanism of both skin and brain disorders by mTORC1

Tuberous sclerosis complex (TSC) is an inherited disorder caused by the constitutive activation of mTORC1 as a result of the inactivation of causative gene *TSC1* or *TSC2*. The disease is characterized by skin disorders including hamartomas, hypomelanotic macules and central nervous system abnormalities such as epilepsy and autism. The mechanisms of hypomelanotic macules, epilepsy and autism remain unknown, although that of hamartomas are known. To elucidate the common mechanisms described above, we made *MITF*-conditional knock out, *Mitf-M*-cre *Tsc2*$^{flox-/-}$, mice, which developed vitiligo and epilepsy. We elucidated that abnormal ER stress which results in mitochondrial oxidative stress occurred in both neurons in the brain and melanocytes in the skin of the mice, and demonstrated that these abnormalities were restored by the inhibition of mTORC1.

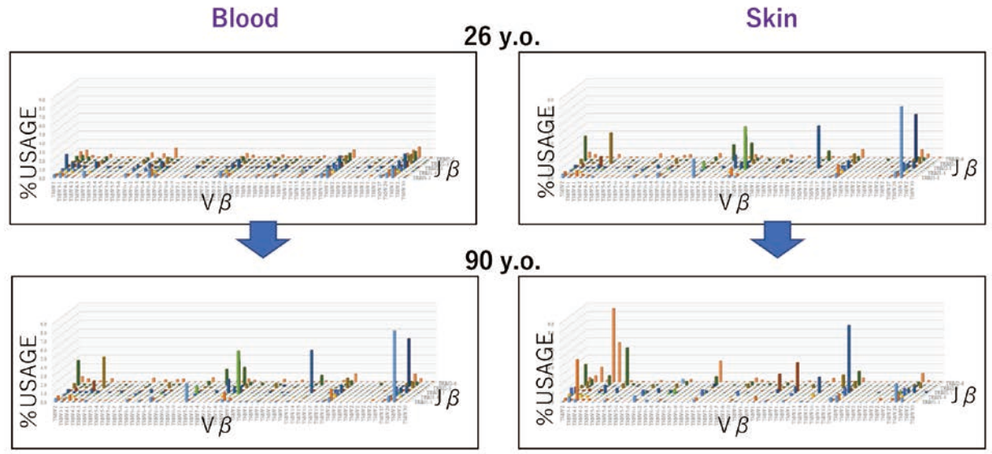


Figure. Difference in T lymphocyte antigen receptor repertoire between the blood and skin from the youth and elderly.
T cells were harvested from the blood and skin from people of different ages, and the repertoire of T cell antigen receptors were analyzed. Representative results from 26-year-old and 90-year-old subjects. (Koguchi-Yoshioka et al. submitted.)

Recent Publications

- Inoue M, Tanboon J, Hirakawa S, Komaki H, Fukushima T, Awano H, Tajima T, Yamazaki K, Hayashi R, Mori T, Shibuya K, Yamanoi T, Yoshimura H, Ogawa T, Katayama A, Sugai F, Nakayama Y, Yamaguchi S, Hayashi S, Noguchi S, Tachimori H, Okiyama N, Fujimoto M, Nishino I. Association of dermatomyositis sine dermatitis and with anti-nuclear matrix protein 2 autoantibodies. JAMA Neurol. (2020) in press.
- Okiyama N, Yamaguchi Y, Kodera M, Hamaguchi Y, Yokozeki H, Ishiguro N, Fujimoto M. Distinct histopathologic patterns of finger eruptions in dermatomyositis based on myositis-specific autoantibody profiles. JAMA Dermatol.155, 1080-1082 (2019).
- Matsushita T, Kobayashi T, Mizumaki K, Kano M, Sawada T, Tennichi M, Okamura A, Hamaguchi Y, Iwakura Y, Hasegawa M, Fujimoto M, Takehara K. BAFF inhibition attenuates fibrosis in scleroderma by modulating the regulatory and effector B cell balance. Sci. Adv.4, eaas9944 (2018).
- Saito A, Okiyama N, Kubota N, Nakamura Y, Fujisawa Y, Watanabe R, Ishitsuka Y, Bleackley RC, Fujimoto M. Blockade of granzyme B remarkably improves mucocutaneous diseases with keratinocyte death in interface dermatitis. J. Invest. Dermatol. 138, 2079-2083 (2018).

Innate Immune Systems

Kazuyo Moro, Ph.D

Professor	Kazuyo Moro
Postdoctoral Fellow	1
Support Staff	1

Group 2 innate lymphoid cells (ILC2) play crucial roles in type 2 immune responses.

ILC2 are multifunctional innate lymphocytes that play roles in type 2 immunity and tissue homeostasis at barrier surfaces of the body. ILC2 are localized to the adipose tissue, lung, colon, skin, bone marrow, and other peripheral tissues and monitoring pathogen invasion. Upon invasion with helminth, damaged epithelial cells release IL-33 and IL-25, which activate ILC2 and initiate IL-5 and IL-13 production. IL-5 and IL-13 induce type 2 immune responses including eosinophilia and mucin production from goblet cells. Furthermore, there is increasing evidence that ILC2 are strongly associated with allergic diseases such as asthma and atopic dermatitis. Cysteine proteases derived from mites and pollen strongly induce IL-33 release from epithelial cells, resulting in the induction of excess ILC2-mediated immune responses, and cause allergic symptoms. Therefore, the treatment of allergic diseases targeting ILC2 is expected as a promising approach (Figure 1) .

ILC2 increase susceptibility to allergy in response to environmental factors.

T cell antigens derived from mites and pollen are known to be a trigger of allergic diseases. On the other hand, allergic diseases can also be caused by environmental factors such as drugs, stress, exercise and cold weather that do not act as T cell antigens. However, how environmental factors cause allergy symptoms is unclear. We found that ILC2 produced IL-4 in response to environmental stress, and resulted in the secretion of IgE from B1 cells without antigen stimulation. We also revealed that IgE from B1 cells promoted the survival of FcεRIα^+ cells such as mast cells and basophils, leading to the increase of allergic susceptibility. Consistently, the IgE-mediated accumulation of FcεRIα^+ cells induces severe allergic symptoms through the amplification of ILC2-mediated responses via cysteinyl leukotriene (CysLT) upon IL-33 stimulation. These findings suggest that ILC2, B1 cells, and FcεRIα^+ cells compose a positive feedback loop. Taken together, our research demonstrated that ILC2 play a crucial role in the pathogenesis of allergic diseases in response to environmental factors (Figure 2).

Screening of inhibitors for ILC2 function from marine microbe extract

There is accumulating evidence that ILC2 are responsible for the development of allergy symptoms. Moreover, recent studies demonstrated the involvement of ILC2 in various diseases such as obesity, rheumatism, and idiopathic pulmonary fibrosis, indicating the possibility that ILC2 is a therapeutic target for allergic diseases as well as various diseases. Inhibitors for ILC2 function, however, have not yet been developed. Therefore, to develop drugs for these diseases, we screened for an inhibitor for ILC2 using the library of marine microbe extracts received from Nagasaki university as part of the 'Screening of ILC2 activator and inhibitor from marine microbe extract library' project. To date, 480 types of extracts have been screened *in vitro* and some candidates for ILC2 inhibitors have been found. The ILC2 selectivity and cytotoxicity of these extracts as well as its effects *in vivo* will be assessed and further the effective component of the extract will be defined with the aim of developing an ILC2 therapeutic agent (Figure 3).

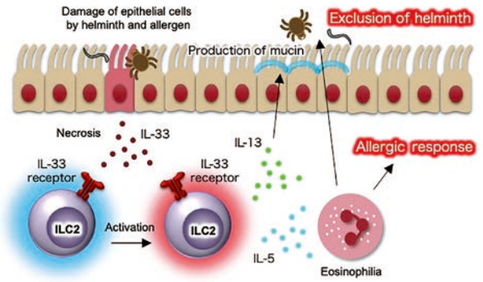


Figure 1. Invasion of helminth induce the release of IL-33 from epithelial cells via necrosis. IL-33 induces IL-5 and IL-13 production from ILC2 to induce strong type 2 immune responses. ILC2-derived IL-5 and IL-13 eliminate parasites by inducing eosinophilia and mucin production, respectively.

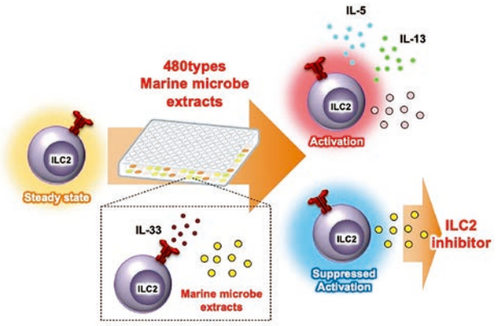


Figure 3. Inhibitor of ILC2 function was screened for using library of marine microbe extracts. ILC2 are cultured with 480 types of marine microbe extract under IL-33 stimulation, some extracts suppressed ILC2 proliferation as well as IL-5 and IL-13 production from ILC2, suggesting that these extracts have a potential to suppress ILC2 functions.

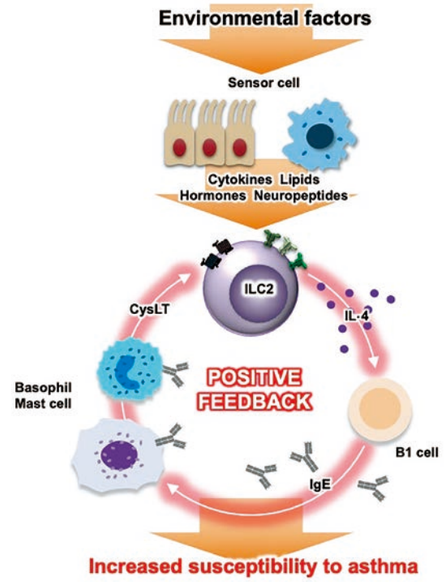


Figure 2. IL-4 derived from ILC2 in response to environmental factors promotes IgE production from B1 cells. This IgE enhances survival of mast cells and basophils and cause severe allergy upon IL-33 stimulation. Moreover, Cysteinyl leukotriene (CysLT) released by mast cells and basophils induces IL-4 production from ILC2, suggesting that these innate immune cells compose positive feedback machinery to increase susceptibility to allergy.

Recent Publications

- Kobayashi T, Ricardo-Gonzalez RR, Moro K. Skin-resident innate lymphoid cells - Cutaneous innate guardians and regulators. Trends Immunol. 41(2), 100-112, doi:10.1016/j.it.2019.12.004 (2020).
- Sasaki T, Moro K, Kubota T, Kubota N, Kato T, Ohno H, Nakae S, Saito H, Koyasu S. Innate lymphoid cells in the induction of obesity. Cell Rep. 28(1), 202-217.e7, doi:10.1016/j.celrep.2019.06.016 (2019).
- Kabata H, Moro K, Koyasu S. The group 2 innate lymphoid cell (ILC2) regulatory network and its underlying mechanisms. Immunol. Rev. 286 (1), 37-52 (2018).
- Koga S, Hozumi K, Hirano KI, Yazawa M, Terooatea T, Minoda A, Nagasawa T, Koyasu S, Moro K. Peripheral PDGFRalpha (+) gp38 (+) mesenchymal cells support the differentiation of fetal liver-derived ILC2. J. Exp. Med. 215 (6), 1609-1626 (2018).
- Moro K, Kabata H, Tanabe M, Koga S, Takeno N, Mochizuki M, Fukunaga K, Asano K, Betsuyaku T, Koyasu S. Interferon and IL-27 antagonize the function of group 2 innate lymphoid cells and type 2 innate immune responses. Nat. Immunol. 17 (1), 76-86 (2016).

Human Immunology (Single Cell Immunology)

James Badger Wing, PhD

Associate Professor	James Badger Wing
Support Staff	1

Background

The Human Immunology (single cell immunology) lab was established in November of 2019 and our work focuses on both single cell biology and its applications to human immunology. In particular, we have an established interest in regulatory T cells (Tregs) expressing the transcription factor Foxp3, which play a critical role in the control of immune homeostasis. Recently it has become clear that a specialized subset of Tregs, T-follicular regulatory cells, (Tfr) have a particular role in the control of T-follicular helper (Tfh) cell-driven germinal center (GC) responses responsible for antibody production. Recent results strongly suggest that Tfr are critical for the regulation of autoantibody production, vaccine response and the production of high affinity IgE responsible for allergies. However, due to their recent discovery in 2011 our understanding of Tfr formation and function still has many gaps. Previously, we and others have demonstrated that Tfr have a high frequency in human blood and lymphoid organs, often making up as many as 50% of total Tregs. Partly because of this combination of high frequency and role in the control of antibody production Tfr are believed to be important to human disease. However, very little is known about changes to their phenotype and function in autoimmune disease patients since the first comprehensive descriptions of their phenotype in humans were carried out by us and others in 2017. The status and subtypes of germinal center resident Tfr in human tonsils in either non-inflamed tonsillar hypertrophy or IgA nephropathy patients are largely unknown at this time. A clear goal of the field is to determine how to manipulate a specific subset of Tregs as for example enhancement of Tfr function would allow control of autoimmune antibody production without affecting the role of Tregs in tumor Immunosurveillance.

Mass Cytometry and single cell immunology

Tregs have key roles in multiple sites and recent advances in single cell biology have made it clear that Tregs show a great range of heterogeneity of states in autoimmunity and cancer. We specialize in the use of single cell technologies in order to better understand the function and phenotype of Treg cells and other cells in the human immune system. In particular mass cytometry (CyTOF) is a technique that allows us to assess the protein expression of millions of cells in a single experiment producing large datasets allowing the detailed comparison of Immune cells in different donors and tissue sites. Additionally, in collaboration with other groups at both IFReC and abroad we are developing novel techniques to combine sequencing based protein analysis with epigenetic analysis with the broader aim of combining these powerful, but cell limited, techniques with the collection of larger CyTOF datasets for multi-omic analysis. This would be greatly beneficial since it allows an understanding of how epigenetic modifications affect protein expression on a single cell level.

Application of single cell immunology to Tregs

Using the CyTOF technique, we have recently found that many Tfr in the blood and tonsils of humans have a naïve phenotype. This unique combination of a memory like Tfr phenotype with naïve characteristics raises the possibility that it is possible to take advantage of both of these factors since in most cases memory like cells do not expand easily in culture. However, we find that naïve like Tfr are expandable in culture and can retain their memory characteristics, indicating that the mass production of these cells as a novel cell therapy may be possible. We are continuing to investigate this point. We believe that further profiling of these cells in both healthy and autoimmune donors and improving ways to experimentally manipulate them will provide both novel insights into any defects in their function in these settings.

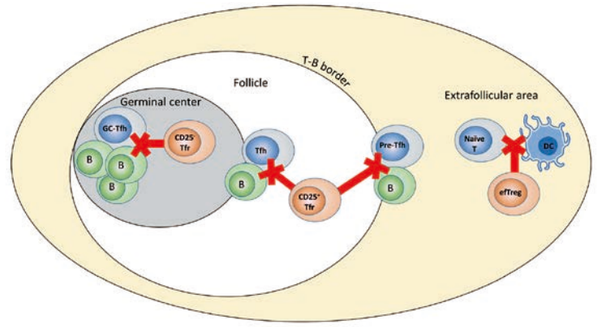


Figure 1. Multiple roles of Tfr cells in the regulation of humoral immunity.

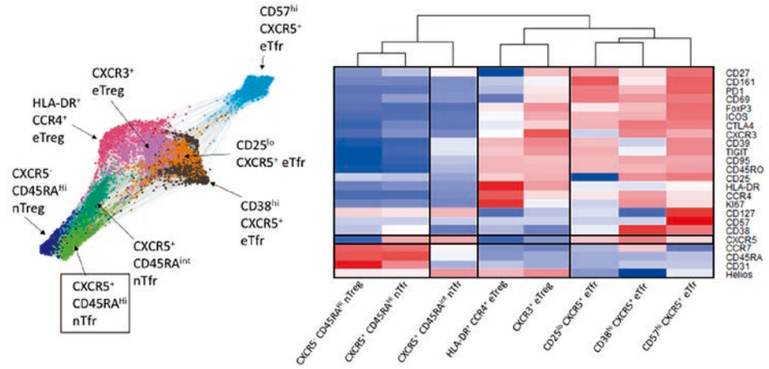


Figure 2. CyTOF analysis of human immune Tregs.

Recent Publications

- Sakaguchi S, Mikami N, Wing JB, Tanaka A, Ichiyama K, Ohkura N. Regulatory T cells and human disease. Annu. Rev. Immunol. 38 (2020) online ahead of print.
- Wing JB, Tanaka A, Sakaguchi S. Human FOXP3+ regulatory T cell heterogeneity and function in autoimmunity and cancer. Immunity 50 (2), 302-316 (2019).
- Ha D, Tanaka A, Kibayashi T, Tanemura A, Sugiyama D, Wing JB, Lim EL, Teng KWW, Adeegebe 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. Proc. Natl. Acad. Sci. USA. 116 (2), 609-618 (2019).
- Wing JB, Tay C, Sakaguchi S. Control of regulatory T cells by co-signal molecules. Co-signal Molecules in T Cell Activation, 179-210 (2019).
- Wing JB, Tekgüç M, Sakaguchi S. Control of germinal center responses by T-follicular regulatory cells. Front. Immunol. 9, 1910 (2018).

Human Immunology (Single Cell Genomics)

Daisuke Okuzaki, PhD

Associate Professor	Daisuke Okuzaki
Visiting Scientist	2

The Laboratory of Human Immunology was established in November 2019 for the purpose of accelerating the application of single cell sequencing technology to improve the connection between fundamental science research and clinical applications. In strategic alliances with top-level immunology research institutes as well as the Graduate School of Medicine and Faculty of Medicine, Osaka university, the lab also utilizes the facilities of the Genome Information Research Center of Research Institute for Microbial Diseases. Last year, multiple equipment assets including single cell resolution sequencing systems capable of sequencing molecules from tens of thousands of cells simultaneously (Chromium (10xGenomics), a BD Rhapsody Express Single-Cell Analysis System, cutting-edge next generation sequencers (Illumina NovaSeq 6000) with the highest throughput currently available in the industry, and a large-scale supercomputer server for the storage and processing of experiment data were introduced, making IFReC one of the few research centers in the country capable of conducting NGS experiments on tens of thousands of samples. A research paper (Hasegawa et al. Nat. Immunol. 2019) that was published in 2019 was based on such single-cell sequencing experiments. Furthermore, experiment data for over one hundred samples was generated in collaboration with the various research laboratories in IFReC. We are currently developing a single-cell gene expression profile based system for novel immune cell type identification as well as the most comprehensive database of immune cells in Japan. The database will include the data generated from different independent research groups as well as from publicly available resources, and will be made available to the collaborating researchers for conducting integration, annotation, and data mining as shown in the figure.

In addition, we developed a pipeline called UNAGI or UNAnnotated Gene Identifier, which processes long-read sequencing data obtained from nanopore sequencing, and compared it with the standard Illumina pipeline by studying the *Saccharomyces cerevisiae* transcriptome in full-length cDNA samples generated from two different biological samples: haploid and diploid cells (Al Kadi et al. Funct. Integr. Genomics 2020). The result outperformed the Illumina pipeline and FLAIR in transcript reconstruction and further contributed to the discovery of 3877 unannotated transcripts including 1282 intergenic transcripts.

In another study (Okuzaki et al. Cancer Sci. 2020), we elucidated the process of cell transformation driven by c-Src, a tyrosine kinase found upregulated in various human cancers by analyzing the expression profiles of miRNAs in a doxycycline-inducible Src expression system. We found that miRNA (miR)-129-1-3p was downregulated in the early phase of c-Src-induced cell transformation and that the reexpression of miR-129-1-3p disrupted c-Src-induced cell transformation. In addition, miR-129-1-3p downregulation was tightly associated with tumor progression in human colon cancer cells/tissues. Results of these studies demonstrate the value of our development of robust algorithms for high throughput sequencing data analysis as well as the potential benefits of the generated data to the scientific community. The sequencing data from our lab can provide not only insight into gene expression profiles in correlation to human physiology but also into novel transcriptome findings in bacteria or other non-model organisms, studies of non-coding RNAs, and into the pathology roles of post transcript-regulator such as miRNAs.

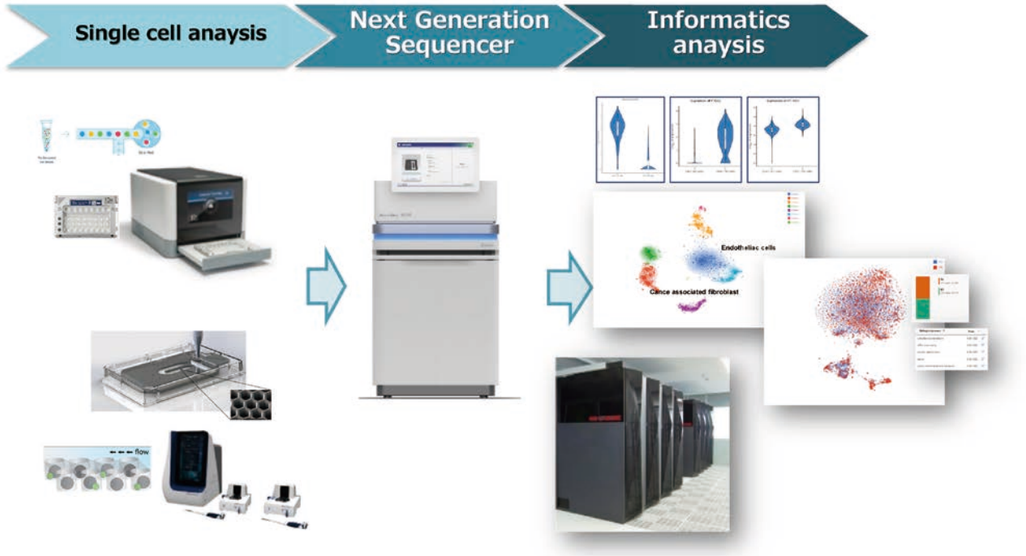


Figure. Overview of the research process.

Recent Publications

- Al Kadi M., et al. UNAGI: an automated pipeline for nanopore full-length cDNA sequencing uncovers novel transcripts and isoforms in yeast. Funct. Integr. Genomics, doi: 10.1007/s10142-020-00732-1 (2020).
- Okuzaki D., et al. c-Src promotes tumor progression through downregulation of microRNA-129-1-3p. Cancer Sci. 111, 418-428, doi: 10.1111/cas.14269 (2020).
- Hasegawa T., et al. Identification of a novel arthritis-associated osteoclast precursor macrophage regulated by FoxM1. Nat. Immunol. 20, 1631-1643, doi: 10.1038/s41590-019-0526-7 (2019).
- Okuzaki D., et al. FCN1 (M-ficolin), which directly associates with immunoglobulin G1, is a molecular target of intravenous immunoglobulin therapy for Kawasaki disease. Sci. Rep. 7, 11334, doi: 10.1038/s41598-017-11108-0 (2017).
- Okuzaki D., et al. Microarray and whole-exome sequencing analysis of familial Behçet's disease patients. Sci. Rep. 6, 19456, doi: 10.1038/srep19456 (2016).

Single Molecule Imaging

Toshio Yanagida, PhD
Ben Seymour, MD/PhD

Professor	Toshio Yanagida Ben Seymour
Postdoctoral Fellow	1
Support Staff	2

The central focus of our IFReC research is trying to unravel the symptomatology of inflammatory disease. In diseases such as inflammatory arthritis, it is well recognised that the most troubling symptoms are fatigue and pain. Fatigue in particular is presumed to be triggered by the acute inflammatory response, such as by blood-brain communication of cytokines. However, what makes fatigue such a difficult problem is that it becomes treatment resistant: even when the acute inflammatory process is controlled with therapy, such as immunologics, the symptoms persist. Therefore some process in the brain leads to the maintenance of symptoms, and this severely limits the clinical effectiveness, in terms of holistic metrics such as quality of life, of immunologics.

We have been studying the brain in patients with inflammatory arthritis, and animal models thereof. In humans, we are able to directly quantify fatigue symptoms, to try and unravel the component neural processes with which it is related. We do this by studying a set of behavioural tasks that tap into these core components, and use sophisticated computational models to decompose neural operations, and then map these in the brain using brain imaging. This process we term computomic brain mapping.

In our results attained over this last year, we have been able to make substantial progress in identifying the core fatigue computational phenotype. In summary, when probing learning and motivational behaviour, patients have a clear enhancement of sensitivity to punishments. This enhancement strongly correlated with specific clinical measures of fatigue. And in particular, this shows a strong correlation with serum levels of IL-6. Our current research now is attempting to localise the effective interaction between IL-6 and fatigue-related behaviour in the brain, based on neuroimaging data.

At the same time, we have also been developing functional biomarkers, using machine learning tools, for fatigue (and pain) based on resting-state brain imaging, in both humans and rodent models. These have been successful in finding discriminative classifiers. However, components of these classifiers typically correlate poorly with specific computational or inflammatory (cytokine) metrics. This suggests that resting-state brain imaging may have a limited ability to identify mechanistic components of disease/symptom specific phenotypes, and emphasises the importance of using targeting behaviour-based approaches to pinpoint immune-brain interactions.

Beyond this, our other research has involved looking at other (non-inflammatory) clinical conditions using machine-learning based brain biomarkers. And more broadly, we have been looking at basic mechanisms of motivational, pain and learning, to provide the fundamental insights necessary to probe inflammation-linked psychopathology.

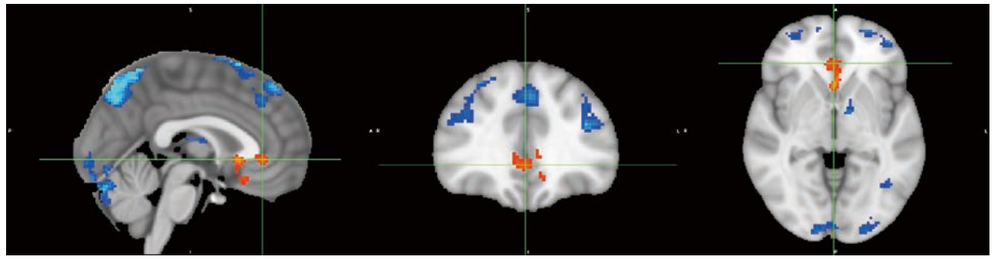


Figure 1. In this experiment, we applied computational models of human motivation and decision-making to deconstruct the brain processes involved behaviour, with the aim to identify regions that are more active in fatigue in inflammatory arthritis and therefore implicated in generating the fatigue phenotype. This figure illustrates pregenual and subgenual anterior cingulate cortex as robustly activated, which strongly implicates them in fatigue.

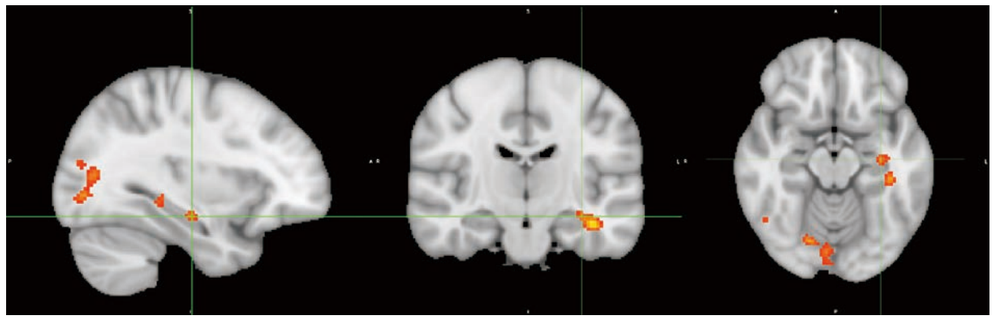


Figure 2. In patients with active inflammatory arthritis, serum IL-6 levels are raised, and strongly modulates the prediction error (which drives human motivated learning) specifically in the hippocampus - a brain region closely related to human memory and consciousness.

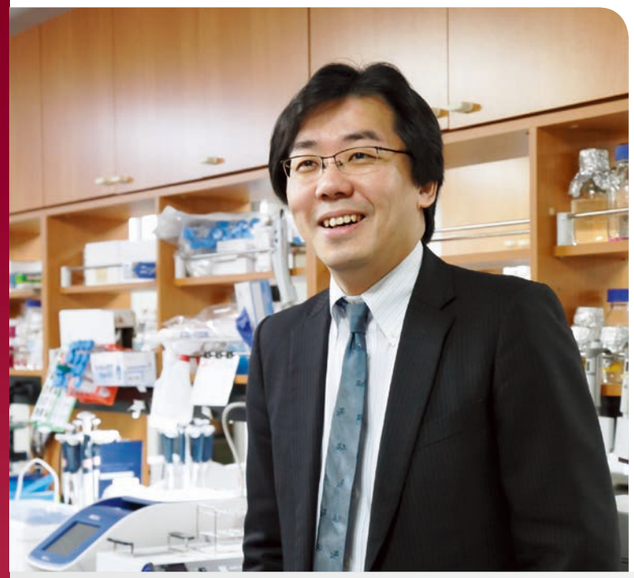
Recent Publications

- Takenaka S, Kan S, Seymour B, Makino T, Sakai Y, Kushioka J, Tanaka H, Watanabe Y, Shibata M, Yoshikawa H and Kaito T. Resting-state amplitude of low-frequency fluctuation is a potentially useful prognostic functional biomarker in cervical myelopathy. Clin.Orthop. Relat.Res. doi: 10.1097/CORR.0000000000001157 (2020).

- Ichikawa N, Lisi G, Yahata N, Okada G, Takamura M, Hashimoto RI, Yamada T, Yamada M, Suhara T, Moriguchi S, Mimura M, Yoshihara Y, Takahashi H, Kasai K, Kato N, Yamawaki S, Seymour B, Kawato M, Morimoto J and Okamoto Y. Primary functional brain connections associated with melancholic major depressive disorder and modulation by antidepressants. Scientific Reports 10(1),1-12 (2020).

- Taylor JE, Lau H, Seymour B, Nakae A, Sumioka H, Kawato M and Koizumi A. An evolutionarily threat-relevant odor strengthens human fear memory. Frontiers in Neuroscience. doi: 10.3389/fnins.2020.00255 (2020).

Immunology and Cell Biology



Masaru Ishii, MD/PhD

Professor	Masaru Ishii
Associate Professor	Keizo Nishikawa Junichi Kikuta
Assistant Professor	Takao Sudo Yutaka Uchida
Postdoctoral Fellow	1
Research Assistant	2
Support Staff	3

1. 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*, 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*.

2. 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 a 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.

3. Splitting the “AtoM” in search of a treatment for rheumatoid arthritis

Inside live bones, “osteoclasts” and “osteoblasts” are constantly generated. The former break down bones while the latter synthesize them. Under well-balanced bone destruction and formation processes, bones continually destroy and form themselves. In the latest study, we developed a unique technology to collect and analyze cells from arthritic joints, successfully identifying a new type of bone-destroying osteoclast that contributes to rheumatoid arthritis (RA), called Arthritis-associated osteoclastogenic Macrophages, or “AtoMs” (Nat. Immunol. 2019). We found that the arthritis-inducing osteoclasts have properties and origins that are distinct from normal osteoclasts involved in bone metabolism. By specifically inhibiting the differentiation and functions of arthritis-inducing osteoclasts without affecting normal osteoclasts, their discovery will aid in the establishment of breakthrough therapy and the development of anti-RA drugs.

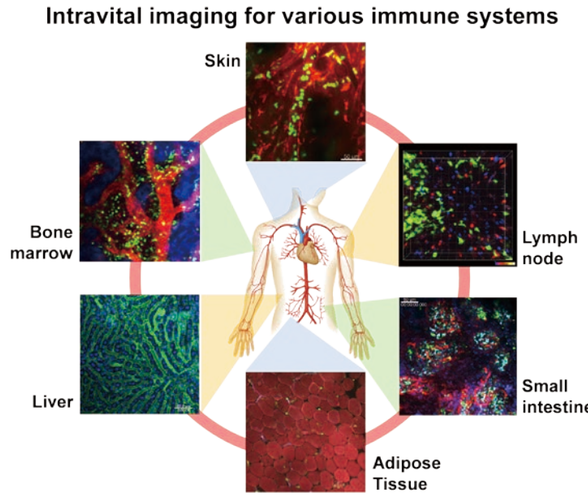


Figure. *In vivo* cellular dynamics in various immune systems. Immune cells are high dynamic and interconnecting various tissues and organs, by forming a ‘soft-wired’ network. We are elucidating the basic principle controlling the dynamic nature of immune cells by visualizing *in vivo* behaviors with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

Recent Publications

- Hasegawa T., et al. Identification of a novel arthritis-associated osteoclast precursor macrophage regulated by FoxM1. Nat. Immunol. 20, 1631-43 (2019).
- 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).
- Nishikawa K., et al. Dnmt3a regulates osteoclast differentiation by coupling to an S-adenosyl methionine-producing metabolic pathway. Nat. Med. 21, 281-7 (2015).

Nuclear Medicine

Jun Hatazawa, MD/PhD

Professor	Jun Hatazawa
Associate Professor	Hiroki Kato
Assistant Professor	Tadashi Watabe
Research Assistant	3

Glioblastoma is one of the most intractable malignancies with a 5-year survival rate of 9.8% even when combination therapy by surgical removal, chemotherapy, and external radiation was applied.

Radionuclide therapy with alpha particles targeting intractable cancers is now being developed in Osaka University. Alpha particles have a strong cell killing effect because of high radiation quality factor. To realize this, the key issues are 1) large amount of production of alpha particles such as actinium-225 (physical half-life:10 day) and Astatine-211 (physical half-life: 7.2 hour) effective for clinical use, 2) cancer cell specific delivery of alpha particle-bound molecules, 3) an evaluation of normal tissue damage. We are now developing astatine-211 labeled phenylalanine (^{211}AtPhe) as a therapeutic agent because of a cancer specific nature as L-type amino acid transporter 1 (Watabe T, et al. Oncotarget 2020), and fluorine-18 labeled fluoro-phenylalanine (^{18}FPhe) (Hatazawa J. et al. Ann. Nucl. Med. 1994; Ogawa T. et al. Eur. J. Nucl. Med. 1996) and fluorine-18 labeled fluoro-borono-phenylalanine (^{18}FBPA) (Shimosegawa E. et al. Ann. Nucl. Med 2016; Beshr R. et al. Ann. Nucl. Med. 2018; Romanov V. et al. Ann. Nucl. Med. 2020) as diagnostic imaging agents with PET.

Astatine-211 was produced by means of AVF Cyclotron facilities in the Research Center for Nuclear Physics, Osaka University, by ^{209}Bi(α,2n)^{211}At nuclear reaction (Ikeda H. et al. Appl. Radiat. Isot. 2018). The metallic Bi target was irradiated by accelerated alpha particles. The produced ^{211}At was separated from the bismuth target and purified via dry distillation. The irradiated Bi target was put in a quartz column and heated up to 850°C using an electric tubular furnace under mixed helium and oxygen gas flow. The evaporated ^{211}At was then swept out from the quartz column with the mixed gas flow and passed through a Teflon tube which was cooled by ice water to trap evaporated ^{211}At. After collection of ^{211}At on the Teflon trap tube for approximately 20 minutes, the trapped ^{211}At was dissolved in approximately 100μL of distilled water (Figure 1) (Watabe T. et al. J. Nucl. Med. 2019).

Treatment effect of ^{211}AtPhe on glioblastoma cells was investigated in mice bearing C6 glioma xenografts and GL261 allografts. The tumor sizes (mm3) were monitored using a caliper. In C6 glioma xenograft model, a delay in tumor growth was found depending on radioactivity of the injected ^{211}AtPhe dose. In controls, tumor volume reached 4 mm^3 at 7 days after injection of saline, whereas it reached 4 mm^3 at 18 days, 25 days, and 30 days after 0.1 MBq, 0.5 MBq, and 1.0 MBq injection of ^{211}AtPhe, respectively (Figure 2).

The whole-body distribution of ^{211}AtPhe was investigated in normal male ICR mice (n=6, 9 weeks old, mean body weight=37.7g). The major organs (brain, thyroid gland, salivary gland, heart, lung, esophagus, liver, stomach, small intestine, large intestine, kidney, adrenal gland, pancreas, spleen, and testes) and blood. Thyroid gland and stomach showed relatively higher accumulation than other organs. There was no significant difference in body weight between controls and ^{211}AtPhe treated mice.

These preclinical studies suggested that ^{211}AtPhe has potential to treat glioblastoma. Further histopathological studies are required before proceeding to the clinical trial.

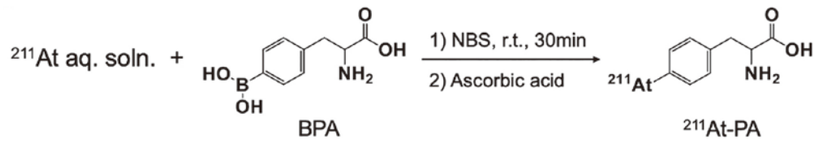


Figure 1. Reaction for the labeling of para-borono-L-phenylalanine with ^{211}At.

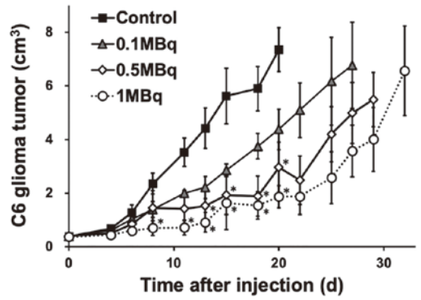
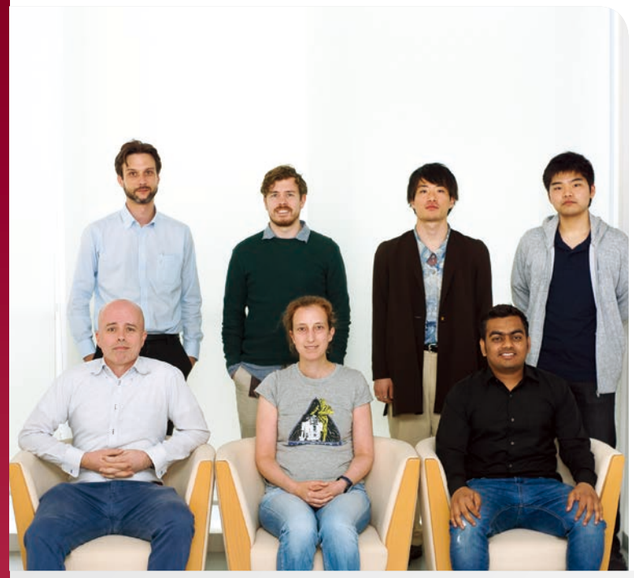


Figure 2. Tumor growth suppression effect by ^{211}AtPhe.

Recent Publications

- Watabe T., et al. Targeted alpha therapy using astatine (^{211}At)-labeled phenylalanine: A preclinical study in glioma bearing mice. Oncotarget 11, 1399-1398 (2020).
- Liu Y., et al. Preclinical evaluation of radiation-induced toxicity I targeted alpha therapy using [211At]NaAt in mice: a revisit. Trans. Oncol. 13(4), 100757 (2020).
- Watabe T., et al. Enhancement of 211At uptake via the sodium iodide symporter by the addition of ascorbic acid in targeted α-therapy of thyroid cancer. J. Nucl. Med. 60, 1301-1307 (2019).
- Beshr R., et al. Preliminary feasibility study on differential diagnosis between radiation-induced cerebral necrosis and recurrent brain tumor by means of [^{18}F]fluoro-borono-phenylalanine PET/CT. Ann. Nucl. Med. 32, 702-708 (2018).
- Ikeda H., et al. Application of astatine-210: Evaluation of astatine distribution and effect of pre-injected iodide in whole body of normal rats. Appl. Radiat. Isot. 139, 251-255 (2018).
- Shimosegawa E., et al. Assessment of 10B concentration in boron neutron capture therapy: potential of image-guided therapy using 18FBPA. Ann. Nucl. Med. 30, 749-755 (2016).

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, 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.

More specifically, we use a combination of label-free methods: Raman scattering that provides molecular signatures from a target cell, and quantitative phase imaging that provides a full quantitative map of the cell morphology, and it turns out that this information is quite related to conditions of interest in the cell. Both of these data sets can be collected in a few seconds or shorter, from a single cell, providing paired measurements relating to the molecular components and the shape-based features. The composition of a cell can vary not only due to the conditions of interest, such as protein expression in response to a stimulus, but also due to inherent biological variability. Similarly, the morphology of cells can be seen to vary significantly from cell to cell when observing a culture dish. Amongst all this inherent cell-to-cell variability however, lie certain key features, both in molecular composition and morphological features that can distinguish cell properties such as the activation state. We previously showed (Pavillion et al, PNAS 2018) that in a macrophage cell line, the response to LPS could be determined from the Raman-derived compositional features, or from the morphological features. The Raman-derived cell composition could in general discern LPS-based activation with a higher degree of accuracy than could be determined from the morphology. Since cell morphology can be thought of as a “later” indicator of cellular change, in the sense that signaling molecules should be expressed or depleted before the overall cell structure shows visible change, we believe both of these techniques are valuable in single-cell analysis. Additionally, both can be paired to maximize the amount of information that can be obtained from a single-cell without modifying it or labeling it.

A recent extension of this approach led to some intriguing results. One question was whether the above analysis would function equally well in primary cells rather than a cell line. We then considered macrophages derived from the mouse peritoneal cavity. Experimentally, to obtain sufficient numbers of cells for statistically relevant analysis then requires multiple donor mice, with the complication that mouse-to-mouse variations could then become important. Turning this complication into a point of interest, we could then ask to what degree the experimental parameters such as mouse-to-mouse variation occur, and how strong such variations are in comparison to the changes that occur with immune activation. This led to the first comprehensive label-free study of how individual peritoneal cavity macrophages respond to LPS, how the procedures for obtaining them (i.e. resident or elicted) affect the responses, and how the different experimental parameters affect the robustness of the analysis. This used single-cell measurements, and spanned more than 30,000 cells in total. The results showed that the label-free analysis could determine single cell LPS response, whether it was resident or had been recruited into the peritoneal cavity, and for cells derived from some of the mice, the donor mouse could even be determined (ie could inherently recognize which donor mice were aberrant). Even when the cell population is highly heterogenous, the methods we developed can still identify cell phenotypes and activation.

Finally, we saw the first results in the study of the nature and causes of neutrophil extracellular traps (NETS) in neutrophils using a combination of fluorescent-labeled imaging flow cytometry, and unlabeled Raman imaging. While NETs have been known for several years now, there remain many unknown factors in the generation of NETs and whether different NET types are formed in different conditions. To study this, we first concentrated on producing reliable protocols to determine NET generation rates when stimulated or inhibited by several distinct endocytotic pathways, and found that one limitation in the study of formation conditions is the lack of sufficient methods to robustly quantify NET formation, and this turns out to be essential for our future research. NETs can, however, be quantified by building metrics based on fluorescence intensities and area in imaging flow cytometry, or by careful analysis of Raman scattering intensities and signal areas in Raman NET imaging.

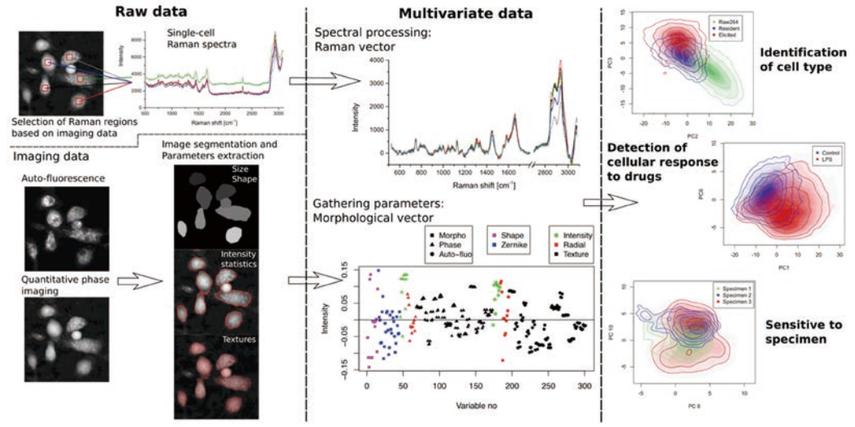


Figure. Label-free single cell analysis. Laser-based Raman analysis of single cells provides features that can be used to identify cell type, drug response, or even donor-specific characteristics. Using cell morphology derived from label-free imaging can provide additional sensitivity to single cell variations. Cell phenotypes and pathway-specific functional changes can be determined from the non-invasive non-labelling methods we developed.

Recent Publications

- Pavillon N and Smith NI. Immune cell type, cell activation, and single cell heterogeneity revealed by label-free optical methods. Sci. Rep. 9, 17054 (2019).
- Lelliott PM, Momota M, Shibahara T, Lee MSJ, Smith NI, Ishii KJ, Coban C. Heparin induces neutrophil elastase-dependent vital and lytic NET formation. Int. Immunol. 32(5), 359-368 (2020).
- 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).
- 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).

Chemical Imaging Techniques

Kazuya Kikuchi, PhD

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Assistant Professor	Masafumi Minoshima
Postdoctoral Fellow	1
Research Assistant	2
Support Staff	1

In Vivo Imaging with Fluorescent Probes Revealed the Dynamics and Function of Osteoclast Proton Pumps

In vivo two-photon fluorescence imaging is a powerful modality to monitor cell dynamics deep within the tissues and organs with high temporal and subcellular resolutions. Small-molecule-based fluorescent probes are promising tools as their fluorescence-sensing properties can be tuned for the optimization of their biomolecular functions and facile administration by injection. For longitudinal monitoring of the protein functions in real-time with high signal/background contrast, the fluorescent probes should have a quick response and be photostable at the targeted tissues *in vivo*. In this study, we aimed to develop a pH-activatable small-molecular probe for multicolor intravital imaging to reveal the functions of osteoclast proton pumps during osteoclastic bone resorption. Osteoclast proton pumps, which are vacuolar H^+-ATPases (V-type H^+-ATPases), are extremely involved in the secretion of several protons to dissolve bone minerals. So far, the mechanism by which mature osteoclasts regulate the localization of the proton pumps upon bone resorption is still unclear due to the lack of spatiotemporal information on acidic compartments.

We initially synthesized and evaluated pH-activatable fluorescence properties of a series of rhodamine spirolactams Rh-1–3 based on a rhodamine-B dye. The fluorescence spectra of Rh-1–3 at different pH regions revealed that all rhodamine derivatives showed a fluorescence increase at the lowered pH regions. The pK_a values of Rh-1–3 ranged from 4.9 to 5.9, which is suitable for imaging acidic areas created by mature osteoclasts. Next, the ring-opening reactions (fluorescence activation) of the spirolactam were monitored by assessing the absorption transition of the dyes upon an immediate pH jump from pH 8.0 to 4.0. Remarkably, Rh-2 quickly turned into fluorescent form when the solution acidified. Because of the suitable pK_a and the rapid fluorescence activation response, Rh-2 was selected as a dye structure for detecting the area of bone acidification *in vivo*. Then we synthesized a small-molecular probe Red-pHocas (red pH-activatable fluorescent probe for osteoclast activity sensing) by the conjugation of Rh-2 with two alendronates containing bone-targeting bisphosphonate groups (Figure, left).

Then, Red-pHocas was administrated subcutaneously to fluorescent reporter mice, where green fluorescent protein (GFP) is expressed under the promoter of the V-type H^+ATPase $a3$ subunit ($a3$-GFP mice) for 3 days then the intravital imaging of calvaria bone tissue was performed using a two-photon excitation microscopy under anesthesia. Fluorescence images were acquired after processing the spectral unmixing algorithms that separate distinct fluorescence signals derived from GFP, Red-pHocas, and second-harmonic generation (SHG) of collagen fibers in bones. We found that the fluorescence signals of Red-pHocas in the local area on the bone surface overlapped with osteoclast localizations, indicating it selectively detected the lowered pH regions upon bone resorption by activated osteoclasts (Figure, middle).

Moreover, we monitored the dynamics of osteoclast proton pumps with the acidic compartments simultaneously over a long time. We found that the osteoclast proton pumps were sparsely distributed at the membrane and intracellular compartments in the osteoclast where few acidic regions were detected. Concomitant with the accumulation of the osteoclast proton pumps on the bone surface, the acidic compartments emerged and spread over the bone surface covered by the osteoclast. Thereafter, the area of acidic compartments on the bone surface gradually decreased without the localization change of the osteoclast proton pumps, indicating that the osteoclast was in an inactive, non-resorbing state. The image analysis revealed that bone acidification was associated with proton pump accumulation to the bone surface.

We further applied our imaging system to the *in vivo* quantitative evaluation of drugs targeting osteoclast proton pumps. Afrer administration of Bafilomycin A1, a known proton pump inhibitor, we found the decrease of the red fluorescent signals in the whole area of view. These results demonstrated that our imaging approach can spatiotemporally analyze the drug efficacies in intact tissues and provide a useful tool for the development of drugs against bone diseases and inhibitors of trafficking pathways of proton pumps.

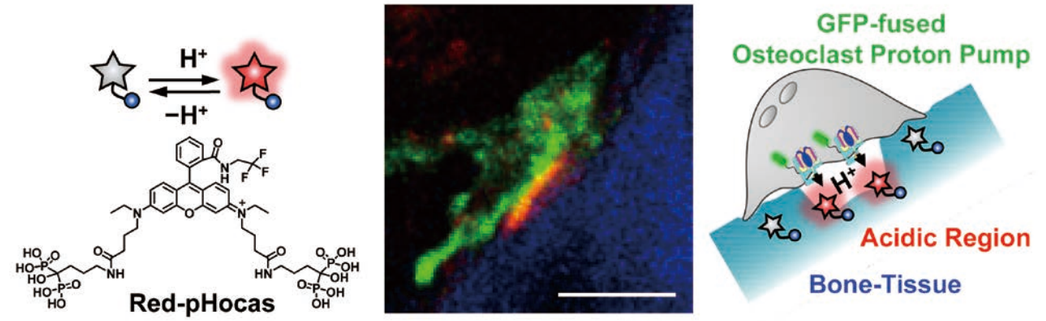

Figure. (left) Chemical structure of Red-pHocas. (middle) Two-photon fluorescence image of osteoclasts in bone tissues. Green, GFP fused proton pumps; red, Red-pHocas; blue, bone tissue. Scale bar: 20 μm. (right) Schematic illustrations of proton pump dynamics and acidic regions in osteoclasts.

Recent Publications

- Kumar N, Hori Y, Nishiura M & Kikuchi K. Rapid no-wash labeling of PYP-tag proteins with reactive fluorogenic ligands affords stable fluorescent protein conjugates for long-term cell imaging studies. Chemical Science 11, 3694-3701 (2020).
- Gao J, Hori Y, Takeuchi O & Kikuchi K. Live-cell imaging of protein degradation utilizing designed protein-tag mutant and fluorescent probe with turn-off switch. Bioconjugate Chem. 31, 577-583 (2020).
- Imoto T, Kawase A, Minoshima M, Yokoyama T, Bito H & Kikuchi K. Photolytic release of a caged inhibitor of an endogenous transcription factor enables optochemical control of CREB-mediated gene expression. Org. Lett., 22, 22-25 (2020).
- Gao J, Hori Y, Shimomura T, Bordy M, Hasserodt J & Kikuchi K. Development of fluorogenic probes for rapid high-contrast imaging of transient nuclear localization of sirtuin 3. ChemBioChem 21, 656-662 (2020).
- Minoshima M, Kikuta J, Omori Y, Seno S, Suehara R, Maeda H, Matsuda H, Ishii M & Kikuchi K. *In vivo* multicolor imaging with fluorescent probes revealed the dynamics and function of osteoclast proton pumps. ACS Cent. Sci. 5, 1059-1066 (2019).

Immune Response Dynamics

Kazuhiro Suzuki, MD/PhD

Professor	Kazuhiro Suzuki
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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 β_2-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 β_2-adrenergic receptor and chemokine receptors, we identified a protein complex consisting of copper metabolism MURR1 domain-containing (COMMD) 3 and COMMD8 (COMMD3/8 complex), the functions of which have been totally unclear (Figure A). This year, we demonstrated that the COMMD3/8 complex plays an important role in chemoattractant receptor signaling and humoral 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 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 trimeric 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 (Figure B). 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* (Figure C). Additionally, deficiency of COMMD3 or COMMD8 in B cells severely impaired humoral immune responses (Figure D and Figure E). 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.

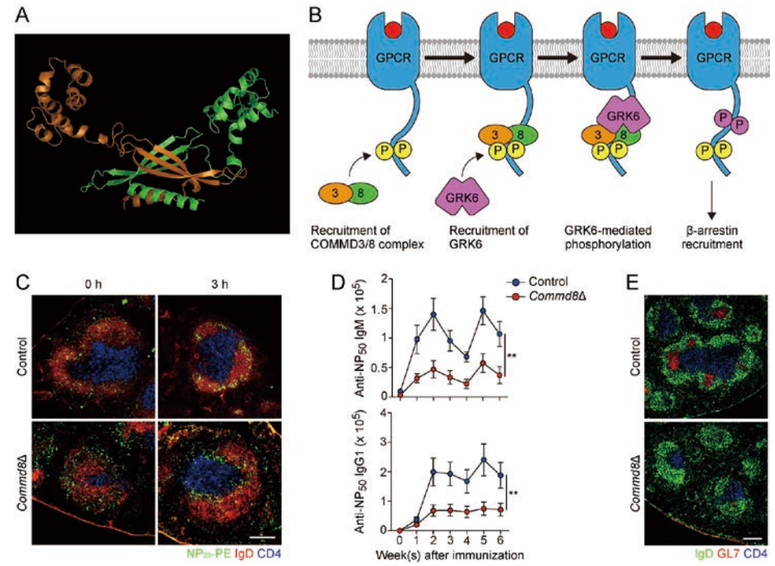

Figure. Role of the COMMD3/8 complex in GPCR signaling and immune responses.
(A) *In silico* modeling for the structure of the COMMD3/8 complex. COMMD3, orange; COMMD8, green. (B) Proposed role of the COMMD3/8 complex in GRK6 recruitment to GPCRs. (C) COMMD8-deficient (*Commd8Δ*) B cells show a defect in migration toward the outer follicle at 3 h after immunization. (D and E) B cell-specific deficiency of COMMD8 severely impairs the antibody response (D) and development of germinal centers (E). 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. 216, 1630-1647 (2019).
- Nakai A and Suzuki K. Adrenergic control of lymphocyte trafficking and adaptive immune responses. Neurochem. Int. 130, 104320 (2019).
- Suzuki K and Nakai A. Immune modulation by neuronal electric shock waves. J. Allergy Clin. Immunol. 141, 2022-2023 (2018).
- 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 β_2-adrenergic receptors. J. Exp. Med. 211, 2583-2598 (2014).

Systems Immunology

Daron M. Standley, PhD

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Research Assistant	4
Visiting Scientist	1
Support Staff	1

In 2019 our lab reached several important milestones in the analysis of adaptive immune receptors, especially in the context of single cell sequencing of B and T cells. We also had a generally productive year in collaborative projects (Metwally H. et al. Sci. Signal 2020; Lee Y. et al. Life Sci. Alliance 2020; Fukushima K. et al. Immunity 2020; Yamasoba D. et al. Nat. Microbiol. 2019; Takeda K. et al. J. Allergy Clin. Immunol. 2019). For general protein functional analysis, we developed a pipeline for predicting protein-nucleotide binding sites in 3D using MAFFT and DASH (Rozewicki J. et al. Nucleic Acids Res. 2019). As shown in Figure 1, the new pipeline dramatically reveals the conservation of nucleotide binding sites on homologs of Regnase-1 (Rozewicki. J. et al. Meth. Mol. Biol. in press).

With regard to repertoire analysis, the first major milestone was to release a state-of-the-art tool for lymphocyte receptor modeling (Repertoire Builder; Schritt, D. et al. Mol. Sys. Des. Eng. 2019). What is different about Repertoire Builder in comparison to other available tools is that it constructs 3D models of B cell receptors or T cell receptors in a high-throughput manner, without loss of accuracy. This makes it possible to model every BCR or TCR in a 10x Chromium VDJ experiment in under 30 minutes.

What the resulting structures then allow you to do, is measure similarities across clones and even across donors. InterClone (Xu Z. et al. Mol. Sys. Des. Eng. 2019) carries out such comparisons using machine learning algorithms that cluster receptors likely to target the same antigen and epitope. Preliminary tests on anti-HIV antibodies achieved a specificity (1- False Positive rate) of over 99% with a sensitivity (True Positive rate) of 62%. A flowchart of InterClone is shown in Figure 2.

The release of Repertoire Builder and InterClone were followed by tools that construct models of TCRs with peptide-MHC complexes and antibodies with their cognate antigens. ImmuneScape (Li S. et al. Meth. Mol. Biol. 2019) makes use of known TCR-peptide-MHC complex structures and models a given TCR-peptide-MHC sequence triplet. It is fast and accurate, and, to our knowledge the first tool of its kind.

Adapt (Davila et al. in prep) is a tool for building antibody-antigen complex models. Because antibodies can bind antigens on any exposed surface, we can not use structural templates as we did with ImmuneScape. Instead, we first predict the possible antibody-antigen interface residues (paratope and epitope, respectively) using machine learning algorithms and then dock the antibody antigen 3D models using the predicted paratopes and epitopes as a guide. Starting from realistic models of unbound antibodies and antigens with known structure, we carried out leave-one-out cross validation. We found that both the sampling and scoring of the Adapt docking pipeline were significantly improved over simply using docking alone. Furthermore, we obtained a 4% improvement in the average area under the receiver-operating characteristic curve for the final epitope prediction (0.80) as compared with the initial prediction (0.76), suggesting that the Adapt epitope predictions do indeed reflect antibody binding specificity.

A web server has been prepared, Adapt (https://sysimm.org/adapt/), that accepts antibody- antigen pairs in the form of either sequences or structures and returns predicted paratope and epitope residues along with high-scoring docked poses. A flowchart of Adapt is shown in Figure 3.

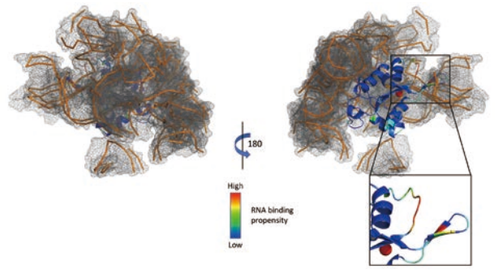

Figure 1. Distribution of nucleotides around homologs of Regnase-1 as revealed by MAFFT-DASH.

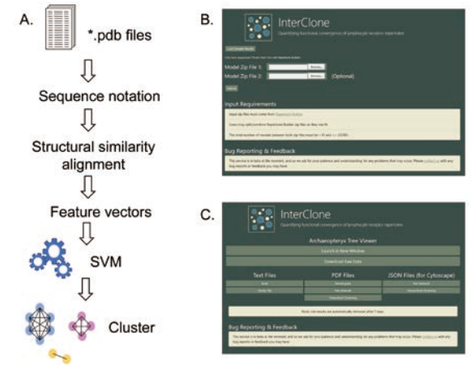

Figure 2. InterClone: Lymphocyte receptor functional clustering.

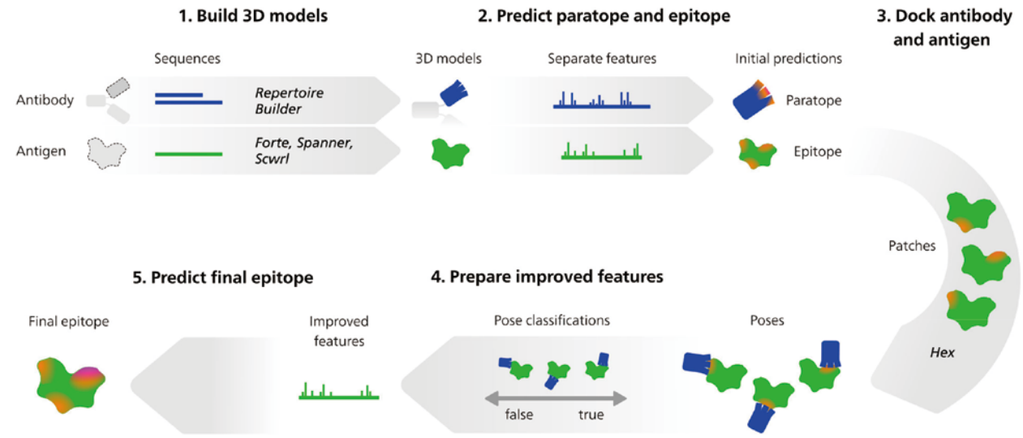

Figure 3. Adapt: a new way to predict antibody-antigen complex structures.

Recent Publications

- Xu Z., et al. Functional clustering of B cell receptors using sequence and structural features. Mol. Syst. Des. Eng. 4, 769-778 (2019).
- 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 e1115, doi: 10.1016/j.jaci.2018.07.006 (2019).
- Schritt D., et al. Repertoire Builder: high-throughput structural modeling of B and T cell receptors. Mol. Syst. Des. Eng. 4, 761-768 (2019).
- Rozewicki J, Li S, Amada KM, Standley DM & Katoh K. MAFFT-DASH: integrated protein sequence and structural alignment. Nucleic Acids Res. 47, W5-W10, doi: 10.1093/nar/gkz342 (2019).
- Li S., et al. Structural modeling of lymphocyte receptors and their antigens. Meth. Mol. Biol. 2048, 207-229, doi: 10.1007/978-1-4939-9728-2_17 (2019).

Statistical Immunology

Yukinori Okada, MD/PhD

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Research Assistant	5
Support Staff	1

Goal of our laboratory

Genetic backgrounds of individuals have substantial impacts on 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 from millions of subjects, and successfully identified comprehensive catalogues of genetic risk loci of immune-related diseases. However, little is known regarding how to develop 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.

Metagenome-wide association study of gut microbiome revealed disease-specific feature of rheumatoid arthritis

Microbiomes play substantial roles in homeostasis and biology of a variety of human diseases through interaction with the host. Metagenome-wide association studies (MWAS) utilizing whole-genome shotgun sequencing is a promising tool to elucidate microbiome etiology. We conducted an initial MWAS of rheumatoid arthritis in Japan. Phylogenetic case–control association tests showed high abundance of multiple species belonging to the genus Prevotella (e.g., *Prevotella salivae*) in the RA case metagenome (Figure 1). Gene functional assessments showed that the abundance of one redox reaction–related gene (R6FCZ7) was significantly decreased in the RA metagenome compared to controls. Our study also identified a population-specific link of the molecular pathways between the metagenome and host genome (Kishikawa T. et al. Ann. Rheum. Dis. 2020).

Characterizing natural selection pressure and evolution of the Japanese population

The history of human beings, namely natural selection pressure and evolution, is embedded in the genome data of current human populations. We conducted a genome-wide scan of natural selection pressure in the Japanese population by using large-scale genome-wide association study (GWAS) data of >170,000 Japanese individuals. We identified 29 loci with significant selection signatures. Phenome-wide enrichment analysis identified alcohol consumption and obesity as key traits for evolution, while bread consumption and hand grip strength were identified for Europeans (Yasumizu Y. et al. Mol Biol Evol 2020). Dimension reduction of the Japanese GWAS data enlightened the population structures, which were clustered into “mainland (Hondo)”, and “non-mainland (Ryukyu and Hokkaido-Ainu)” (Figure 2). This study demonstrated the value of the combinatory approach to applying both linear and non-linear machine-learning methods (Sakaue S. et al. Nat. Commun. 2020).

Trans-biobank polygenic risk score analysis identified causal biomarkers for lifespan

Identification of modifiable biomarkers related to lifespan is one of the ultimate goals of human genome studies. Polygenic risk score (PRS) is a genetic risk of an individual which aggregates genotype risk of genome-wide SNPs. We developed a novel statistical genetics method which utilizes PRS as an instrumental variable to infer causality on the target phenotype. Through trans-ethnic biobank collaboration collecting ~700,000 worldwide individuals, we identified high blood pressure and obesity as causal biomarkers responsible for lifespan (Sakaue S. et al. Nat. Med. 2020).

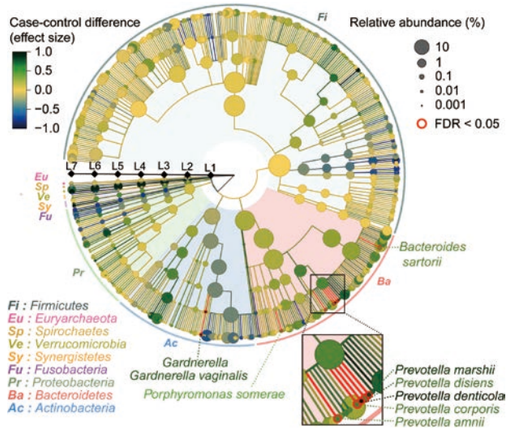


Figure 1. Metagenome-wide association study (MWAS) of gut microbiome of rheumatoid arthritis in the Japanese population.

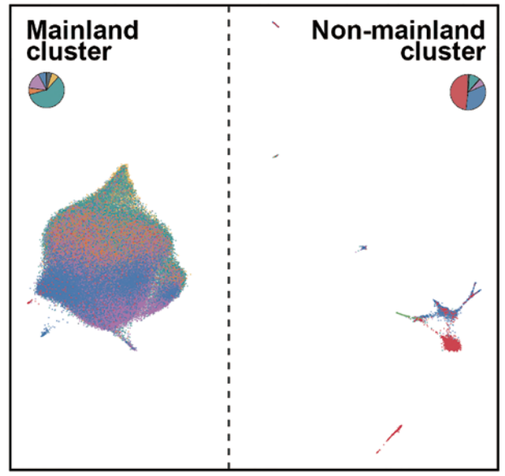


Figure 2. Machine learning-based deconvolution of the genome-wide association study (GWAS) data of the Japanese population.

Recent Publications

- Sakaue S., et al. Trans-biobank analysis with 676,000 individuals elucidates the association of polygenic risk scores of complex traits with human lifespan. Nat. Med. 26, 542-548 (2020).
- Sakaue S., et al. Dimensionality reduction reveals fine-scale structure in the Japanese population with consequences for polygenic risk prediction. Nat. Commun.11, 1569 (2020).
- Matoba N., et al. GWAS of 165,084 Japanese individuals identified nine loci associated with dietary habits. Nat. Hum. Behav. 4, 308-316 (2020).
- Yasumizu Y., et al. Genome-wide natural selection signatures are linked to genetic risk of modern phenotypes in the Japanese population. Mol. Biol. Evol. 37, 1306-1316 (2020).
- Kishikawa T., et al. A metagenome-wide association study of gut microbiome revealed novel etiology of rheumatoid arthritis in the Japanese population. Ann. Rheum. Dis. 79,103-111 (2020).

Quantitative Immunology

Associate Professor Diego Diez

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

Development of computational methods

Single cell genomics technologies have changed how we understand cell heterogeneity and identity but 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. UMAP or PCA plot). Our method, called *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 important issue is how to combine different datasets produced by different laboratories using different technologies. We are developing methods that leverage techniques from engineering control to estimate and correct batch effects. A key focus of our method is on preserving as much as possible the original cell population structure.

Mathematical modeling

The large number of datasets accumulating from single cell genomics experiments opens the door to approaches that study the immune system from a more theoretical perspective. 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 use linear regression to model immune transcriptional regulatory networks. 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. Using the expression level of the regulators as a proxy for their activities we apply these methods to study the differentiation of immune cells. We use information from trajectory and RNA velocity to infer the differentiation pathway along which to estimate transcriptional regulatory networks.

Applications to immunology

We are interested in studying how transcriptional regulatory networks control immune cell differentiation.

We collaborate with the Host Defense laboratory to study the role of the RNase 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 also study the role of Regnase 1 in NK cell development. Here we use 10x genomics whole transcriptomic and feature barcoding to quantify the expression level of a selected number of proteins from murine splenocytes.

We collaborate with the Experimental Immunology laboratory and the Systems Immunology laboratory to study T cell development in SKG mice. In this project we use data from 10x genomics single cell transcriptomics and TCR immune repertoire to investigate how TCR signaling impacts T cell development. We use computational methods to identify T cell signaling pathways driving the development of autoimmunity.

We collaborate with the Immune Regulation laboratory to study the role of CD4 T cells in human Eosinophilic Chronic Rhinosinusitis. Here we combine single cell RNA sequencing with TCR immune repertoire profiling to identify pathogenic T cells involved in chronic inflammation that may be responding to bacterial antigens.

Our group is using single cell genomics to study an increasing number of topics, including NKT cell differentiation in mice and IgA nephropathy in humans, using a combination of single cells transcriptomics, feature barcoding, and (TCR and BCR) immune profiling.

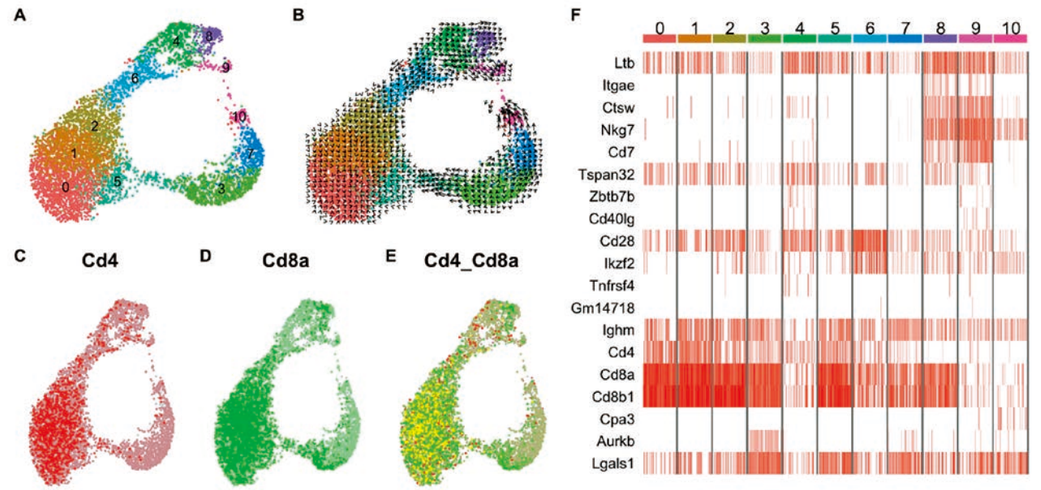


Figure. Single cell RNA-seq of thymocytes gives insight into T cell development: A) clustering, B) RNA velocity C-E) expression of differentiation markers, F) differential expression.

Recent Publications

- Vandenbon A, Diez D. singleCellHaystack: A clustering-independent method for finding differentially expressed genes in single-cell transcriptome data. bioRxiv 557967; doi: https://doi.org/10.1101/557967 (2019).
- Nakai W, Yoshida T, Diez D, Miyatake Y, Nishibu T, Imawaka N, Naruse K, Sadamura Y, Hanayama R. A novel affinity-based method for the isolation of highly purified extracellular vesicles. Sci. Rep. 6(1), 33935. doi: 10.1038/srep33935 (2016).
- Bahrini I, Song JH, Diez D. & Hanayama R. Neuronal exosomes facilitate synaptic pruning by up-regulating complement factors in microglia. Sci. Rep. 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 9th NIF Winter School on Advanced Immunology

Date January 20-24, 2020
Venue Awaji Yumebutai International Conference Center, Hyogo / Grand Cube Osaka – Osaka International Convention Center, Osaka (The 11th International Symposium of IFReC)

The program comprised a four-day lecture course on Awaji Island and a one-day international symposium at the Osaka International Convention Center. Fifty-four young researchers from around the world, selected from 261 applicants of 58 nationalities, learned cutting-edge immunology from fifteen world-renowned senior researchers at the course and from ten up-and-coming and senior researchers at the symposium. Three young researchers from companies collaborating with IFReC also attended and joined in the discussions. The Winter School was highly evaluated by both participants and lecturers as an excellent opportunity for networking with young peers through active discussions and communications as well as for its high level of science. Four participants were awarded the best presentation awards and had an opportunity to give a presentation at the International Symposium of IFReC. Dr. Chris Schiering, who was a participant at a previous winter school in 2014, was invited to the symposium as a speaker and as a role model for the young researchers. The NIF Winter School on Advanced Immunology has been organized jointly with the Singapore Immunology Network (SIgN) since FY2011. However, this was the last Winter School jointly organized with SIgN due to reform of SIgN, while IFReC will continue to hold the Winter School.

Lecturer	Title
Nobuyuki Takakura (IFReC, Osaka University, Japan)	Endothelial stem cell population in health and diseases
Eran Elinav (The Weizmann Institute of Science, Israel)	Host micro biome interactions in health and disease
Kiyoshi Takeda (IFReC, Osaka University, Japan)	Regulation of intestinal homeostasis
Olaf Rötzschke (SIgN, Singapore)	Dissection of allergic reactions in an allergen-saturated environment
Paul Kubes (University of Calgary, Canada)	Imaging the innate immune system in sterile injury and infections
Florent Ginhoux (SIgN, Singapore)	Macrophage and dendritic cell biology: From development to functions
Tatiana Kisseleva (University of California San Diego, USA)	IL-17 signaling in steatotic hepatocytes and macrophages promotes alcoholic liver disease-induced hepatocellular carcinoma
Sidonia Fagarasan (Riken IMS, Japan)	Impact of PD-1 deficiency on microbiome and brain
Kazuyo Moro (IFReC, Osaka University, Japan)	The role of group 2 innate lymphoid cells in idiopathic interstitial pneumonias
Joachim Schultze (University of Bonn, Germany)	Plasticity within the myeloid cell system
Lisa Ng (SIgN, Singapore)	Immune mechanisms of arbovirus pathogenesis: Strategies for immunotherapies
Laurent Rénia (SIgN, Singapore)	Rosetting of *Plasmodium*-infected red blood cells: severity factor or escape mechanisms?
Oliver Bannard (University of Oxford, UK)	Selection events in germinal centers
Akihiko Yoshimura (Keio University, Japan)	Neural damage and repair after ischemic brain injury by innate and adaptive immunity
Shimon Sakaguchi (IFReC, Osaka University, Japan)	Treg-Up or Treg-Down to control immune responses

Immunology at the Forefront - The 11th International Symposium of IFReC

Date January 24, 2020
Venue Osaka International Convention Center (Grand Cube Osaka), Osaka, Japan

This symposium provided an excellent opportunity to share the hottest academic research achievements in the world with researchers in Japan and to contribute to advancing immunology research at IFReC. Talented young scientists, who had recently published remarkable research results, in addition to several well-established senior researchers were invited to the symposium as speakers. The symposium attracted 187 participants of which 104 were from overseas. The participants of the 9th Winter School on Advanced Immunology also attended the symposium and joined in the active discussion. The successful interactions among researchers at the symposium laid the groundwork for future international collaborations.

Speaker	Title
Chair: James Badger Wing (IFReC, Osaka University, Japan)	
Art Weiss (University of California San Francisco, USA)	New Insights into TCR Ligand Discrimination
Motoko Y. Kimura (Chiba University, Japan)	CD69 Biology and Pathology
François Legoux (Institut Curie, France)	Microbial Metabolites Control the Thymic Development of Mucosal Associated Invariant T cells
Chair: Masahiro Yamamoto (IFReC, Osaka University, Japan)	
Thirumala-Devi Kanneganti (St. Jude Children's Research Hospital, USA)	Targeting the Inflammasome for the Treatment of Inflammatory and Infectious Diseases
Cevayir Coban (The University of Tokyo / IFReC, Osaka University, Japan)	Host-*Plasmodium* Interactions and the Sterile Immunity Against Malaria: Where Are We?
Olivia Majer (Free University of Berlin, Germany)	Cellular Mechanisms of Self Versus Non-Self Discrimination by Nucleic Acid-Sensing TLRs
Chair: Katsumori Segawa (IFReC, Osaka University, Japan)	
Marc Beyer (German Center for Neurodegenerative Diseases (DZNE), Germany)	Role of Special-AT-Rich Binding Protein 1 for the Differentiation and Function of $CD4^+$ T-Cells
Justin Taylor (University of Washington, USA)	B Cells Engineered to Express Pathogen-Specific Antibodies Protect Against Infection
Chris Schiering (Imperial College London, UK)	Aryl Hydrocarbon Receptor in Immunity and Metabolism
Ken Murphy (Washington University in St. Louis, USA)	High IRF8 Levels Engage AICE-dependent Enhancers for cDC1 Identity

The 1st UCL-OU Joint Symposium on Immunology

Date June 27-28, 2019
Venue Taniguchi Memorial Hall, Suita Campus, Osaka University

The UCL-OU Joint Symposium was organized and held jointly with University College London (UCL), which is one of the Global Knowledge Partners of Osaka University. This partnership is expected to promote a collaboration in the field of immunology. Such collaboration with UCL will lead to advancing research at IFReC into acquired immunology. Presentations by 10 UCL researchers and 11 IFReC researchers were made in two days for an audience of 158 participants. After the symposium, UCL researchers visited IFReC laboratories to discuss further collaboration. Director Kiyoshi Takeda of IFReC and Dr. Hans Stauss of UCL agreed that the next joint symposium would be held in London in 2020. After the joint symposium, Osaka University and UCL concluded an Academic Exchange Agreement in October 2019.

Day1

Speaker	Title
Chair: Masako Kohyama (IFReC, Osaka University, Japan)	
Tomohiro Kurosaki (IFReC, Osaka University, Japan)	Function of Tet Proteins in B cell tolerance
Claudia Mauri (University College London, UK)	Is rheumatoid arthritis a disease of the gut?
Hisashi Arase (IFReC, Osaka University, Japan)	MHC class II-induced misfolded proteins are targets for autoimmune diseases
Kazuhiro Suzuki (IFReC, Osaka University, Japan)	GPCR-mediated control of humoral immune responses
Chair: Wataru Ise (IFReC, Osaka University, Japan)	
Lucy Walker (University College London, UK)	Identifying targets of CTLA-4 regulation *in vivo*
David Sansom (University College London, UK)	Understanding CTLA-4 interactions with CD80 and CD86
Takashi Nagasawa (IFReC, Osaka University, Japan)	Bone marrow microenvironmental niches for hematopoietic stem cells and immune cells
Benedict Seddon (University College London, UK)	Novel functions for inflammatory signalling in T cell development
Chair: Takeshi Inoue (IFReC, Osaka University, Japan)	
Masaru Ishii (IFReC, Osaka University, Japan)	Dynamics of immune cell migration and interaction visualized by intravital microscopy
Siobhan Burns (University College London, UK)	Human Immunity: Lessons from Primary Immunodeficiency
Shigekazu Nagata (IFReC, Osaka University, Japan)	Phosphatidylserine-dependent engulfment of apoptotic cells
Sho Yamasaki (IFReC, Osaka University, Japan)	Sensing environmental lipids and metabolites via immune receptors

Day2

Speaker	Title
Chair: Katsumori Segawa (IFReC, Osaka University, Japan)	
Shizuo Akira (IFReC, Osaka University, Japan)	The role of endoribonuclease Regnase-1 in inflammation and immune response
Masahiro Yamamoto (IFReC, Osaka University, Japan)	Cell-intrinsic host defense against intracellular pathogens and its inflammation
Mahdad Noursadeghi (University College London, UK)	Systems level analysis of immunopathology in tuberculosis
Chair: Eiji Umemoto (IFReC, Osaka University, Japan)	
Kiyoshi Takeda (IFReC, Osaka University, Japan)	Regulation of intestinal immune responses by microbiota-derived metabolites
Lizzy Rosser (University College London, UK)	Regulation of arthritic B cell responses by gut microbes and metabolites
Mala Maini (University College London, UK)	Living in the Liver: constraints on adaptive immunity
Chair: James Wing (IFReC, Osaka University, Japan)	
Ronjon Chakraverty (University College London, UK)	Autoimmunity in graft-versus-host disease
Shimon Sakaguchi (IFReC, Osaka University, Japan)	Conversion of conventional T cells into Treg cells
Hans Stauss (University College London, UK)	Genetic engineering of T cell specificity and function

IFReC Seminars

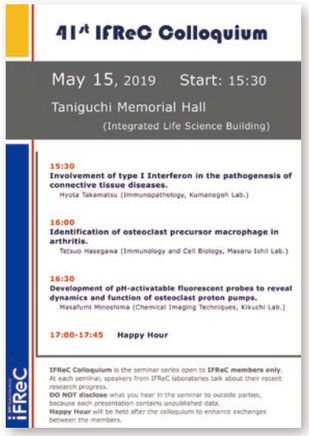
IFReC holds seminars throughout the year with speakers from a variety of disciplines including immunology, imaging, and informatics with the aim of promoting collaborative research, as well as to inspire and educate the next generation of scientists. Since its establishment, IFReC has held more than a hundred seminars, which have served as a forum for effective interaction between researchers beyond academic disciplines. In FY2019, we held a number of seminars by guest speakers including world-class scientists such as Gabriel Núñez and Frederic Geissmann.

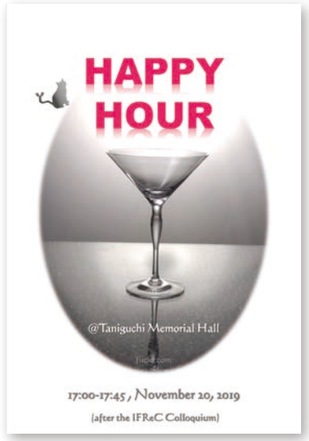
Date	Talk Title	Speaker
August 19. 2019	Regulation of body cavity immunity	Yasutaka Okabe (Institute for Frontier Life and Medical Sciences, Kyoto University, Japan)
September 9, 2019	Pyroptosis: from innate immunity to cancer	Feng Shao (Deputy Director for Academic Affairs, National Institute of Biological Sciences, Beijing, China)
November 26, 2019	Host-microbiota interactions in health and disease	Gabriel Núñez (Paul de Kruif Endowed Professor, Department of Pathology, University of Michigan, USA)
November 29, 2019	Peripheral immune control of latent virus infection	Norifumi Iijima (National Institutes of Biomedical Innovation, Health and Nutrition, Japan)
December 16, 2019	The macrophage biology	Frederic Geissmann (William E. Snee Chair of Immunology, Memorial Sloan Kettering Cancer Center, USA)
January 31, 2020	Introduction for RNA-Seq data analysis	Hidemasa Bono (Database Center for Life Science, Japan)
February 6, 2020	Phosphoinositides as key regulators of membrane organization	Fubito Nakatsu (Graduate School of Medical and Dental Sciences, Niigata University, Japan)

IFReC Colloquia

"IFReC Colloquia" is a series of discussion meetings for IFReC members held once every other month. At each colloquium, speakers from three 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 exchanges among IFReC members in an informal setting. These events serve as a platform to promote interdisciplinary research and deeper understanding of the many research activities conducted in IFReC. In FY2019, IFReC Colloquia were held in odd-numbered months except March 2020 due to the impact of COVID-19.










Date		Title	Speakers
41st	May 15, 2019	Involvement of type I Interferon in the pathogenesis of connective tissue diseases.	Hyota Takamatsu (Immunopathology, Kumanogoh Lab)
		Identification of osteoclast precursor macrophage in arthritis.	Tetsuo Hasegawa (Immunology and Cell Biology, Masaru Ishii Lab)
		Development of pH-activatable fluorescent probes to reveal dynamics and function of osteoclast proton pumps.	Masafumi Minoshima (Chemical Imaging Techniques, Kikuchi Lab)
42nd	July 24, 2019	Germinal-center mediated memory B cell development is driven by cooperation of metabolic fitness and survival.	Takeshi Inoue (Lymphocyte Differentiation, Kurosaki Lab)
		Misfolded protein / MHC class II complex in autoimmune diseases.	Tadahiro Suenaga (Immunochemistry, Arase Lab)
		Paroxysmal nocturnal hemoglobinuria and Inherited GPI deficiency caused by the mutations in PIGB gene.	Yoshiko Murakami (Immunoglycobiology, Kinoshita Lab)
43rd	September 18, 2019	B cell-specific MyD88 is involved in the adjuvanticity of a TLR-independent particulate adjuvant.	Michelle Sue Jann Lee (Malaria Immunology, Coban Lab)
		Negative regulation of T cell activation through phosphatases.	Takashi Saito (Cell Signaling, Saito Lab)
		IL-17-induced phosphorylation of Regnase-1 is critical in controlling target mRNA stability and inflammatory response.	Hiroki Tanaka (Host Defense, Akira Lab)
44th	November 20, 2019	Single cell RNA-seq analysis of regulatory T cells for drug discovery.	Naganari Ohkura (Experimental Immunology, Sakaguchi Lab)
		B cells in allergic and autoimmune diseases.	Manabu Fujimoto (Cutaneous Immunology, Fujimoto Lab)
		TRPM5 negatively modulates innate-like response of LPS-stimulated B lymphocytes.	Taiki Sakaguchi (Mucosal Immunology, Takeda Lab)
45th	January 29, 2020	STAT1 displays functionally distinct phosphorylation upon LPS stimulation.	Hozaifa Saad Hassan Metwally (Immune Regulation, Kishimoto Lab)
		ILC2 a therapeutic target for type 2 immune diseases.	Kazuyo Moro (Innate Immune Systems, Moro Lab)
		Initial phospholipid-dependent Irgb6 targeting to Toxoplasma gondii vacuoles mediates host defense.	Youngae Lee (Immunoparasitology, Yamamoto Lab)



Science Café on the Edge

"Science Café on the Edge," organized by IFReC in a relaxing café-like atmosphere, provides the general public with an opportunity to participate in informal talks with IFReC researchers about science, from basic knowledge to advanced research achievements. We hope to promote communications between scientists and the general public as well as promote a greater understanding of science technology through the direct conversations with IFReC researchers. In FY2019, IFReC held the event four times.

Forefront of "Car-T cell therapy," a novel type of cancer immunotherapy

Guest speaker Dr. Naoki HOSEN (Department of Cancer Stem Cell Biology, Graduate School of Medicine, Osaka University)
Date 2 May, 2019
Venue 1st floor, Techno-Alliance Building, Suita Campus, Osaka University


The exquisite harmony within you – cross-talk between intestinal bacteria and immune cells

Guest speaker Dr. Eiji UMEMOTO (Immune Regulation, Graduate School of Medicine, Osaka University)
Date 16 October, 2019
Venue Art Area B1 (Naniwabashi station, Keihan Line)


Resistant bacteria spreading around the world

Guest speaker Dr. Yukihiro AKEDA (Section of Bacterial Drug Resistance Research, Research Collaboration Center on Emerging and Re-emerging Infections, Osaka University)
Date 6 November, 2019
Venue Art Area B1 (Naniwabashi station, Keihan Line)

What is nuclear medicine? – from image diagnosis to cancer therapy –

Guest speaker Dr. Tadashi WATABE (Department of Nuclear Medicine and Tracer Kinetics, Graduate School of Medicine, Osaka University)
Date 8 December, 2019
Venue A lecture room, 1st floor, Lecture Building, School of Medicine, Suita Campus, Osaka University

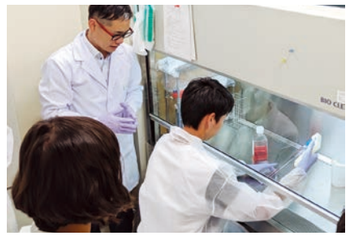
Visits

Senior high school at Komaba, University of Tsukuba "Research on Kansai Area"

Date 22 May, 2019
Visited Lab Oncogene Research, IFReC (Prof. Masato OKADA)
Stem Cell Biology and Developmental Immunology, IFReC (Prof. Takashi NAGASAWA)


Nara SSH (Super Science High School) science tour

Date 1 August, 2019
Lecturer Dr. Masako KOHYAMA (Immunochemistry, IFReC)
Laboratory training Host Defense, Experimental Immunology, Live Immuno-Imaging Facility




School visit

Date 18 November, 2019
Visited school Hiroshima Municipal Motomachi Senior High School
Lecturer Prof. Kiyoshi TAKEDA (Mucosal Immunology, IFReC)


Winter School 2019 for high school faculties

Date 26 December, 2019
Lecturer Prof. Shizuo AKIRA (Host Defense, IFReC)
Prof. Hisashi ARASE (Immunochemistry, IFReC)
Prof. Sho YAMASAKI (Molecular Immunology, IFReC)

Collaboration with KNOWLEDGE CAPITAL

Knowledge Capital CHO-Gakko (Super-School) Series

This is a program organized by Knowledge Capital under the theme of “Beyond schools and even beyond oneself” for learning about inspiration in research and its process and also for thinking and talking together with participants. It allows persons without expert knowledge to attend and provides an opportunity to connect the general public with researchers belonging to universities, companies and research institutions. In FY2019, a total of seven Knowledge Capital Super-School events were held and jointly organized by the Public Relations Office (Graduate School of Medicine at Osaka University) and the Institute for Integrated Cell-Material Sciences (iCeMS at Kyoto University).

「Osaka University × Knowledge Capital」Cutting edge medical research at Osaka University

School 1: Early detection of pancreatic cancer

Guest speaker Dr. Masamitsu KONNO (Frontier Science for Cancer and Chemotherapy, Graduate School of Medicine, Osaka University)
Date 11 April, 2019
Venue CAFE Lab., 1st floor, North building, Grand Front Osaka

School 2: Manufacturing antibodies

Guest speaker Dr. Takeshi INOUE (Lymphocyte Differentiation, IFReC, Osaka university)
Date 15 May, 2019
Venue CAFE Lab., 1st floor, North building, Grand Front Osaka

School 3: Sports medicine for the Tokyo 2020 Olympic and Paralympic Games

Guest speaker Dr. Ken NAKATA (Medicine for Sports and Performing Arts, Graduate School of Medicine, Osaka University)
Date 19 June, 2019
Venue CAFE Lab., 1st floor, North building, Grand Front Osaka

School 4: Toxoplasmosis and immunity

Guest speaker Dr. Miwa SASAI (Immunoparasitology, IFReC, Osaka university)
Date 17 July, 2019
Venue CAFE Lab., 1st floor, North building, Grand Front Osaka

「WPI × Knowledge Capital」KANSAI WPI series

School 1: The progression of an illness caused by functional and structural abnormality of capillaries

Guest speaker Dr. Nobuyuki TAKAKURA (Signal Transduction, IFReC, Osaka university)
Date 25 January, 2020
Venue CAFE Lab., 1st floor, North building, Grand Front Osaka

School 2: Crazing: printing without using ink!

Guest speaker Dr. Masateru ITO (Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University)
Date 1 February, 2020
Venue CAFE Lab., 1st floor, North building, Grand Front Osaka

School 3: Revealing immune and inflammatory dynamics with the latest intravital imaging technology

Guest speaker Dr. Masaru ISHII (Immunology and Cell Biology, IFReC, Osaka university)
Date 8 February, 2020
Venue CAFE Lab., 1st floor, North Building, Grand Front Osaka

Workshop Fes.

As a third type of venue intended for education in addition to schools and homes, Knowledge Capital regularly organizes workshops in collaboration with universities and private companies.

We hope to offer a foundation upon which children can develop their creativity based on their own experiences gained through their participation in workshops of various themes.

In FY2019, IFReC participated in Workshop Fes. twice, once in summer and once in autumn.

IFReC Immunology Lab – Learning through games about the mechanism protecting our bodies

Date 3-4 August, 2019
Venue CAFE Lab., 1st floor, North building, Grand Front Osaka
Date 23-24 November, 2019
Venue Event space, 4th floor, North Building, Grand Front Osaka

Osaka University Co-Creation Day

Date November 11, 2019
Venue LaLaport EXPOCITY

Osaka University held Osaka University Co-Creation Day@EXPOCITY on Saturday, 30 November, 2019 to expand and deepen interactions among private companies, local governments, and the local community.

About 5,000 visitors such as families with kids, married couples, and groups attended and the event ended successfully.

Under the theme of “IFReC Immunology Lab,” visitors to IFReC’s booth participated in a fill-in-the-blank quiz regarding cancer therapy and diagnostic methods shown on the displayed posters and a game using models of blood cells, and viewed slides of normal and colorectal cancer tissues under a microscope.

“Kagaku Zanmai” in Aichi

Date December 27, 2019
Venue NINS Okazaki Conference Center, Aichi
Host Okazaki High-school, Aichi prefecture

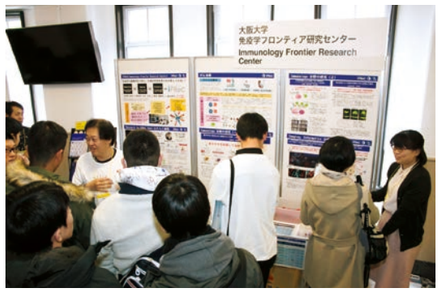
“Kagaku Zanmai (addiction to science)” is an advanced initiative called “Aichi model”. In this program, the high schools in Aichi prefecture that are actively engaged in science education, collaborate with universities and research institutes to conduct research and presentation. At the annual meeting of Kagaku Zanmai 2019, WPI institutes organized the presentation booth for demonstrating the top level researches in Japan.

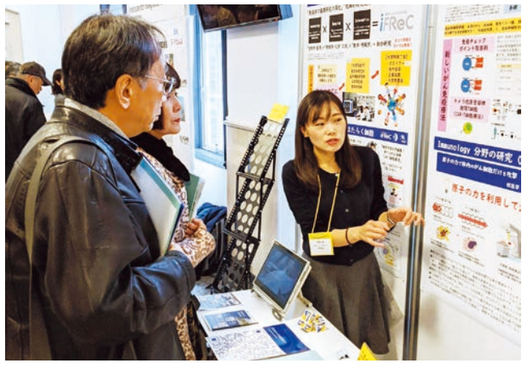
Super Science High School Student Fair

The Super Science High School (SSH) Student Fair 2019 was held on August 7-8 at Kobe International Exhibition Hall. IFReC together with other WPI institutes operated collaborative booths at the event. This event is an important opportunity for presenting WPI information to SSH students from around the country.

WPI Science Symposium

The 8th WPI Science Symposium was held at the Yasuda Auditorium on the Hongo Campus of The University of Tokyo on January 12, 2020. The theme was “The Power of Mathematics: Bridging Worlds Together.” IFReC and other WPI institutes used this event to publicize their research achievements at each booth.





Nature Jobs Career Expo in London & NY

The Nature journal (© 2020 Springer Nature) has been hosting international career fairs to connect talented researchers to job opportunities and provide them with career information and advice. IFReC had a booth exhibition at the Nature Careers Live in London (October 3) and in New York (October 19-20). At both booths, IFReC introduced their research activities and recruited outstanding young researchers for the Advanced Postdoc Program with the aim of advancing research and improving the level of research in Japan by promoting the global circulation of talented researchers.

AAAS 2020 Annual Meeting

Osaka University including IFReC participated in the annual meeting of the American Association for the Advancement of Science (AAAS 2020) on February 13-16, 2020 in Seattle, USA. The AAAS is the largest international non-profit organization in the world for the advancement of science. The annual meeting of the AAAS is a widely recognized scientific event, with various networking opportunities for researchers, journalists, government officials, and citizens. The main theme for AAAS 2020 was "ENVISIONING TOMORROW'S EARTH." In his plenary address, Bill Gates (Bill & Melinda Gates Foundation/Microsoft founder) talked about the obligations of the wealthy, including himself, to the global environment.

At AAAS 2020, the staff from Osaka University including IFReC organized an exhibition booth introducing the university's research projects, research environments, recruitment information (e.g., advanced postdoc at IFReC), etc. IFReC also exhibited a booth at "Family Science Days." The event featured a number of hands-on activities and demonstrations for families and kids. IFReC's booth focused on observing cancer tissues and classifying immune cells, and was enthusiastically received by visitors all day.

Information

Japanese Language Class

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

IFReC has continued holding the classes since 2012, and to date, the cumulative total is over 300 participants.

We offer two lecture-style classes, one is for an elementary to a pre-intermediate level, and another is for an intermediate to an advanced level. Class members learn verb and adjective conjugations, basic Japanese grammar, vocabulary of each level, Kanji, etc., through provided class materials and group work.

Voice from IFReC Japanese class participant

Thanks to IFReC for challenging me to learn the Japanese language. The class taught me so much about Japanese culture as well as language and helped me to grow a lot. Teachers teach us from the heart, and staffs are very kind and willing to help us adjust to life in Japan. I was happy with the class and had very nice experiences. Thanks again.

— Youngae, who had participated in the class for a decade.

Major Awards

Shimon SAKAGUCHI

- The Order of Cultural Merit of Japan
- The Paul Elrich and Ludwig Darmstaedter Prize, Germany
- The German Immunoogy Prize
- Honorary Doctorate from University of Birmingham

Each prize was given for his outstanding achievements on the discovery of the Regulatory T cell and elucidating its roles in autoimmune diseases, allergies, and cancers.

(Photo: Keio University)

Tadamitsu KISHIMOTO

- The 2019 Keio Medical Science Prize

The reason for selection and the major achievement is “IL-6: from molecule to medicine”.

Shigekazu NAGATA

- The 2019 ECDO Honorary Award

The research on “Phosphatidylserine-dependent efferocytosis and entosis” is highly appreciated.

Kiyoshi TAKEDA

- The Mochida Memorial Academic Award

“Discovering the mechanism of maintenance of intestinal homeostasis”

MOCHIDA

Ken J. ISHII

- JSI Award

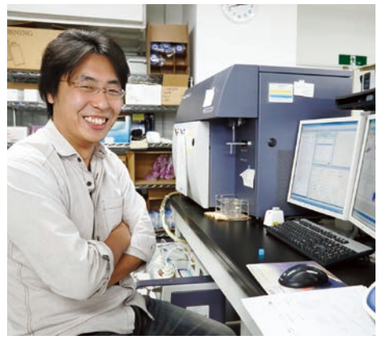
“Mechanism of vaccine adjuvant and its clinical application”

JSI

Takeshi INOUE

- JSI Young Investigator Award

“Elucidation of molecules that control B cell differentiation and activation”



Katsumori SEGAWA

- Osaka University Prize

Kazutaka KATOH & Daron M. STANDLEY

- The 8th in “The Most Cited Articles of the Heisei Period” ranking in all the research fields

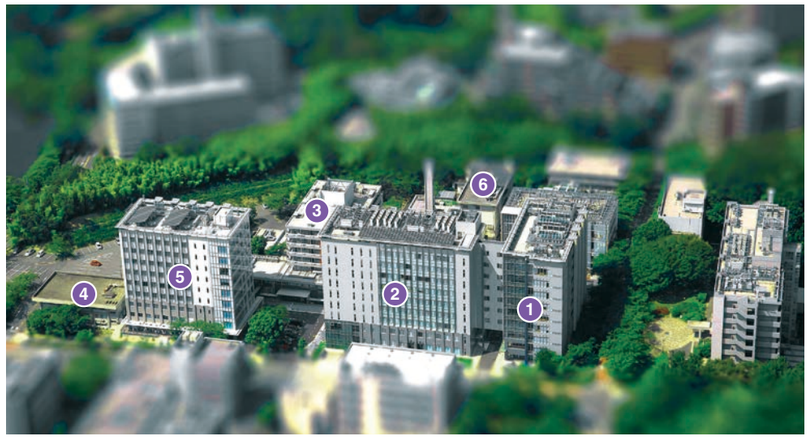
“MAFFT multiple sequence alignment software version 7: improvements in performance and usability” Mol.Biol.Evol.(2013).

Clarivate Analytics

Common Facilities (IFReC, RIMD, Animal Resource Center)

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.



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

Network Administration Office

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

Live immuno-imaging facility

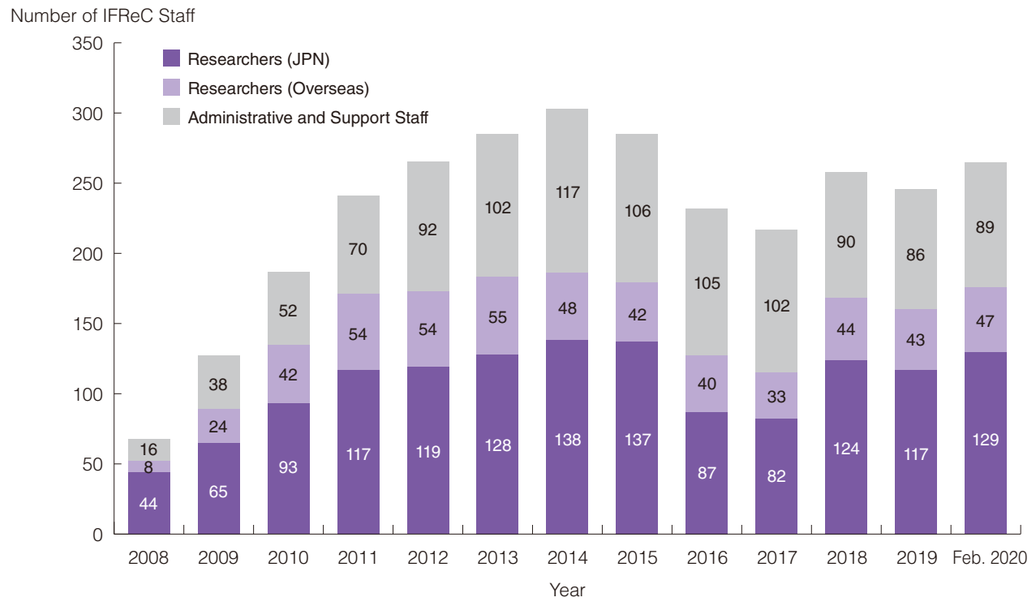
- SPF animal experiment facility with 11.7T MRI, in vivo imager & two-photon microscope.

Core Instrumentation Facility

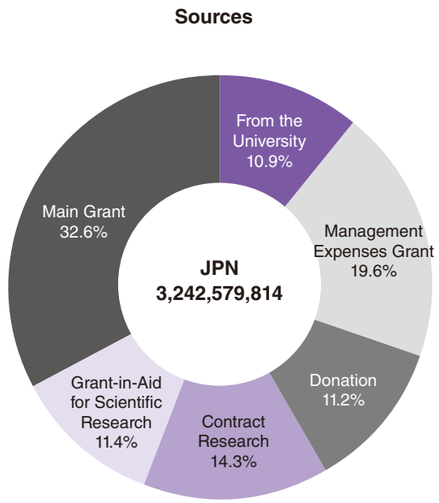
- Basic and advanced instruments
- In-house service
- DNA sequencing, cell sorting, electron microscopy, mass spectrometry and next-generation sequencing analysis
- Radio isotope facility

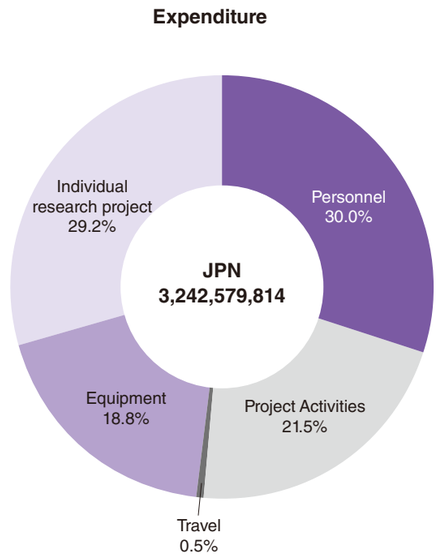
Composition & Finance

Composition



Finance





Advanced Postdoc Program

Around the world, leading research institutions are in fierce competition to discover excellent young researchers. IFReC has, therefore, established the Advanced Postdoc system, which offers outstanding young researchers opportunities to work with field-leading researchers in IFReC as well as to conduct their own research under their own merit. Under this system, IFReC has employed seven excellent postdoctoral fellows in 2018 and 2019. They were assigned to laboratories in IFReC with an internationally competitive salary and research funds (three million JPY per year) to conduct original research.

IFReC Advanced Postdoctoral fellows

Grant for Next Generation Principal Investigators

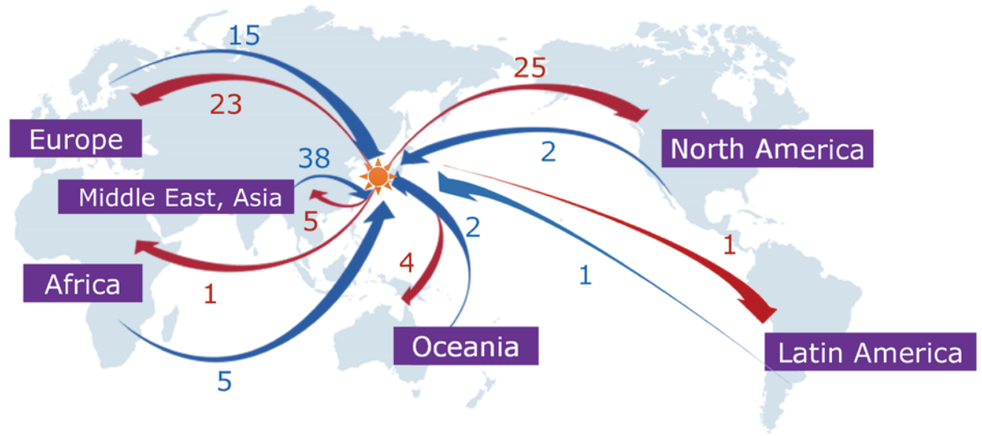
This program aims to foster the next generation of principal investigators at IFReC. In particular, challenging research that will contribute to the development of immunology or basic research that has the potential to create a new field of study in immunology is selected. IFReC has selected three PIs for FY2018-2020 and two PIs for FY2019-2021, and provides continuous research funding for a period of three fiscal years.

The grant is expected to generate excellent research achievements, raise the international recognition of the next generation of PIs, and contribute to the acquisition of external research funding required to operate the laboratories.

Unique Support Programs for Young Researchers

To strengthen our international research network and our basis for international collaborative research, IFReC has established two kinds of financial support programs for researchers. The first one is the “IFReC Kishimoto Foundation Fellowship,” which has been used to invite international researchers to Osaka. The second one is the “Program for International Circulation of Young Talented Researchers” for those who wish to participate in overseas research activities. These programs aim to develop the practical skills and abilities of young researchers in international collaborative research and to develop their network with researchers overseas. Since 2009, over 120 researchers have received the grants.

Number of the grant recipients (FY2009-2019)



Overseas ⇨ Osaka 63*

IFReC Kishimoto Foundation Fellowship

*Based on nationality

Osaka ⇨ Overseas 59

Program for International Circulation of Young Talented Researchers

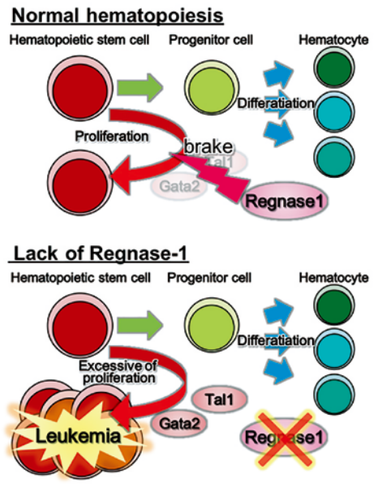
Selected Articles

Regnase-1-mediated post-transcriptional regulation is essential for hematopoietic stem and progenitor cell homeostasis.

Hiroyasu Kidoya, Fumitaka Muramatsu, Teppei Shimamura, Weizhen Jia, Takashi Satoh, Yumiko Hayashi, Hisamichi Naito, Yuya Kunisaki, Fumio Arai, Masahide Seki, Yutaka Suzuki, Tsuyoshi Osawa, Shizuo Akira, and Nobuyuki Takakura.

***Nature Communications* 10: 1072 (2019)**

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. HSPC fate 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. Nobuyuki Takakura (Signal Transduction, IFReC/RIMD) and his research group show that Regnase-1 regulates self-renewal of HSPCs through modulating the stability of Gata2 and Tal1 mRNA. In addition, they found that dysfunction of Regnase-1 leads to the rapid onset of abnormal hematopoiesis. Thus, the data reveal that Regnase-1-mediated post-transcriptional regulation is required for HSPC maintenance and suggest that it represents a leukemia tumor suppressor.

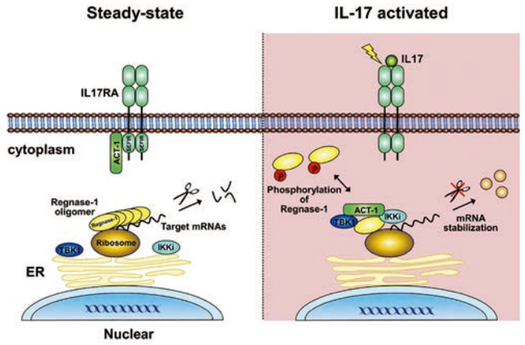


Phosphorylation-dependent Regnase-1 release from endoplasmic reticulum is critical in IL-17 response.

Hiroki Tanaka, Yasunobu Arima, Daisuke Kamimura, Yuki Tanaka, Noriyuki Takahashi, Takuya Uehata, Kazuhiko Maeda, Takashi Satoh, Masaaki Murakami, and Shizuo Akira.

***J Exp Med* 216: 1431-1449 (2019)**

Regnase-1 (also known as Zc3h12a or MCPIP-1) is an endoribonuclease involved in mRNA degradation of inflammation-associated genes. Regnase-1 is inactivated in response to external stimuli through post-translational modifications including phosphorylation, yet the precise role of phosphorylation remains unknown. Hiroki Tanaka, Shizuo Akira (Host Defense, IFReC) and their research group demonstrated that interleukin (IL)-17 induces phosphorylation of Regnase-1 in an Act1-TBK1/IKKi–dependent manner, especially in nonhematopoietic cells. Phosphorylated Regnase-1 is released from the endoplasmic reticulum (ER) into the cytosol, thereby losing its mRNA degradation function, which leads to expression of IL-17 target genes. By using CRISPR/Cas-9 technology, they generated Regnase-1 mutant mice, in which IL-17–induced Regnase-1 phosphorylation is completely blocked. Mutant mice (Regnase-1$^{AA/AA}$ and Regnase-1$^{\Delta CTD/\Delta CTD}$) were resistant to the IL-17–mediated inflammation caused by T helper 17 (Th17) cells *in vivo*. Thus, Regnase-1 plays a critical role in the development of IL-17–mediated inflammatory diseases via the Act1-TBK1-IKKi axis, and blockade of Regnase-1 phosphorylation sites may be promising for treatment of Th17-associated diseases.

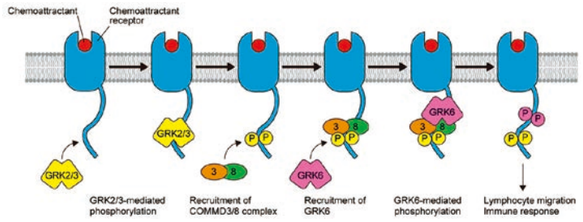


The COMMD3/8 complex determines GRK6 specificity for chemoattractant receptors.

Akiko Nakai, Jun Fujimoto, Haruhiko Miyata, Ralf Stumm, Masashi Narazaki, Stefan Schulz, Yoshihiro Baba, Atsushi Kumanogoh, and Kazuhiro Suzuki.

***J Exp Med* 216: 1630-1647 (2019)**

Lymphocyte migration is mediated by G protein-coupled receptors (GPCRs) that respond to chemoattractive molecules, represented by chemokines. 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. However, the molecular mechanism that determines the specificity of GRK targeting is poorly understood. Kazuhiro Suzuki (Immune Response Dynamics, IFReC) and his group identified a protein complex consisting of copper metabolism MURR1 domain-containing (COMMD) 3 and COMMD8 (COMMD3/8 complex) as an adaptor that selectively recruits a specific GRK to chemoattractant receptors and promotes lymphocyte migration.

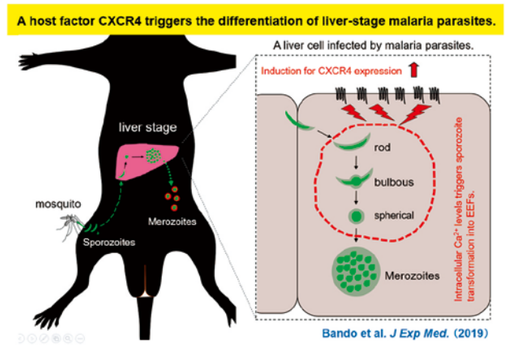


CXCR4 regulates *Plasmodium* development in mouse and human hepatocytes.

Hironori Bando, Ariel Pradipta, Shiroh Iwanaga, Toru Okamoto, Daisuke Okuzaki, Shun Tanaka, Joel Vega-Rodríguez, Youngae Lee, Ji Su Ma, Naoya Sakaguchi, Akira Soga, Shinya Fukumoto, Miwa Sasai, Yoshiharu Matsuura, Masao Yuda, Marcelo Jacobs-Lorena, and Masahiro Yamamoto.

***J Exp Med* 216: 1733-1748 (2019)**

The liver stage of the etiological agent of malaria, *Plasmodium*, is obligatory for successful infection of its various mammalian hosts. Differentiation of the rod-shaped sporozoites of *Plasmodium* into spherical exoerythrocytic forms (EEFs) via bulbous expansion is essential for parasite development in the liver. However, little is known about the host factors regulating the morphological transformation of *Plasmodium* sporozoites in this organ. The research group of Masahiro Yamamoto (Immunoparasitology, IFReC/RIMD) showed that sporozoite differentiation into EEFs in the liver involves protein kinase C ζ-mediated NF-κB activation, which robustly induces the expression of C-X-C chemokine receptor type 4 (CXCR4) in hepatocytes and subsequently elevates intracellular Ca^{2+} levels, thereby triggering sporozoite transformation into EEFs. Blocking CXCR4 expression by genetic or pharmacological intervention profoundly inhibited the liver stage development of the *P. berghei* rodent malaria parasite and the human *P. falciparum* parasite also. Collectively, the experiments show that CXCR4 is a key host factor for *Plasmodium* development in the liver, and CXCR4 warrants further investigation for malaria prophylaxis.

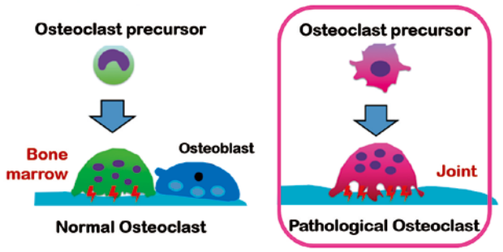


Identification of a novel arthritis-associated osteoclast precursor macrophage regulated by FoxM1.

Tetsuo Hasegawa, Junichi Kikuta, Takao Sudo, Yoshinobu Matsuura, Takahiro Matsui, Szandor Simmons, Kosuke Ebina, Makoto Hirao, Daisuke Okuzaki, Yuichi Yoshida, Atsushi Hirao, Vladimir V. Kalinichenko, Kunihiro Yamaoka, Tsutomu Takeuchi, and Masaru Ishii.

Nature Immunology 20: 1631-1643 (2019)

Masaru Ishii (Immunology and Cell Biology, IFReC/Graduate School of Medicine) show osteoclasts in pannus originate exclusively from circulating bone marrow-derived cells and not from locally resident macrophages. They identify murine $CX_3CR1^{hi}Ly6C^{int}F4/80^{+}I\text{-}A^{+}/I\text{-}E^{+}$ macrophages (termed here "arthritis-associated osteoclastogenic macrophages [AtoMs]") as the osteoclast precursor (OP)-containing population in the inflamed synovium, comprising a subset distinct from conventional OPs in homeostatic bone remodeling. Tamoxifen-inducible Foxm1 deletion suppressed the capacity of AtoMs to differentiate into osteoclasts *in vitro* and *in vivo*. Furthermore, synovial samples from human rheumatoid arthritis (RA) patients contained $CX_3CR1^{+}HLA\text{-}DR^{hi}CD11c^{+}CD80^{-}CD86^{+}$ cells that corresponded to mouse AtoMs, and human osteoclastogenesis was inhibited by the FoxM1 inhibitor, thiostrepton, constituting a potential target for RA treatment.

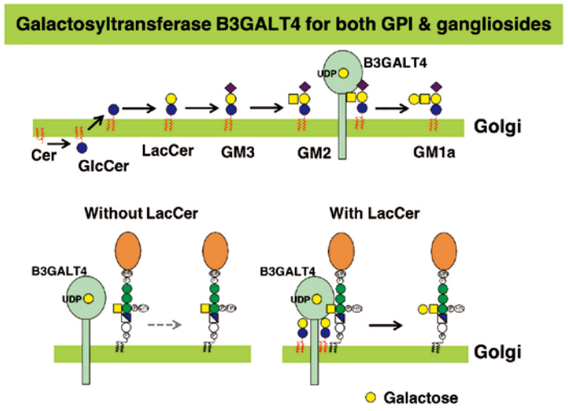


Cross-talks of glycosylphosphatidylinositol biosynthesis with glycosphingolipid biosynthesis and ER-associated degradation.

Yicheng Wang, Yusuke Maeda, Yi-Shi Liu, Yoko Takada, Akinori Ninomiya, Tetsuya Hirata, Morihisa Fujita, Yoshiko Murakami, and Taroh Kinoshita.

Nature Communications 11: 860 (2020)

Glycosylphosphatidylinositol (GPI) is a glycolipid for post-translational modification of many cell-surface proteins in eukaryotic cells. The structure of the core backbone of GPI is conserved whereas the structural variation of GPI anchors is introduced by side-chain modifications. In some mammalian GPI-APs, the N-acetylgalactosamine side-chain linked to the first mannose is further modified with galactose by an unknown galactosyltransferase (GPI-Gal-T). The research group of Taroh Kinoshita (Immunoglycobiology, IFReC/RIMD) performed genome-scale CRISPR-Cas9 knockout screening for GPI-Gal-T and found that B3GALT4, known as GM1 synthase, is GPI-Gal-T. They also demonstrated the requirement of lactosylceramide for efficient galactosylation of GPI side chain. In addition, we show that GPI biosynthesis in the endoplasmic reticulum (ER) is regulated by ER-associated degradation system to prevent GPI accumulation. Thus, our work demonstrates cross-talks of GPI biosynthesis with glycosphingolipid biosynthesis and the ER quality control system.

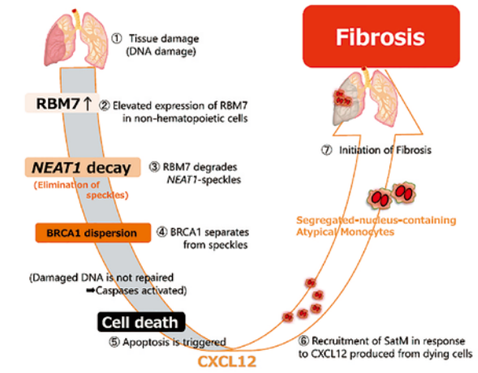


Dysregulated expression of the nuclear exosome targeting complex component RBM7 in non-hematopoietic cells licenses the development of fibrosis.

Kiyoharu Fukushima, Takashi Satoh, Fuminori Sugihara, Yuki Sato, Toru Okamoto, Yuichi Mitsui, Sachiyo Yoshio, Songling Li, Satoshi Nojima, Daisuke Motooka, Shota Nakamura, Hiroshi Kida, Daron M. Standley, Eiichi Morii, Tatsuya Kanto, Motoko Yanagita, Yoshiharu Matsuura, Takashi Nagasawa, Atsushi Kumanogoh, and Shizuo Akira.

Immunity 52: 542-556 (2020)

Fibrosis is an incurable disorder of unknown etiology. Segregated-nucleus-containing atypical monocytes (SatMs) discovered by the authors are critical for the development of fibrosis. The research group of Satoh and Shizuo Akira (Host Defense, IFReC) examined the mechanisms that recruit SatMs to pre-fibrotic areas. A screen based on cytokine expression in the fibrotic lung revealed that the chemokine Cxcl12, which is produced by apoptotic nonhematopoietic cells, was essential for SatM recruitment. Analyses of lung tissues at fibrosis onset showed increased expression of Rbm7, a component of the nuclear exosome targeting complex. Rbm7 deletion suppressed bleomycin-induced fibrosis and at a cellular level, suppressed apoptosis of nonhematopoietic cells. Mechanistically, Rbm7 bound to noncoding (nc) RNAs that form subnuclear bodies, including Neat1 speckles. Dysregulated expression of Rbm7 resulted in the nuclear degradation of Neat1 speckles, the dispersion of the DNA repair protein BRCA1, and the triggering of apoptosis. Thus, Rbm7 in epithelial cells plays a critical role in the development of fibrosis by regulating ncRNA decay and thereby the production of chemokines that recruit SatMs.

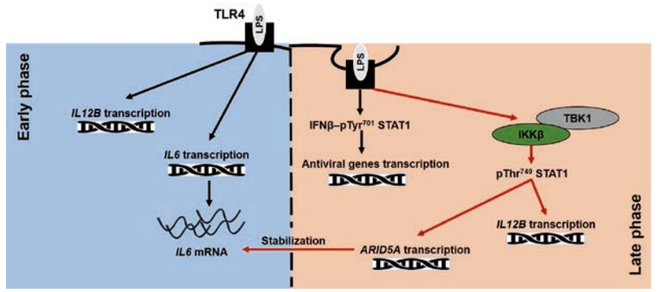


Noncanonical STAT1 phosphorylation expands its transcriptional activity into promoting LPS-induced IL-6 and IL-12p40 production.

Hozaifa Metwally, Toshio Tanaka, Songling Li, Gyanu Parajuli, Sujin Kang, Hamza Hanieh, Shigeru Hashimoto, Jaya P. Chalise, Yohannes Gemechu, Daron M. Standley, and Tadamitsu Kishimoto.

Science Signaling 13: eaay0574 (2020)

The lipopolysaccharide (LPS)–induced endocytosis of Toll-like receptor 4 (TLR4) is an essential step in the production of interferon-β (IFN-β), which activates the transcription of antiviral response genes by STAT1 phosphorylated at Tyr701. Hozaifa Saad Hassan Metwally, Tadamitsu Kishimoto (Immune Regulation, IFReC) and the research group showed that noncanonical phosphorylation in response to LPS confers STAT1 with distinct DNA binding and gene-regulatory properties that promote both IL12B expression and IL6 mRNA stabilization. Their study provides a potential mechanism for how TLR4 endocytosis regulate proinflammatory cytokine production.



Publications

1 Adachi, Y; Tonouchi, K; Nithichanon, A; Kuraoka, M; Watanabe, A; Shinnakasu, R; Asanuma, H; Ainai, A; Ohmi, Y; Yamamoto, T; Ishii, KJ; Hasegawa, H; Takeyama, H; Lertmemongkolchai, G; Kurosaki, T; Ato, M; Kelsoe, G; Takahashi, Y. Exposure of an occluded hemagglutinin epitope drives selection of a class of cross-protective influenza antibodies. Nature Communications 10, 3883 (2019).

2 Akamatsu, M; Mikami, N; Ohkura, N; Kawakami, R; Kitagawa, Y; Sugimoto, A; Hirota, K; Nakamura, N; Ujihara, S; Kurosaki, T; Hamaguchi, H; Harada, H; Xia, GL; Morita, Y; Aramori, I; Narumiya, S; Sakaguchi, S. Conversion of antigen-specific effector/memory T cells into Foxp3-expressing T-reg cells by inhibition of CDK8/19. Science Immunology 4, eaaw2707 (2019).

3 Akiyama, M; Ishigaki, K; Sakaue, S; Momozawa, Y; Horikoshi, M; Hirata, M; Matsuda, K; Ikegawa, S; Takahashi, A; Kanai, M; Suzuki, S; Matsui, D; Naito, M; Yamaji, T; Iwasaki, M; Sawada, N; Tanno, K; Sasaki, M; Hozawa, A; Minegishi, N; Wakai, K; Tsugane, S; Shimizu, A; Yamamoto, M; Okada, Y; Murakami, Y; Kubo, M; Kamatani, Y. Characterizing rare and low-frequency height-associated variants in the Japanese population. Nature Communications 10, 4393 (2019).

4 Akizuki, S; Ishigaki, K; Kochi, Y; Law, SM; Matsuo, K; Ohmura, K; Suzuki, A; Nakayama, M; Iizuka, Y; Koseki, H; Ohara, O; Hirata, J; Kamatani, Y; Matsuda, F; Sumida, T; Yamamoto, K; Okada, Y; Mimori, T; Terao, C. PLD4 is a genetic determinant to systemic lupus erythematosus and involved in murine autoimmune phenotypes. Annals of the Rheumatic Diseases 78, 509-518 (2019).

5 Amariuta, T; Luo, Y; Gazal, S; Davenport, EE; van de Geijn, B; Ishigaki, K; Westra, HJ; Teslovich, N; Okada, Y; Yamamoto, K; Price, AL; Raychaudhuri, S. IMPACT: Genomic annotation of cell-state-specific regulatory elements inferred from the epigenome of bound transcription factors. American Journal of Human Genetics 104, 879-895 (2019).

6 Aoki, M; Watabe, T; Nagamori, S; Naka, S; Ikeda, H; Kongpracha, P; Horitsugi, G; Kanai, Y; Shimosegawa, E; Kanai, Y; Hatazawa, J. Distribution of LAT1-targeting PET tracer was independent of the tumor blood flow in rat xenograft models of C6 glioma and MIA PaCa-2. Annals of Nuclear Medicine 33, 394-403 (2019).

7 Arase, N; Tanemura, A; Jin, H; Nishioka, M; Aoyama, Y; Oiso, N; Matsunaga, K; Suzuki, T; Nishigori, C; Kawamura, T; Shimizu, T; Ito, A; Fukai, K; Abe, Y; Yang, LL; Tsuruta, D; Takeoka, K; Iwatani, Y; Hidaka, Y; Nishida, M; Yamauchi-Takihara, K; Arase, H; Fujimoto, M; Katayama, I. Autoantibodies detected in patients with vitiligo vulgaris but not in those with rhododendrol-induced leukoderma. Journal of Dermatological Science 95, 80-83 (2019).

8 Argilaguet, J; Pedragosa, M; Esteve-Codina, A; Riera, G; Vidal, E; Peligero-Cruz, C; Casella, V; Andreu, D; Kaisho, T; Bocharov, G; Ludewig, B; Heath, S; Meyerhans, A. Systems analysis reveals complex biological processes during virus infection fate decisions. Genome Research 29, 907-919 (2019).

9 Bando, H; Lee, Y; Sakaguchi, N; Pradipta, A; Sakamoto, R; Tanaka, S; Ma, JS; Sasai, M; Yamamoto, M. Toxoplasma effector GRA15-dependent suppression of IFN-gamma-induced antiparasitic response in human neurons. Frontiers In Cellular and Infection Microbiology 9, 140 (2019).

10 Bando, H; Pradipta, A; Iwanaga, S; Okamoto, T; Okuzaki, D; Tanaka, S; Vega-Rodriguez, J; Lee, Y; Ma, JS; Sakaguchi, N; Soga, A; Fukumoto, S; Sasai, M; Matsuura, Y; Yuda, M; Jacobs-Lorena, M; Yamamoto, M. CXCR4 regulates Plasmodium development in mouse and human hepatocytes. Journal of Experimental Medicine 216, 1733-1748 (2019).

11 Boocock, J; Leask, M; Okada, Y; Matsuo, H; Kawamura, Y; Shi, YY; Li, CG; Mount, DB; Mandal, AK; Wang, WQ; Cadzow, M; Gosling, AL; Major, TJ; Horsfield, JA; Choi, HK; Fadason, T; O'Sullivan, J; Stahl, EA; Merriman, TR. Genomic dissection of 43 serum urate-associated loci provides multiple insights into molecular mechanisms of urate control. Human Molecular Genetics 29, 923-943 (2020).

12 Braganza, CD; Shibata, K; Fujiwara, A; Motozono, C; Sonoda, KH; Yamasaki, S; Stocker, BL; Timmer, MSM. The effect of MR1 ligand glyco-analogues on mucosal-associated invariant T (MAIT) cell activation. Organic & Biomolecular Chemistry 17, 8992-9000 (2019).

13 Canete, PF; Sweet, RA; Gonzalez-Figueroa, P; Papa, I; Ohkura, N; Bolton, H; Roco, JA; Cuenca, M; Bassett, KJ; Sayin, I; Barry, E; Lopez, A; Canaday, DH; Meyer-Hermann, M; Doglioni, C; de St Groth, BF; Sakaguchi, S; Cook, MC; Vinuesa, CG. Regulatory roles of IL-10-producing human follicular T cells. Journal of Experimental Medicine 216, 1843-1856 (2019).

14 Chalise, JP; Hashimoto, S; Parajuli, G; Kang, SJ; Singh, SK; Gemechu, Y; Metwally, H; Nyati, KK; Dubey, PK; Zaman, MMU; Nagahama, Y; Hamza, H; Masuda, K; Kishimoto, T. Feedback regulation of Arid5a and Ppar-gamma 2 maintains adipose tissue homeostasis. Proceedings of the National Academy of Sciences USA 116, 15128-15133 (2019).

15 Clark, DW; Okada, Y; Moore, KHS; Mason, D et al. Associations of autozygosity with a broad range of human phenotypes. Nature Communications 10, 4957 (2019).

16 Cossarizza, A; Chang, HD; Radbruch, A; Acs, A; Adam, D et al. Guidelines for the use of flow cytometry and cell sorting in immunological studies (second edition). European Journal of Immunology 49, 1457-1973 (2019).

17 DeVorkin, L; Pavey, N; Carleton, G; Comber, A; Ho, C; Lim, J; McNamara, E; Huang, HC; Kim, P; Zacharias, LG; Mizushima, N; Saitoh, T; Akira, S; Beckham, W; Lorzadeh, A; Moksa, M; Cao, Q; Murthy, A; Hirst, M; DeBerardinis, RJ; Lum, JJ. Autophagy regulation of metabolism is required for $CD8^+$ T cell anti-tumor immunity. Cell Reports 27, 502 (2019).

18 Endo, T; Mikedis, MM; Nicholls, PK; Page, DC; de Rooij, DG. Retinoic acid and germ cell development in the ovary and testis. Biomolecules 9, 775 (2019).

19 Fatoba, O; Ohtake, Y; Itokazu, T; Yamashita, T. Immunotherapies in Huntington's disease and alpha-Synucleinopathies. Frontiers in Immunology 11, 337 (2020).

20 Fisch, D; Bando, H; Clough, B; Hornung, V; Yamamoto, M; Shenoy, AR; Frickel, EM. Human GBP1 is a microbe-specific gatekeeper of macrophage apoptosis and pyroptosis. Embo Journal 38, e100926 (2019).

21 Fujimoto, K; Kawaguchi, Y; Shimohigoshi, M; Gotoh, Y; Nakano, Y; Usui, Y; Hayashi, T; Kimura, Y; Uematsu, M; Yamamoto, T; Akeda, Y; Rhee, JH; Yuki, Y; Ishii, KJ; Crowe, SE; Ernst, PB; Kiyono, H; Uematsu, S. Antigen-specific mucosal immunity regulates development of intestinal bacteria-mediated diseases. Gastroenterology 157, 1530 (2019).

22 Fujita, Y; Yamashita, T. The effects of leptin on glial cells in neurological diseases. Frontiers in Neuroscience 13, 828 (2019).

23 Fukushima, K; Satoh, T; Sugihara, F; Sato, Y; Okamoto, T; Mitsui, Y; Yoshio, S; Li, SL; Nojima, S; Motooka, D; Nakamura, S; Kida, H; Standley, DM; Morii, E; Kanto, T; Yanagita, M; Matsuura, Y; Nagasawa, T; Kumanogoh, A; Akira, S. Dysregulated expression of the nuclear exosome targeting complex component Rbm7 in nonhematopoietic cells licenses the development of fibrosis. Immunity 52, 542 (2020).

24 Gao, J; Hori, Y; Nishiura, M; Bordy, M; Hasserodt, J; Kikuchi, K. Engineered protein-tag for rapid live-cell fluorogenic visualization of proteins by anionic probes. Chemistry Letters 49, 232-235 (2020).

25 Gao, JC; Hori, Y; Shimomura, T; Bordy, M; Hasserodt, J; Kikuchi, K. Development of fluorogenic probes for rapid high-contrast imaging of transient nuclear localization of sirtuin 3. Chembiochem 21, 656-662 (2020).

26 Hamaguchi, M; Muramatsu, R; Fujimura, H; Mochizuki, H; Kataoka, H; Yamashita, T. Circulating transforming grow factor-beta 1 facilitates remyelination in the adult central nervous system. Elife 8, e41869 (2019).

27 Hasegawa, T; Ishii, M. Visualizing bone tissue in homeostatic and pathological conditions. Proceedings of the Japan Academy Series B-Physical and Biological Sciences 96, 43-49 (2020).

28 Hasegawa, T; Kikuta, J; Sudo, T; Matsuura, Y; Matsui, T; Simmons, S; Ebina, K; Hirao, M; Okuzaki, D; Yoshida, Y; Hirao, A; Kalinichenko, VV; Yamaoka, K; Takeuchi, T; Ishii, M. Identification of a novel arthritis-associated osteoclast precursor macrophage regulated by FoxM1. Nature Immunology 20, 1631 (2019).

29 Hashimoto, S; Furukawa, S; Hashimoto, A; Tsutaho, A; Fukao, A; Sakamura, Y; Parajuli, G; Onodera, Y; Otsuka, Y; Handa, H; Oikawa, T; Hata, S; Nishikawa, Y; Mizukami, Y; Kodama, Y; Murakami, M; Fujiwara, T; Hirano, S; Sabe, H. ARF6 and AMAP1 are major targets of KRAS and TP53 mutations to promote invasion, PD-L1 dynamics, and immune evasion of pancreatic cancer. Proceedings of the National Academy of Sciences USA 116, 17450-17459 (2019).

30 Hayashi, Y; Jia, WZ; Kidoya, H; Muramatsu, F; Tsukada, Y; Takakura, N. Galectin-3 inhibits cancer metastasis by negatively regulating integrin beta 3 expression. American Journal of Pathology 189, 900-910 (2019).

31 Herppich, S; Toker, A; Pietzsch, B; Kitagawa, Y; Ohkura, N; Miyao, T; Floess, S; Hori, S; Sakaguchi, S; Huehn, J. Dynamic imprinting of the Treg cell-specific epigenetic signature in developing thymic regulatory T cells. Frontiers in Immunology 10, 2382 (2019).

32 Hochsmann, B; Murakami, Y; Osato, M; Knaus, A; Kawamoto, M; Inoue, N; Hirata, T; Murata, S; Anliker, M; Eggermann, T; Jager, M; Floettmann, R; Hollein, A; Murase, S; Ueda, Y; Nishimura, JI; Kanakura, Y; Kohara, N; Schrezenmeier, H; Krawitz, PM; Kinoshita, T. Complement and inflammasome overactivation mediates paroxysmal nocturnal hemoglobinuria with autoinflammation. Journal of Clinical Investigation 129, 5123-5136 (2019).

33 Hori, Y; Kikuchi, K. Chemical tools with fluorescence switches for verifying epigenetic modifications. Accounts of Chemical Research 52, 2849-2857 (2019).

34 Hossain, MM; Tsuzuki, T; Sakakibara, K; Imaizumi, F; Ikegaya, A; Inagaki, M; Takahashi, I; Ito, T; Takamatsu, H; Kumanogoh, A; Negishi, T; Yukawa, K. PlexinA1 is crucial for the midline crossing of callosal axons during corpus callosum development in BALB/cAJ mice. Plos One 14, e0221440 (2019).

35 Ikeda-Matsuo, Y; Miyata, H; Mizoguchi, T; Ohama, E; Naito, Y; Uematsu, S; Akira, S; Sasaki, Y; Tanabe, M. Microsomal prostaglandin E synthase-1 is a critical factor in dopaminergic neurodegeneration in Parkinson's disease. Neurobiology of Disease 124, 81-92 (2019).

36 Ikumi, K; Odanaka, M; Shime, H; Imai, M; Osaga, S; Taguchi, O; Nishida, E; Hemmi, H; Kaisho, T; Morita, A; Yamazaki, S. Hyperglycemia is associated with psoriatic inflammation in both humans and mice. Journal of Investigative Dermatology 139, 1329 (2019).

37 Imanishi, T; Saito, T. T cell co-stimulation and functional modulation by innate signals. Trends in Immunology 41, 200-212 (2020).

38 Imoto, T; Kawase, A; Minoshima, M; Yokoyama, T; Bito, H; Kikuchi, K. Photolytic release of a caged inhibitor of an endogenous transcription factor enables optochemical control of CREB-mediated gene expression. Organic Letters 22, 22-25 (2020).

39 Imoto, T; Muramatsu, M; Miyasaka, H; Mizukami, S; Kikuchi, K. Improvement in photostability of fluorescein by lanthanide Ions based on energy transfer-based triplet state quenching. Chemistry Letters 48, 1181-1184 (2019).

40 Ishifune, C; Tsukumo, S; Maekawa, Y; Hozumi, K; Chung, DH; Motozono, C; Yamasaki, S; Nakano, H; Yasutomo, K. Regulation of membrane phospholipid asymmetry by Notch-mediated flippase expression controls the number of intraepithelial $TCR\alpha\beta^+CD8\alpha\alpha^+$ T cells. Plos Biology 17, e3000262 (2019).

41 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. $PD\text{-}1^+$ regulatory T cells amplified by PD-1 blockade promote hyperprogression of cancer. Proceedings of the National Academy of Sciences USA 116, 9999-10008 (2019).

42 Kang, SJ; Tanaka, T; Narazaki, M; Kishimoto, T. Targeting interleukin-6 signaling in clinic. Immunity 50, 1007-1023 (2019).

43 Kashiyama, N; Miyagawa, S; Fukushima, S; Kawamura, T; Kawamura, A; Yoshida, S; Eiraku, S; Harada, A; Matsunaga, K; Watabe, T; Toda, K; Hatazawa, J; Sawa, Y. MHC-mismatched allotransplantation of induced pluripotent stem cell-derived cardiomyocyte sheets to improve cardiac function in a primate ischemic cardiomyopathy model. Transplantation 103, 1582-1590 (2019).

44 Kawamura, Y; Nakaoka, H; Nakayama, A; Okada, Y; Yamamoto, K et al. Genome-wide association study revealed novel loci which aggravate asymptomatic hyperuricaemia into gout. Annals of the Rheumatic Diseases 78, 1430-1437 (2019).

45 Kayama, H; Tani, H; Kitada, S; Opasawatchai, A; Okumura, R; Motooka, D; Nakamura, S; Takeda, K. BATF2 prevents T-cell-mediated intestinal inflammation through regulation of the IL-23/IL-17 pathway. International Immunology 31, 371-383 (2019).

46 Kishikawa, T; Maeda, Y; Nii, T; Motooka, D; Matsumoto, Y; Matsushita, M; Matsuoka, H; Yoshimura, M; Kawada, S; Teshigawara, S; Oguro, E; Okita, Y; Kawamoto, K; Higa, S; Hirano, T; Narazaki, M; Ogata, A; Saeki, Y; Nakamura, S; Inohara, H; Kumanogoh, A; Takeda, K; Okada, Y. Metagenome-wide association study of gut microbiome revealed novel aetiology of rheumatoid arthritis in the Japanese population. Annals of the Rheumatic Diseases 79, 103-111 (2020).

47 Kitazume-Taneike, R; Taneike, M; Omiya, S; Misaka, T; Nishida, K; Yamaguchi, O; Akira, S; Shattock, MJ; Sakata, Y; Otsu, K. Ablation of Toll-like receptor 9 attenuates myocardial ischemia/reperfusion injury in mice. Biochemical and Biophysical Research Communications 515, 442-447 (2019).

48 Kiyozumi, D; Nakano, I; Sato-Nishiuchi, R; Tanaka, S; Sekiguchi, K. Laminin is the ECM niche for trophoblast stem cells. Life Science Alliance 3, e201900515 (2020).

49 Knaus, A; Kortrum, F; Kleefstra, T; Stray-Pedersen, A; Dukic, D; Murakami, Y; Gerstner, T; van Bokhoven, H; Iqbal, Z; Horn, D; Kinoshita, T; Hempe, MA; Krawitz, PM. Mutations in PIGU impair the function of the GPI transamidase complex, causing severe intellectual disability, epilepsy, and brain anomalies. American Journal of Human Genetics 105, 395-402 (2019).

50 Kobari, S; Kusakabe, T; Momota, M; Shibahara, T; Hayashi, T; Ozasa, K; Morita, H; Matsumoto, K; Saito, H; Ito, S; Kuroda, E; Ishii, KJ. IL-33 is essential for adjuvant effect of hydroxypropyl-beta-cyclodexrin on the protective intranasal influenza vaccination. Frontiers in Immunology 11, 360 (2020).

51 Kubagawa, H; Honjo, K; Ohkura, N; Sakaguchi, S; Radbruch, A; Melchers, F; Jani, PK. Functional roles of the IgM Fc receptor in the immune system. Frontiers in Immunology 10, 945 (2019).

52 Kumar, A; Kumar, H. Long noncoding RNA: TRIMming the viral load. Cellular & Molecular Immunology 16, 843-845 (2019).

53 Kumar, N; Hori, Y; Kikuchi, K. Photoactive yellow protein and its chemical probes: an approach to protein labelling in living cells. Journal of Biochemistry 166, 121-127 (2019).

54 Leach, S; Shinnakasu, R; Adachi, Y; Momota, M; Makino-Okamura, C; Yamamoto, T; Ishii, KJ; Fukuyama, H; Takahashi, Y; Kurosaki, T. Requirement for memory B-cell activation in protection from heterologous influenza virus reinfection. International Immunology 31, 771-779 (2019).

55 Lee, MSJ; Natsume-Kitatani, Y; Temizoz, B; Fujita, Y; Konishi, A; Matsuda, K; Igari, Y; Tsukui, T; Kobiyama, K; Kuroda, E; Onishi, M; Marichal, T; Ise, W; Inoue, T; Kurosaki, T; Mizuguchi, K; Akira, S; Ishii, KJ; Coban, C. B cell-intrinsic MyD88 signaling controls IFN-gamma-mediated early IgG2c class switching in mice in response to a particulate adjuvant. European Journal of Immunology 49, 1433-1440 (2019).

56 Lee, Y; Yamada, H; Pradipta, A; Ma, JS; Okamoto, M; Nagaoka, H; Takashima, E; Standley, DM; Sasai, M; Takei, K; Yamamoto, M. Initial phospholipid-dependent Irgb6 targeting to Toxoplasma gondii vacuoles mediates host defense. Life Science Alliance 3, e201900549 (2020).

57 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 Part A 95A, 565-578 (2019).

58 Leu, C; Stevelink, R; Smith, AW; Goleva, SB; Kanai, M; Ferguson, L; Campbell, C; Kamatani, Y; Okada, Y; Sisodiya, SM; Cavalleri, GL; Koeleman, BPC; Lerche, H; Jehi, L; Davis, LK; Najm, IM; Palotie, A; Daly, MJ; Busch, RM; Lal, D. Polygenic burden in focal and generalized epilepsies. Brain 142, 3473-3481 (2019).

59 Lim, EL; Okkenhaug, K. Phosphoinositide 3-kinase delta is a regulatory T-cell target in cancer immunotherapy. Immunology 157, 210-218 (2019).

60 Lu, YG; Oura, S; Matsumura, T; Oji, A; Sakurai, N; Fujihara, Y; Shimada, K; Miyata, H; Tobita, T; Noda, T; Castaneda, JM; Kiyozumi, D; Zhang, Q; Larasati, T; Young, SAM; Kodani, M; Huddleston, CA; Robertson, MJ; Coarfa, C; Isotani, A; Aitken, RJ; Okabe, M; Matzuk, MM; Garcia, TX; Ikawa, M. CRISPR/Cas9-mediated genome editing reveals 30 testisenriched genes dispensable for male fertility in mice. Biology of Reproduction 101, 501-511 (2019).

61 Maeda, Y; Takeda, K. Host-microbiota interactions in rheumatoid arthritis. Experimental and Molecular Medicine 51, 150 (2019).

62 Maeda, Y; Tsuda, T; Takeda, Y; Koyama, S; Hayama, Y; Nojima, S; Kimura, T; Ito, D; Takamatsu, H; Kang, SJ; Nishide, M; Morimoto, K; Hosokawa, T; Kinehara, Y; Kato, Y; Nakatani, T; Takeda, K; Hayama, M; Obata, S; Akazawa, H; Shikina, T; Inohara, H; Kumanogoh, A. SEMA4A promotes eosinophil survival and contributes to eosinophil-mediated allergic diseases. Allergology International 68, 274-276 (2019).

63 Maekawa, A; Arase, N; Tamai, K; Nomura, T; Kiyohara, E; Wataya-Kaneda, M; Arase, H; Katayama, I; Fujimoto, M. Case of epidermolytic ichthyosis with impairment of pulmonary function and exacerbated skin manifestations in a late middle-aged adult. Journal of Dermatology 46, e480e482 (2019).

64 Martin, AR; Kanai, M; Kamatani, Y; Okada, Y; Neale, BM; Daly, MJ. Clinical use of current polygenic risk scores may exacerbate health disparities. Nature Genetics 51, 584-591 (2019).

65 Mastellos, DC; Blom, AM; Connolly, ES; Daha, MR; Geisbrecht, BV; Ghebrehiwet, B; Gros, P; Hajishengallis, G; Holers, VM; Huber-Lang, M; Kinoshita, T; Mollnes, TE; Montgomery, RA; Morgan, BP; Nilsson, B; Pio, R; Ricklin, D; Risitano, AM; Taylor, RP; Mantovani, A; Ioannidis, JPA; Lambris, JD. 'Stealth' corporate innovation: an emerging threat for therapeutic drug development. Nature Immunology 20, 1409-1413 (2019).

66 Masuda, T; Low, SK; Akiyama, M; Hirata, M; Ueda, Y; Matsuda, K; Kimura, T; Murakami, Y; Kubo, M; Kamatani, Y; Okada, Y. GWAS of five gynecologic diseases and cross-trait analysis in Japanese. European Journal of Human Genetics 28, 95-107 (2020).

67 Matoba, N; Akiyama, M; Ishigaki, K; Kanai, M; Takahashi, A; Momozawa, Y; Ikegawa, S; Ikeda, M; Iwata, N; Hirata, M; Matsuda, K; Kubo, M; Okada, Y; Kamatani, Y. GWAS of smoking behaviour in 165,436 Japanese people reveals seven new loci and shared genetic architecture. Nature Human Behaviour 3, 471-477 (2019).

68 Matoba, N; Akiyama, M; Ishigaki, K; Kanai, M; Takahashi, A; Momozawa, Y; Ikegawa, S; Ikeda, M; Iwata, N; Hirata, M; Matsuda, K; Murakami, Y; Kubo, M; Kamatani, Y; Okada, Y. GWAS of 165,084 Japanese individuals identified nine loci associated with dietary habits. Nature Human Behaviour 4, 308-316 (2020).

69 Matoba, T; Imai, M; Ohkura, N; Kawakita, D; Ijichi, K; Toyama, T; Morita, A; Murakami, S; Sakaguchi, S; Yamazaki, S. Regulatory T cells expressing abundant CTLA-4 on the cell surface with a proliferative gene profile are key features of human head and neck cancer. International Journal of Cancer 144, 2811-2822 (2019).

70 Matsuda, A; Asada, Y; Suita, N; Iwamoto, S; Hirakata, T; Yokoi, N; Ohkawa, Y; Okada, Y; Yokomizo, T; Ebihara, N. Transcriptome profiling of refractory atopic keratoconjunctivitis by RNA sequencing. Journal of Allergy and Clinical Immunology 143, 1610 (2019).

71 Matsumura, T; Endo, T; Isotani, A; Ogawa, M; Ikawa, M. An azoospermic factor gene, Ddx3y and its paralog, Ddx3x are dispensable in germ cells for male fertility. Journal of Reproduction and Development 65, 121-128 (2019).

72 Matsumura, T; Ikebe, T; Arikawa, K; Hosokawa, M; Aiko, M; Iguchi, A; Togashi, I; Kai, S; Ohara, S; Ohara, N; Ohnishi, M; Watanabe, H; Kobayashi, K; Takeyama, H; Yamasaki, S; Takahashi, Y; Ato, M. Sequential sensing by TLR2 and mincle directs immature myeloid cells to protect against invasive group A streptococcal infection in mice. Cell Reports 27, 561 (2019).

73 Matsuo, T; Hashimoto, M; Sakaguchi, S; Sakaguchi, N; Ito, Y; Hikida, M; Tsuruyama, T; Sakai, K; Yokoi, H; Shirakashi, M; Tanaka, M; Ito, H; Yoshifuji, H; Ohmura, K; Fujii, T; Mimori, T. Strain-specific manifestation of lupus-like systemic autoimmunity caused by Zap70 mutation. Journal of Immunology 202, 3161-3172 (2019).

74 Matsuoka, S; Hashimoto, D; Kadowaki, M; Ohigashi, H; Hayase, E; Yokoyama, E; Hasegawa, Y; Tateno, T; Chen, XZ; Aoyama, K; Oka, H; Onozawa, M; Takeda, K; Akashi, K; Teshima, T. Myeloid differentiation factor 88 signaling in donor T cells accelerates graft-versus-host disease. Haematologica 105, 226-234 (2020).

75 Matsushita, K; Tanaka, H; Yasuda, K; Adachi, T; Fukuoka, A; Akasaki, S; Koida, A; Kuroda, E; Akira, S; Yoshimoto, T. Regnase-1 degradation is crucial for IL-33-and IL-25-mediated ILC2 activation. JCI Insight 5, e131480 (2020).

76 Mesin, L; Schiepers, A; Ersching, J; Barbulescu, A; Cavazzoni, CB; Angelini, A; Okada, T; Kurosaki, T; Victora, GD. Restricted clonality and limited germinal center reentry characterize memory B cell reactivation by boosting. Cell 180, 92 (2020).

77 Metwally, H; Tanaka, T; Li, SL; Parajuli, G; Kang, SJ; Hanieh, H; Hashimoto, S; Chalise, JP; Gemechu, Y; Standley, DM; Kishimoto, T. Noncanonical STAT1 phosphorylation expands its transcriptional activity into promoting LPS-induced IL-6 and IL-12p40 production. Science Signaling 13, eaay0574 (2020).

78 Minoshima, M; Kikuta, J; Omori, Y; Seno, S; Suehara, R; Maeda, H; Matsuda, H; Ishii, M; Kikuchi, K. In vivo multicolor imaging with fluorescent probes revealed the dynamics and function of osteoclast proton pumps. Acs Central Science 5, 1059-1066 (2019).

79 Mizuno, A; Okada, Y. Biological characterization of expression quantitative trait loci (eQTLs) showing tissue-specific opposite directional effects. European Journal of Human Genetics 27, 1745-1756 (2019).

80 Molineros, JE; Looger, LL; Kim, K; Okada, Y; Terao, C et al. Amino acid signatures of HLA Class-I and II molecules are strongly associated with SLE susceptibility and autoantibody production in Eastern Asians. Plos Genetics 15, e1008092 (2019).

81 Morimoto, A; Kikuta, J; Ishii, M. Intravital multiphoton microscopy as a novel tool in the field of immunopharmacology. Pharmacology & Therapeutics 206, UNSP 107429 (2020).

82 Motomura, Y; Kobayashi, T; Moro, K. The Neuropeptide CGRP induces bipolar syndrome in group 2 innate lymphoid cells. Immunity 51, 598-600 (2019).

83 Murakami, M; Tognini, P. The Circadian Clock as an Essential Molecular Link Between Host Physiology and Microorganisms. Frontiers in Cellular and Infection Microbiology 9, 469 (2020).

84 Nagata, S; Sakuragi, T; Segawa, K. Flippase and scramblase for phosphatidylserine exposure. Current Opinion in Immunology 62, 31-38 (2020).

85 Nagatake, T; Hirata, S; Koga, T; Kuroda, E; Kobari, S; Suzuki, H; Hosomi, K; Matsumoto, N; Yanrismet, Y; Shimojou, M; Morimoto, S; Sasaki, F; Ishii, KJ; Yokomizo, T; Kunisawa, J. BLT1 mediates commensal bacteria-dependent innate immune signals to enhance antigen-specific intestinal IgA responses. Mucosal Immunology 12, 1082-1091 (2019).

86 Naito, H; Wakabayashi, T; Ishida, M; Gil, CH; Iba, T; Rahmawati, FN; Shimizu, S; Yoder, MC; Takakura, N. Isolation of tissue-resident vascular endothelial stem cells from mouse liver. Nature Protocols 15, 1066-1081 (2020).

87 Nakai, A; Fujimoto, J; Miyata, H; Stumm, R; Narazaki, M; Schulz, S; Baba, Y; Kumanogoh, A; Suzuki, K. The COMMD3/8 complex determines GRK6 specificity for chemoattractant receptors. Journal of Experimental Medicine 216, 1630-1647 (2019).

88 Nakai, A; Suzuki, K. Adrenergic control of lymphocyte trafficking and adaptive immune responses. Neurochemistry International 130, 104320 (2019).

89 Nakanishi, T; Fujita, Y; Tanaka, T; Yamashita, T. Anti-repulsive guidance molecule-a antibody treatment and repetitive transcranial magnetic stimulation have synergistic effects on motor recovery after spinal cord injury. Neuroscience Letters 709, 134329 (2019).

90 Nakatochi, M; Kanai, M; Nakayama, A; Hishida, A; Kawamura, Y et al. Genome-wide meta-analysis identifies multiple novel loci associated with serum uric acid levels in Japanese individuals. Communications Biology 2, 115 (2019).

91 Nakatsukasa, H; Oda, M; Yin, J; Chikuma, S; Ito, M; Koga-Iizuka, M; Someya, K; Kitagawa, Y; Ohkura, N; Sakaguchi, S; Koya, I; Sanosaka, T; Kohyama, J; Tsukadar, Y; Yamanaka, S; Takamuraenya, T; Lu, QJ; Yoshimura, A. Loss of TET proteins in regulatory T cells promotes abnormal proliferation, Foxp3 destabilization and IL-17 expression. International Immunology 31, 335-347 (2019).

92 Nishi, C; Yanagihashi, Y; Segawa, K; Nagata, S. MERTK tyrosine kinase receptor together with TIM4 phosphatidylserine receptor mediates distinct signal transduction pathways for efferocytosis and cell proliferation. Journal of Biological Chemistry 294, 7221-7230 (2019).

93 Nishida, S; Tsuboi, A; Tanemura, A; Ito, T; Nakajima, H; Shirakata, T; Morimoto, S; Fujiki, F; Hosen, N; Oji, Y; Kumanogoh, A; Kawase, I; Oka, Y; Azuma, I; Morita, S; Sugiyama, H. Immune adjuvant therapy using Bacillus Calmette-Guerin cell wall skeleton (BCG-CWS) in advanced malignancies: A phase 1 study of safety and immunogenicity assessments. Medicine 98, e16771 (2019).

94 Noguchi, E; Akiyama, M; Yagami, A; Hirota, T; Okada, Y; Kato, Z; Kishikawa, R et al. HLA-DQ and RBFOX1 as susceptibility genes for an outbreak of hydrolyzed wheat allergy. Journal of Allergy and Clinical Immunology 144, 1354-1363 (2019).

95 Nyati, KK; Agarwal, RG; Sharma, P; Kishimoto, T. Arid5a regulation and the roles of Arid5a in the inflammatory Response and Disease. Frontiers In Immunology 10, 2790 (2019).

96 Nyati, KK; Zaman, MMU; Sharma, P; Kishimoto, T. Arid5a, an RNA-binding protein in immune regulation: RNA stability, inflammation, and autoimmunity. Trends in Immunology 41, 255-268 (2020).

97 Ogawa, K; Okuno, T; Hosomichi, K; Hosokawa, A; Hirata, J; Suzuki, K; Sakaue, S; Kinoshita, M; Asano, Y; Miyamoto, K; Inoue, I; Kusunoki, S; Okada, Y; Mochizuki, H. Next-generation sequencing identifies contribution of both class I and II HLA genes on susceptibility of multiple sclerosis in Japanese. Journal of Neuroinflammation 16, 162 (2019).

98 Ogawa, K; Stuart, PE; Tsoi, LC; Suzuki, K; Nair, RP; Mochizuki, H; Elder, JT; Okada, Y. A transethnic mendelian randomization study identifies causality of obesity on risk of psoriasis. Journal of Investigative Dermatology 139, 1397-1400 (2019).

99 Oh, JE; Iijima, N; Song, E; Lu, PW; Klein, J; Jiang, RY; Kleinstein, SH; Iwasaki, A. Migrant memory B cells secrete luminal antibody in the vagina. Nature 571, 122 (2019).

100 Oh-hora, M; Lu, XY; Shiokawa, M; Takayanagi, H; Yamasaki, S. Stromal interaction molecule deficiency in T cells promotes spontaneous follicular helper T cell development and causes type 2 immune disorders. Journal of Immunology 202, 2616-2627 (2019).

101 Ohshima, K; Nojima, S; Tahara, S; Kurashige, M; Kawasaki, K; Hori, Y; Taniguchi, M; Umakoshi, Y; Okuzaki, D; Wada, N; Ikeda, J; Fukusaki, E; Morii, E. Serine racemase enhances growth of colorectal cancer by producing pyruvate from serine. Nature Metabolism 2, 81 (2020).

102 Okumura, R; Kodama, T; Hsu, CC; Sahlgren, BH; Hamano, S; Kurakawa, T; Iida, T; Takeda, K. Lypd8 inhibits attachment of pathogenic bacteria to colonic epithelia. Mucosal Immunology 13, 75-85 (2020).

103 Orimo, T; Sasaki, I; Hemmi, H; Ozasa, T; Fukuda-Ohta, Y; Ohta, T; Morinaka, M; Kitauchi, M; Yamaguchi, T; Sato, Y; Tanaka, T; Hoshino, K; Katayama, K; Fukuda, S; Miyake, K; Yamamoto, M; Satoh, T; Furukawa, K; Kuroda, E; Ishii, KJ; Takeda, K; Kaisho, T. Cholera toxin B induces interleukin-1 beta production from resident peritoneal macrophages through the pyrin inflammasome as well as the NLRP3 inflammasome. International Immunology 31, 657-668 (2019).

104 Osa, A; Uenami, T; Naito, Y; Hirata, H; Koyama, S; Takimoto, T; Shiroyama, T; Futami, S; Nakatsubo, S; Sawa, N; Yano, Y; Nagatomo, I; Takeda, Y; Mori, M; Kida, H; Kumanogoh, A. Monitoring antibody binding to T cells in a pembrolizumab-treated patient with lung adenocarcinoma on hemodialysis. Thoracic Cancer 10, 2183-2187 (2019).

105 Osada-Oka, M; Goda, N; Saiga, H; Yamamoto, M; Takeda, K; Ozeki, Y; Yamaguchi, T; Soga, T; Tateishi, Y; Miura, K; Okuzaki, D; Kobayashi, K; Matsumoto, S. Metabolic adaptation to glycolysis is a basic defense mechanism of macrophages for Mycobacterium tuberculosis infection. International Immunology 31, 781-793 (2019).

106 Ozaki, T; Muramatsu, R; Nakamura, H; Kinoshita, M; Kishima, H; Yamashita, T. Proteomic analysis of protein changes in plasma by balloon test occlusion. Journal of Clinical Neuroscience 72, 397-401 (2020).

107 Ozasa, K; Temizoz, B; Kusakabe, T; Kobari, S; Momota, M; Coban, C; Ito, S; Kobiyama, K; Kuroda, E; Ishii, KJ. Cyclic GMP-AMP triggers asthma in an IL-33-dependent manner that is blocked by amlexanox, a TBK1 Inhibitor. Frontiers in Immunology 10, 2212 (2019).

108 Pareek, S; Kurakawa, T; Das, B; Motooka, D; Nakaya, S; Rongsen-Chandola, T; Goyal, N; Kayama, H; Dodd, D; Okumura, R; Maeda, Y; Fujimoto, K; Nii, T; Ogawa, T; Iida, T; Bhandari, N; Kida, T; Nakamura, S; Nair, GB; Takeda, K. Comparison of Japanese and Indian intestinal microbiota shows diet-dependent interaction between bacteria and fungi. Npj Biofilms and Microbiomes 5, 37 (2019).

109 Pavillon, N; Smith, NI. Immune cell type, cell activation, and single cell heterogeneity revealed by label-free optical methods. Scientific Reports 9, 17054 (2019).

110 Reinink, P; Buter, J; Mishra, VK; Ishikawa, E; Cheng, TY; Willemsen, PTJ; Porwollik, S; Brennan, PJ; Heinz, E; Mayfield, JA; Dougan, G; van Els, CA; Cerundolo, V; Napolitani, G; Yamasaki, S; Minnaard, AJ; McCeland, M; Moody, DB; Van Rhijn, I. Discovery of Salmonella trehalose phospholipids reveals functional convergence with mycobacteria. Journal of Experimental Medicine 216, 757-771 (2019).

111 Reja, SI; Minoshima, M; Hori, Y; Kikuchi, K. Development of an effective protein-labeling system based on smart fluorogenic probes. Journal of Biological Inorganic Chemistry 24, 443-455 (2019).

112 Romanov, V; Isohashi, K; Alobthani, G; Beshr, R; Horitsugi, G; Kanai, Y; Naka, S; Watabe, T; Shimosegawa, E; Hatazawa, J. Evaluation of the total distribution volume of F-18-FBPA in normal tissues of healthy volunteers by non-compartmental kinetic modeling. Annals of Nuclear Medicine 34, 155-162 (2020).

113 Rozewicki, J; Li, SL; Amada, KM; Standley, DM; Katoh, K. MAFFT-DASH: integrated protein sequence and structural alignment. Nucleic Acids Research 47, W5-W10 (2019).

114 Ryoden, Y; Fujii, T; Segawa, K; Nagata, S. Functional expression of the P2X7 ATP receptor requires Eros. Journal of Immunology 204, 559-568 (2020).

115 Sakaue, S; Akiyama, M; Hirata, M; Matsuda, K; Murakami, Y; Kubo, M; Kamatani, Y; Okada, Y. Functional variants in ADH1B and ALDH2 are non-additively associated with all-cause mortality in Japanese population. European Journal of Human Genetics 28, 378-382 (2020).

116 Sakaue, S; Hirata, J; Kanai, M; Suzuki, K; Akiyama, M; Too, CL; Arayssi, T; Hammoudeh, M; Al Emadi, S; Masri, BK; Halabi, H; Badsha, H; Uthman, IW; Saxena, R; Padyukov, L; Hirata, M; Matsuda, K; Murakami, Y; Kamatani, Y; Okada, Y. Dimensionality reduction reveals fine-scale structure in the Japanese population with consequences for polygenic risk prediction. Nature Communications 11, 1569 (2020).

117 Sakaue, S; Kanai, M; Karjalainen, J; Akiyama, M; Kurki, M; Matoba, N; Takahashi, A; Hirata, M; Kubo, M; Matsuda, K; Murakami, Y; Daly, MJ; Kamatani, Y; Okada, Y. Trans-biobank analysis with 676,000 individuals elucidates the association of polygenic risk scores of complex traits with human lifespan. Nature Medicine 26, 542-548 (2020).

118 Sakaue, S; Okada, Y. GREP: genome for REPositioning drugs. Bioinformatics 35, 3821-3823 (2019).

119 Sanjo, H; Nakayama, J; Yoshizawa, T; Fehling, HJ; Akira, S; Taki, S. Cutting edge: TAK1 safeguards macrophages against proinflammatory cell death. Journal of Immunology 203, 783-788 (2019).

120 Sasai, M; Yamamoto, M. Decision by injection without infection. Journal of Experimental Medicine 217, e20192145 (2020).

121 Sasai, M; Yamamoto, M. Innate, adaptive, and cell-autonomous immunity against Toxoplasma gondii infection. Experimental and Molecular Medicine 51, 156 (2019).

122 Sato, A; Ono, C; Tamura, T; Mori, H; Izumi, T; Torii, S; Fauzyah, Y; Yamamoto, T; Morioka, Y; Okuzaki, D; Fukuhara, T; Matsuura, Y. Rimonabant suppresses RNA transcription of hepatitis B virus by inhibiting hepatocyte nuclear factor 4 alpha. Microbiology and Immunology, 345-355 (2020).

123 Schmidleithner, L; Thabet, Y; Schonfeld, E; Kohne, M; Sommer, D et al. Enzymatic activity of HPGD in Treg cells suppresses Tconv cells to maintain adipose tissue homeostasis and prevent metabolic dysfunction. Immunity 50, 1232 (2019).

124 Schritt, D; Li, SL; Rozewicki, J; Katoh, K; Yamashita, K; Volkmuth, W; Cavet, G; Standley, DM. Repertoire builder: high-throughput structural modeling of B and T cell receptors. Molecular Systems Design & Engineering 4, 761-768 (2019).

125 Shimizu, Y; Kohyama, M; Yorifuji, H; Jin, H; Arase, N; Suenaga, T; Arase, H. Fc gamma RIIIA-mediated activation of NK cells by IgG heavy chain complexed with MHC class II molecules. International Immunology 31, 303-314 (2019).

126 Sidwell, T; Liao, Y; Garnham, AL; Vasanthakumar, A; Gloury, R; Blume, J; Teh, PP; Chisanga, D; Thelemann, C; Rivera, FD; Engwerda, CR; Corcoran, L; Kometani, K; Kurosaki, T; Smyth, GK; Shi, W; Kallies, A. Attenuation of TCR-induced transcription by Bach2 controls regulatory T cell differentiation and homeostasis. Nature Communications 11, 252 (2020).

127 Simmons, S; Sasaki, N; Umemoto, E; Uchida, Y; Fukuhara, S; Kitazawa, Y; Okudaira, M; Inoue, A; Tohya, K; Aoi, K; Aoki, J; Mochizuki, N; Matsuno, K; Takeda, K; Miyasaka, M; Ishii, M. Highendothelial cell-derived S1P regulates dendritic cell localization and vascular integrity in the lymph node. Elife 8, e41239 (2019).

128 Spiliopoulou, A; Colombo, M; Plant, D; Nair, N; Cui, J et al. Association of response to TNF inhibitors in rheumatoid arthritis with quantitative trait loci for CD40 and CD39. Annals of the Rheumatic Diseases 78, 1055-1061 (2019).

129 Suematsu, R; Miyamoto, T; Saijo, S; Yamasaki, S; Tada, Y; Yoshida, H; Miyake, Y. Identification of lipophilic ligands of Siglec5 and-14 that modulate innate immune responses. Journal of Biological Chemistry 294, 16776-16788 (2019).

130 Takahashi, R; Amano, H; Ito, Y; Eshima, K; Satoh, T; Iwamura, M; Nakamura, M; Kitasato, H; Uematsu, S; Raouf, J; Jakobsson, PJ; Akira, S; Majima, M. Microsomal prostaglandin E synthase-1 promotes lung metastasis via SDF-1/CXCR4-mediated recruitment of $CD11b^{+}Gr1^{+}$MDSCs from bone marrow. Biomedicine & Pharmacotherapy 121, 109581 (2020).

131 Takeuchi, Y; Hirota, K; Sakaguchi, S. Impaired T cell receptor signaling and development of T cell-mediated autoimmune arthritis. Immunological Reviews 294, 164-176 (2020).

132 Takeuchi, Y; Hirota, K; Sakaguchi, S. Synovial tissue inflammation mediated by autoimmune T cells. Frontiers in Immunology 10, 1989 (2019).

133 Tanabe, S; Yamashita, T. Function of lymphocytes in oligodendrocyte development. Neuroscientist 26, 74-86 (2020).

134 Tanaka, A; Nishikawa, H; Noguchi, S; Sugiyama, D; Morikawa, H; Takeuchi, Y; Ha, D; Shigeta, N; Kitawaki, T; Maeda, Y; Saito, T; Shinohara, Y; Kameoka, Y; Iwaisako, K; Monma, F; Ohishi, K; Karbach, J; Jager, E; Sawada, K; Katayama, N; Takahashi, N; Sakaguchi, S. Tyrosine kinase inhibitor imatinib augments tumor immunity by depleting effector regulatory T cells. Journal of Experimental Medicine 217, e20191009 (2020).

135 Tanaka, A; Sakaguchi, S. Targeting Treg cells in cancer immunotherapy. European Journal of Immunology 49, 1140-1146 (2019).

136 Tanuma, M; Kasai, A; Bando, K; Kotoku, N; Harada, K; Minoshima, M; Higashino, K; Kimishima, A; Arai, M; Ago, Y; Seiriki, K; Kikuchi, K; Kawata, S; Fujita, K; Hashimoto, H. Direct visualization of an antidepressant analog using surfaceenhanced Raman scattering in the brain. JCI Insight 5, e133348 (2020).

137 Tobita, T; Kiyozumi, D; Muto, M; Noda, T; Ikawa, M. Lvrn expression is not critical for mouse placentation. Journal of Reproduction and Development 65, 239-244 (2019).

138 Tsuda, T; Nishide, M; Maeda, Y; Hayama, Y; Koyama, S; Nojima, S; Takamatsu, H; Okuzaki, D; Morita, T; Nakatani, T; Kato, Y; Nakanishi, Y; Futami, Y; Suga, Y; Naito, Y; Konaka, H; Satoh, S; Naito, M; Izumi, M; Obata, S; Nakatani, A; Shikina, T; Takeda, K; Hayama, M; Inohara, H; Kumanogoh, A. Pathological and therapeutic implications of eosinophil-derived semaphorin 4D in eosinophilic chronic rhinosinusitis. Journal of Allergy and Clinical Immunology 145, 843 (2020).

139 Tsuji, T; Cheng, J; Tatematsu, T; Ebata, A; Kamikawa, H; Fujita, A; Gyobu, S; Segawa, K; Arai, H; Taguchi, T; Nagata, S; Fujimoto, T. Predominant localization of phosphatidylserine at the cytoplasmic leaflet of the ER, and its TMEM16K-dependent redistribution. Proceedings of the National Academy of Sciences USA 116, 13368-13373 (2019).

140 Tsujioka, H; Yamashita, T. Comparison of gene expression profile of the spinal cord of sprouting-capable neonatal and sprouting-incapable adult mice. Bmc Genomics 20, 619 (2019).

141 Usui, R; Yabe, D; Fauzi, M; Goto, H; Botagarova, A; Tokumoto, S; Tatsuoka, H; Tahara, Y; Kobayashi, S; Manabe, T; Baba, Y; Kurosaki, T; Herrera, PL; Nagashima, K; Ogura, M; Nagashima, K; Inagaki, N. GPR40 activation initiates store-operated Ca^{2+} entry and potentiates insulin secretion via the IP3R1/STIM1/Orai1 pathway in pancreatic beta-cells. Scientific Reports 9, 15562 (2019).

142 van der Peet, PL; Gunawan, C; Watanabe, M; Yamasaki, S; Williams, SJ. Synthetic beta-1,2-mannosyloxymannitol glycolipid from the fungus Malassezia pachydermatis signals through human mincle. Journal of Organic Chemistry 84, 6788-6797 (2019).

143 Wagner, M; Ealey, KN; Tetsu, H; Kiniwa, T; Motomura, Y; Moro, K; Koyasu, S. Tumor-derived lactic acid contributes to the paucity of intratumoral ILC2s. Cell Reports 30, 2743 (2020).

144 Wang, YC; Maeda, Y; Liu, YS; Takada, Y; Ninomiya, A; Hirata, T; Fujita, M; Murakami, Y; Kinoshita, T. Cross-talks of glycosylphosphatidylinositol biosynthesis with glycosphingolipid biosynthesis and ER-associated degradation. Nature Communications 11, 860 (2020).

145 Weber, M; Yamada, N; Tian, X; Bull, SD; Minoshima, M; Kikuchi, K; Mackenzie, AB; James, TD. Sensing peroxynitrite in different organelles of murine RAW264.7 macrophages with coumarin-based fluorescent probes. Frontiers in Chemistry 8, 39 (2020).

146 Xu, ZC; Li, SL; Rozewicki, J; Yamashita, K; Teraguchi, S; Inoue, T; Shinnakasu, R; Leach, S; Kurosaki, T; Standley, DM. Functional clustering of B cell receptors using sequence and structural features. Molecular Systems Design & Engineering 4, 769-778 (2019).

147 Yamada, M; Fujita, Y; Hayano, Y; Hayakawa, H; Baba, K; Mochizuki, H; Yamashita, T. Increased expression of fibronectin leucine-rich transmembrane protein 3 in the dorsal root ganglion induces neuropathic pain in rats. Journal of Neuroscience 39, 7615-7627 (2019).

148 Yamaguchi, T; Teraguchi, S; Furusawa, C; Machiyama, H; Watanabe, TM; Fujita, H; Sakaguchi, S; Yanagida, T. Theoretical modeling reveals that regulatory T cells increase T-cell interaction with antigen-presenting cells for stable immune tolerance. International Immunology 31, 743-753 (2019).

149 Yamamoto, K; Sakaue, S; Matsuda, K; Murakami, Y; Kamatani, Y; Ozono, K; Momozawa, Y; Okada, Y. Genetic and phenotypic landscape of the mitochondrial genome in the Japanese population. Communications Biology 3, 104 (2020).

150 Yamamoto, T; Kanuma, T; Takahama, S; Okamura, T; Moriishi, E; Ishii, KJ; Terahara, K; Yasutomi, Y. STING agonists activate latently infected cells and enhance SIV-specific responses ex vivo in naturally SIV controlled cynomolgus macaques. Scientific Reports 9, 5917 (2019).

151 Yamashita, Y; Suzuki, C; Uchiyama, Y; Nagata, S. Infertility caused by inefficient apoptotic germ cell clearance in Xkr8-deficient male mice. Molecular and Cellular Biology 40, e00402-19 (2020).

152 Yorifuji, H; Arase, N; Kohyama, M; Hirano, T; Suenaga, T; Kumanogoh, A; Arase, H. Transport of cellular misfolded proteins to the cell surface by HLA-B27 free heavy chain. Biochemical and Biophysical Research Communications 511, 862-868 (2019).

153 Zhu, C; Luo, X; Espulgar, WV; Koyama, S; Kumanogoh, A; Saito, M; Takamatsu, H; Tamiya, E. Real-time monitoring and detection of single-cell level cytokine secretion using LSPR technology. Micromachines 11, 107 (2020).

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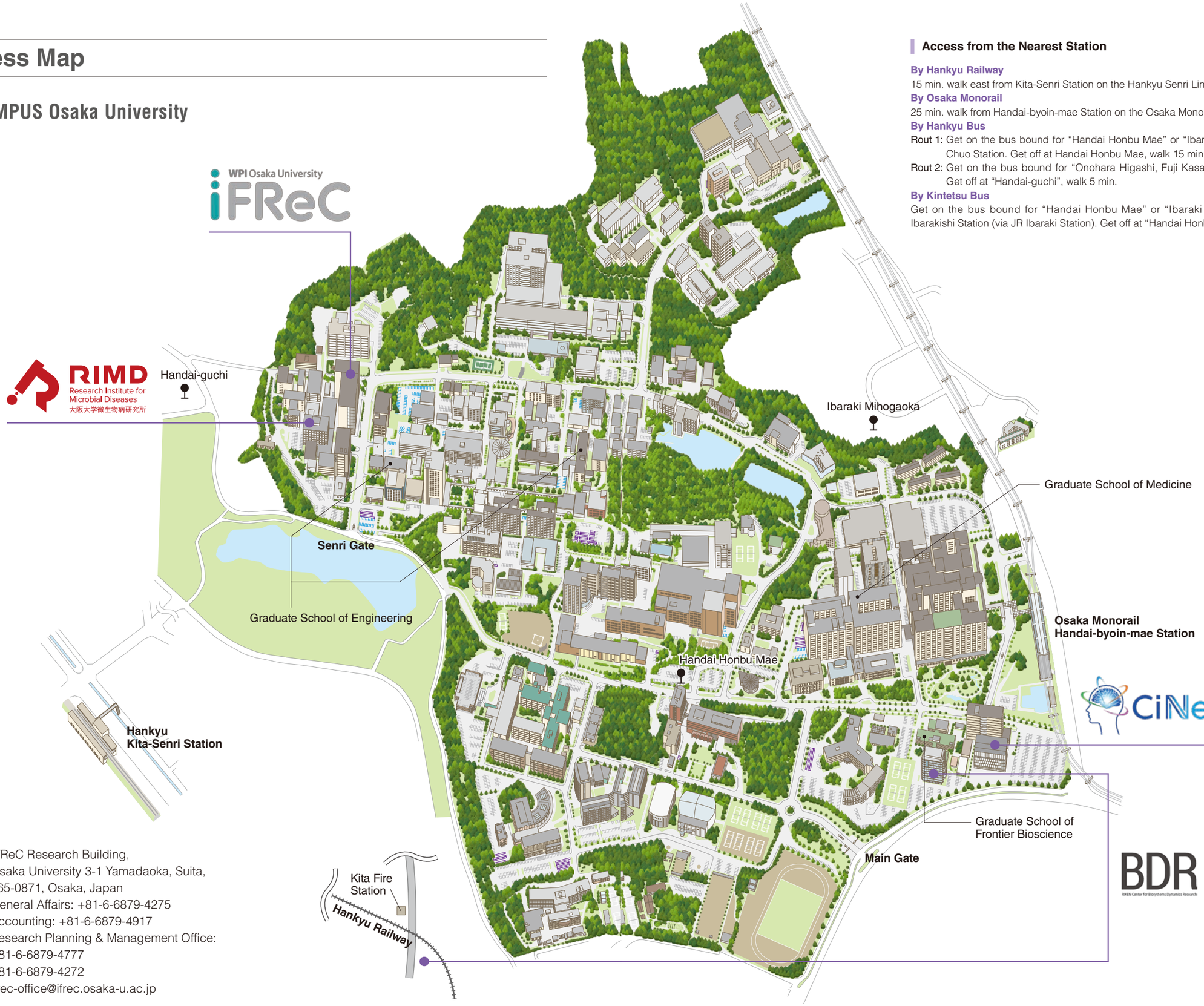

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