WPI Immunology Frontier Research Center 2020-2021

World Premier
International
Research Center

Osaka University

Immunology Frontier Research Center

Annual Report
of IFReC
2020-2021

Osaka University

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Research Center Initiative

WPI Osaka University
iFReC

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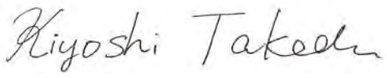
Message from the Director



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 2020-2021.

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

I assumed the directorship of IFReC from Prof. Shizuo Akira in 2019. Soon after that time from around the end of 2019, an infectious disease (COVID-19) caused by a novel coronavirus called SARS-CoV-2 has been spreading throughout the world and has developed into a pandemic.

COVID-19 still runs rampant across the globe, and as of March 2021, travel between countries remains severely restricted. Although the traditional role of scientists involves directly discussing their findings across national borders, the COVID-19 crisis has forced the cancellation or postponement of many academic events, including international symposia and seminars with invited guest speakers. Similarly, many outreach activities such as science cafés or visits by high school students have also been put on hold.

However, using remote conferencing systems, we have gradually started to hold alternative events. In March 2021, we started an online seminar called the “Immuno-Seminar Series” featuring internationally renowned researchers. Of course, we encountered some constraints, such as time zone differences, but we will continue our international collaborations as a WPI research center without interruption. Looking ahead toward the day when the COVID-19 situation is resolved, we are already planning to resume holding international symposia and our annual “Winter School,” an international educational program that has continued for ten years.

As a research center for immunology receiving government support, all members of IFReC recognize the necessity of conducting basic research as our contribution to society to overcome this fundamental problem of COVID-19. Therefore, we launched the “Team Osaka University Research on COVID-19” consortium through which we are promoting a team-based approach to research that surpasses the framework of individual research.

In FY2020, we welcomed two principal investigators. One is Dr. Naoki Hosen, who is a world-renowned researcher in the field of cancer immunology, and the other is Dr. Yasutaka Okabe who is joining our center under the Young Lead Researcher program.

Even in these difficult times, IFReC will continue to contribute to the further advancement of science through research and education.

Efforts of Osaka University and IFReC in the COVID-19 Epidemic

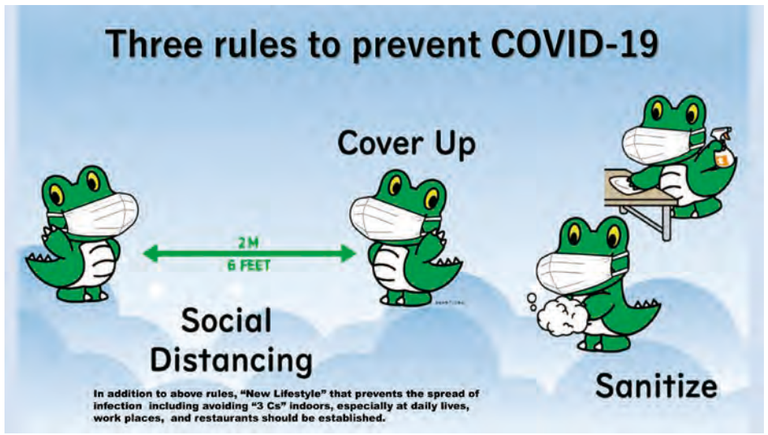
Jun SAKANOUE

Osaka University's response to COVID-19

The number of people infected with the new coronavirus (SARS-CoV-2) has been increasing worldwide since the end of 2019 and has reached 120 million (44,000 in Japan) while the number of deaths has exceeded 2.6 million (8,300 in Japan) (WHO as of March 10, 2021).

Osaka University has planned its response to the "New Era of COVID-19" by establishing the "Osaka University Coronavirus Infectious Disease Task Force" in March 2020, which consists of the university president, department directors, and infectious disease experts. The task force established "Osaka University's COVID-19 Response Levels" and prepared guidelines for infection prevention measures. Telecommuting was introduced for the first time and the administrative office in IFReC has been promoting it more actively than what the university's response levels specify. However, for now, users must provide their own internet environment for telecommuting and telecommuting is expected decline once the coronavirus crisis passes. For students of the university (including international students), tuition reduction and exemption and extension of payment deadlines were offered. The university also provides an environment for online classes and lends out wireless routers. For telecommuters, a consultation service was set up at the Health and Counseling Center to provide them with support. In addition to the above, IFReC also recommends and sponsors twice-monthly PCR tests for the faculty and staff. Fortunately, there have been no COVID-19 cases so far among IFReC members and their close relatives.

Despite the preventive measures taken by Osaka University, the cumulative total number of infected people is 110 (as of March 10, 2021), which is about 0.35% of the total number of its members and does not differ much from the national percentage as a whole. This number includes COVID-19 patients at the university hospital and also the clusters of infections that have occurred among some university students (Osaka University website).



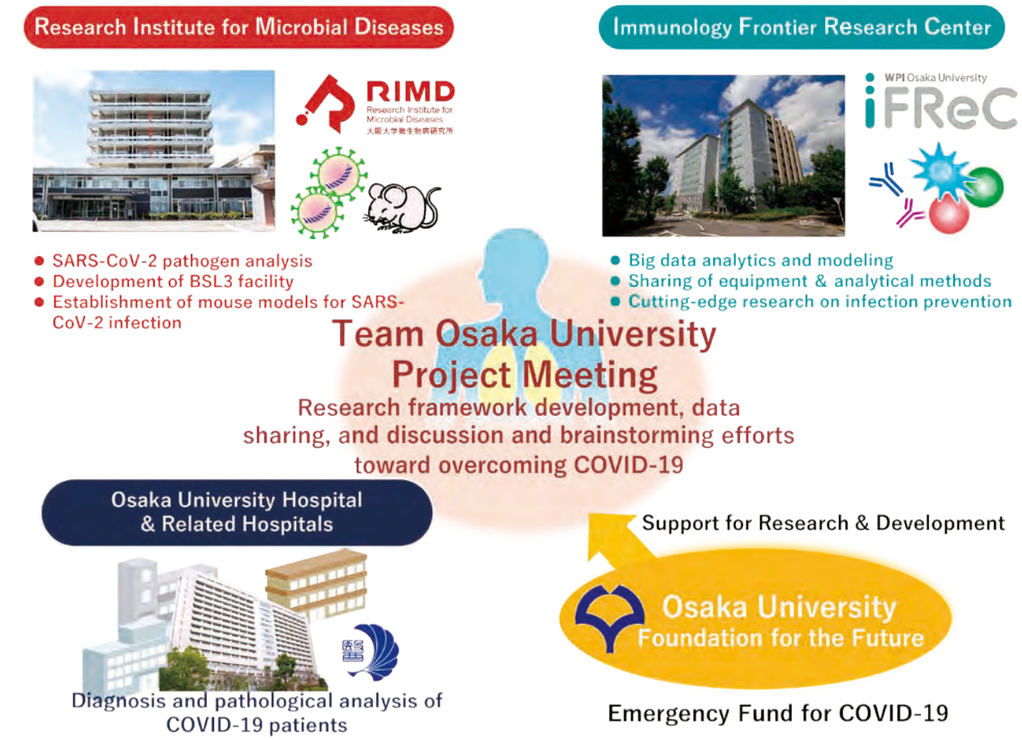
Dr. Wani, a mascot of Osaka University appeals the university's policy.

COVID-19 related research at Osaka University

Osaka University is one of the centers of excellence in the field of infectious diseases and immunology. However, its COVID-19 related research has been unable to produce substantial results and only a few papers on basic research in this field appear on the Osaka University's website after 2020. One of them is the finding by the Laboratory of Immune Regulation at IFReC, which reveals cytokine IL-6 increases in the blood during early COVID-19, and promotes thrombus formation via PAI (plasminogen activator inhibitor) -1, a promoter of blood clotting from blood vessels (Kang et al. PNAS 2020). Osaka University supports the "Sustainable Development Goals (SDGs)" promoted by the United Nations. The aforementioned PNAS paper and another one by the Laboratory of Lymphocyte Differentiation that revealed the differentiation mechanism of memory B cells (Inoue et al. JEM 2020) were selected as good examples for one of the SDGs, "Health for All" (cf. "Selected Articles").

Research Promotion in the New Era of Corona by the "Center for Infectious Disease Education and Research"

Some departments at Osaka University, including IFReC, the Graduate School of Medicine, the University Hospital, the Graduate School of Science, the Research Institute for Microbial Diseases, and the Institute of Industrial Science, are collaborating with organizations outside the university to promote comprehensive research directly related to emerging infectious diseases, including pathological analysis, the development of drugs, preventive vaccines and treatment strategies, and the analysis of infection spread. Furthermore, as a ten-year plan, the university decided to establish a comprehensive research and development center that will work on basic research, clinical research, clinical trials, and practical application, by combining the university's outstanding knowledge in protein science, pathogenic microbiology, genome science, nanotechnology, and information science. Fortunately, the research organizations including IFReC are concentrated on the Suita Campus, which is an ideal environment for a center for the comprehensive research and development on infectious diseases. IFReC is also a member of this "Center for Infectious Disease Education and Research" and is expected to play a leadership role in the center.



Establishing Center for Infectious Disease Education and Research (CIDER)

Organization

Organization Chart

As of March, 2021

Osaka University
International Advanced Research Institute (IARI)

MEXT
WPI Initiative Program Committee
JSPS

WPI Osaka University iFReC

International Scientific Advisory Board

Research Groups
- **Immunology Group**
- **Imaging Group**
- **Informatics Group**
- **Unit for Combined Research Fields**

Director

Management Committee

Board of Representatives

Deputy Directors

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 Pharma)
- Immune-therapeutic Drug Discovery (Otsuka Pharma)
- 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
- University College London, UK
- ImmunoSensation^2, Clusters of Excellence, the Rheinische Friedrich-Wilhelms-University of Bonn, Germany

Administrative Office

General Affairs Section
- Employment and acceptance procedures 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 October, 2020

Akira UKAWA	Director, Center for WPI Center, 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)
Maki KAWAI	Director General, Institute for Molecular Science (IMS)/National Institutes of Natural Sciences (NINS), Japan
Klaus von KLITZING	Director, Max Planck Institute for Solid State Research, Germany Nobel laureate in Physics (1985)
Kiyoshi KUROKAWA	Professor Emeritus, National Graduate Institute for Policy Studies Former president, Science Council of Japan
Chuan Poh LIM	Chairman, Singapore Food Agency (SFA)
Hiroshi MATSUMOTO	President, RIKEN, Japan
Ryozo NAGAI	President, Jichi Medical University, Japan
Ryoji NOYORI (Chair)	Director-General of Center for Research and Development Strategy (CRDS)/Japan Science and Technology Agency (JST) 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, Institute of Research into the Fundamental Laws of the Universe (IRFU/CEA), France

WPI Academy

In 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 October, 2020

Toshio KUROKI	Special Advisor, Research Center for Science Systems, JSPS, Japan

Program Officer for IFReC

Takehiko SASAZUKI	University Professor, Institute for Advanced Study, Kyushu University, Japan

International Scientific Advisory Board for IFReC

As of March, 2021

Lewis LANIER	University of California, San Francisco	Immunology
Anne O'GARRA	The Francis Crick Institute	Immunology
Jeffrey RAVETCH	Rockefeller University	Immunology
Art WEISS	University of California, San Francisco/Howard Hughes Medical Institute	Immunology
Joachim SCHULTZ	DZNE/LIMES Institute, University of Bonn	Imaging

Laboratories

As of March, 2021

Laboratories of IFReC

Host Defense

Shizuo Akira

#innateimmunity
#pathogenrecognition
#macrophage #regnase

Immunoglycobiology

Taroh Kinoshita
Yoshiko Murakami

#gpi-anchor
#paroxysmalnocturnalhemoglobinuria

Immunopathology

Atsushi Kumanogoh

#immunesemaphorin
#autoimmunediseases
#t-cellactivation

Immunochemistry

Hisashi Arase

#mhc #neo-self
#misfoldedprotein
#malaria #covid-19

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 & Immunology

Shigekazu Nagata

#macrophage
#celldeathsignal
#apoptosis

Molecular Neuroscience

Toshihide Yamashita

#centralnervoussystem
#encephalomyelitis

Molecular Immunology

Sho Yamasaki

#lectin
#novelimmunereceptor

Stem Cell Biology and Developmental Immunology

Takashi Nagasawa

#carcell #stemcell
#niche

Aging Biology

Eiji Hara

#aging #sasp
#cancer

Oncogene Research

Masato Okada

#mtor #src

Signal Transduction

Nobuyuki Takakura

#bloodvessels #stemcell
#cancer

Cutaneous Immunology

Manabu Fujimoto

#intractableskindiseases
#allergy

Innate Immune Systems

Kazuyo Moro

#ilc2
#autoimmunediseases

Human Immunology (Single Cell Immunology)

James Badger Wing

#humandisease #singlecell
#treg

Human Immunology (Single Cell Genomics)

Daisuke Okuzaki

#humandisease #singlecell
#genomics

Immune Homeostasis

Yasutaka Okabe

#macrophage
#immunehomeostasis

Cellular Immunotherapy

Naoki Hosen

#cancer
#cartcell

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

Chemical Imaging Techniques

Kazuya Kikuchi

#chemicalbiology
#fluorescentprobe

Immune Response Dynamics

Kazuhiro Suzuki

#adrenergicrceptor
#lymphocytetrafficking
#commd38

Biophotonics

Nicholas Isaac Smith

#labelfree #ramanscattering
#intracellimaging

Systems Immunology

Daron Standley

#immunerepertoire
#receptormodeling

Statistical Immunology

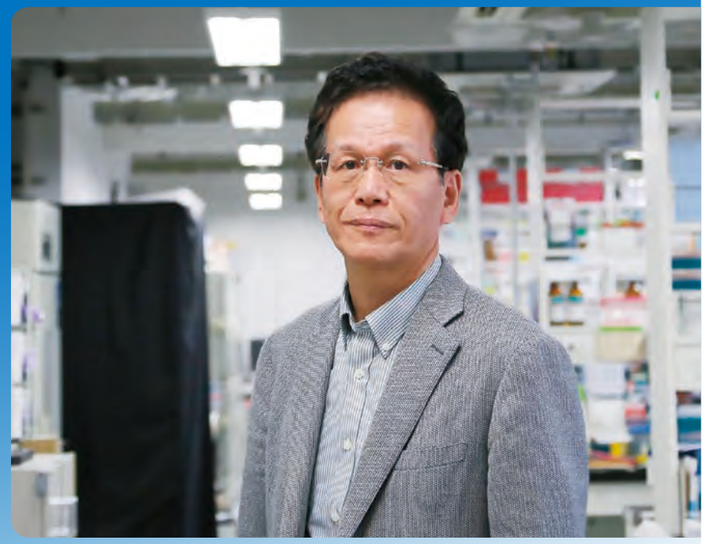
Yukinori Okada

#statisticalgenetics #bigdata
#diseaseriskgenes

Host Defense

Shizuo Akira, MD/PhD

Professor	Shizuo Akira
Associate Professor	Kazuhiko Maeda Takashi Satoh (-July, 2020)
Assistant Professor	Hiroki Tanaka Kanako Kuniyoshi
Postdoctoral Fellows	2
Research Assistants	6
Visiting Scientists	5
Support Staff	5



Our research focuses on the innate immune system, which is an evolutionally conserved host defense mechanism against various pathogens. Innate immune responses are initiated by pattern recognition receptors, 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.

Molecular mechanism of endoribonuclease Regnase-1 in inflammation

Regnase-1 is a member of CCCH-type zinc finger proteins and *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 *IL6* and *IL12 p40* in macrophages and *c-Rel*, *Ox40*, and *IL2* in $CD4^+$ T cells. The Regnase-1 protein is cleaved by MALT1 protease activity 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, and through the use of tissue-specific *Regnase-1*-deficient mice and mutant mice, we promote the understanding of the precious roles of Regnase-1 in immune and non-immune cells.

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 the 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*$^{\Delta IEC}$ mice, which are *Regnase-1*-deficient in IECs, 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.

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

Macrophages consist of at least two subsets. 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 the 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 treatments. The development of fibrosis is associated with activation of monocytes and macrophages. We identified a new macrophage subset where 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 increased in the fibrotic phase, *RBM7*-deletion in nonhematopoietic cells suppresses fibrosis, and 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. Therefore, we believe the inhibition of RBM7 could lead to an effective treatment of fibrosis in patients.

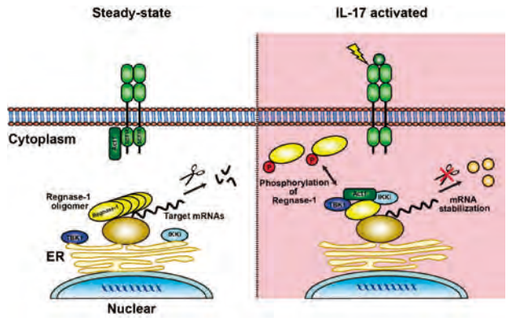


Figure 1. IL-17 induces Regnase-1 phosphorylation in an Act-1/TBK1/IKKi–dependent manner. Phosphorylation of Regnase-1 is released from the endoplasmic reticulum into the cytosols, resulting in loss of the mRNA degradation function.

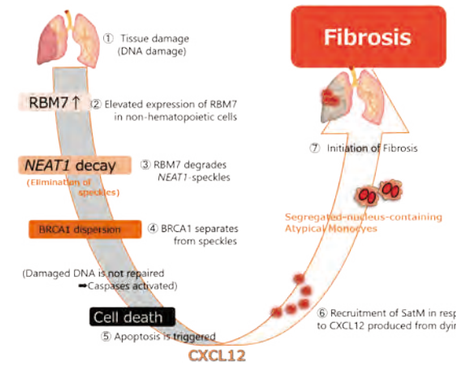


Figure 2. Dysregulated expression of the nuclear exosome targeting complex component Rbm7 in non-hematopoietic cells licenses the development of fibrosis.

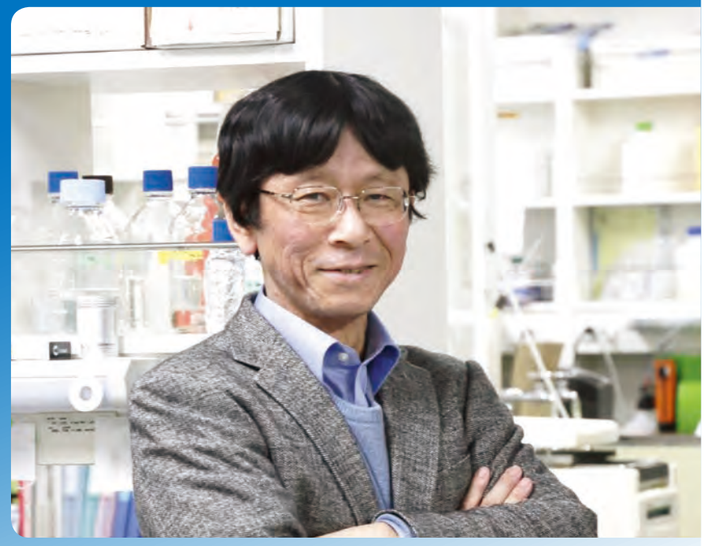
Recent Publications

- Akira S and Maeda K. Control of RNA stability in immunity. Annu. Rev. Immunol. 39: 481-509 (2021).
- Fukushima K, Satoh T, Sugihara F, Sato Y, Okamoto T, Mitsui Y, Yoshio S, Li S, 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 non-hematopoietic cells licenses the development of fibrosis. Immunity 52: 542-556 (2020).
- Tanaka H, Arima Y, Kamimura D, Tanaka Y, Takahashi N, Uehata T, Maeda K, Satoh T, Murakami M, Akira S. Phosphorylation-dependent Regnase-1 release from endoplasmic reticulum is critical in IL-17 response. J. Exp. Med. 216: 1431-1449 (2019).
- Maeda K, Caldez MJ, Akira S. Innate immunity in allergy. Allergy 74: 1660-1674 (2019).
- Nagahama Y, Shimoda M, Mao G, Singh SK, Kozakai Y, Sun X, Motooka D, Nakamura S, Tanaka H, Satoh T, Maeda K, Akira S. Regnase-1 controls colon epithelial regeneration via regulation of mTOR and purine metabolism. Proc. Natl. Acad. Sci. USA. 115: 11036-11041 (2018).

Immunoglycobiology

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

Professor	Taroh Kinoshita Yoshiko Murakami
Postdoctoral Fellows	1
Research Assistants	1
Support Staff	3



PNH is a hematopoietic stem cell (HSC) disorder characterized by complement-mediated hemolysis and thrombosis, and bone marrow failure. In patients with PNH, the affected blood cells are defective in glycosylphosphatidylinositol-anchored proteins (GPI-APs), including the complement regulatory proteins CD55 and CD59. Deficiency of these complement regulatory proteins renders erythrocytes sensitive to complement-mediated destruction and plays a fundamental role in thrombosis. The classic form of PNH (PIGA-PNH) is caused by a somatic mutation of the *PIGA* gene in the HSC and subsequent clonal expansion, in which bone marrow failure is implicated. *PIGA* is involved in the first step of GPI biosynthesis and is the only X-linked gene among 27 genes involved in GPI-AP biosynthesis. A somatic mutation in one allele of *PIGA* is sufficient to generate GPI-AP deficient cells in females as well as males. Recently, in collaboration with Dr. Muus, Dr. Langemeijer and their colleagues in the Netherlands, we reported on PNH caused by a homozygous mutation of the *PIGB* gene in chromosome 15q (PIGB-PNH) (Langemeijer S et al, Blood Adv 2020). We demonstrated that the patient is heterozygous for a loss-of-function variant of *PIGB* and a copy-number neutral loss-of-heterozygosity (CN-LOH) event in an HSC caused homozygosity of the *PIGB* mutation. Almost all blood cells including erythrocytes and granulocytes were GPI-AP deficient, indicating predominant hematopoiesis by the *PIGB* mutant clone. We also found that a 70-kbp deletion previously implicated in clonal hematopoiesis was present in the same chromosome 15q as the *PIGB* mutation, perhaps accounting for dominance of the *PIGB* mutant clone. The patient's mother who is heterozygous for the same *PIGB* mutation and 70-kbp deletion had at least four PNH clones. All of them had CN-LOH of various lengths spanning *PIGB* and a region of the 70-kbp deletion, therefore, characterizing familial PNH. Like patients with PIGT-PNH, the PIGB-PNH patient had recurrent autoinflammatory symptoms such as joint pains and urticaria. We suspect accumulated GPI biosynthetic intermediate plays a role in the inflammatory symptoms.

Studies on inherited GPI deficiencies (IGD)

We have been contributing to the global collaboration studies led by Dr. Campeau in Canada aiming to discover and characterize the first cases of IGD caused by mutations in genes, for which association with IGD has not been established. This year, we published a paper on PIGF-IGD and PIGK-IGD (Nguyen et al. Am J Hum Genet, 2020; Salian et al. Hum Genet, 2021).

Studies on GPI biosynthesis.

Side-chains of some GPI-APs, such as prion proteins, are modified by sialic acid. How sialic acid modification of GPI side-chain occurs has been unknown. In collaboration with Dr. Kobayashi of Hokkaido University and Dr. Nishikaze of the Shimadzu Corporation, we analyzed a human prion protein and found that its GPI side-chain is modified by α2,3-linked N-acetylneuraminic acid (Kobayashi et al. J Biol Chem. 2020). This will allow us to identify an enzyme that mediates the sialylation.

Mechanisms regulating the levels of GPI biosynthesis have been unclear. Using a convenient method to determine cellular GPI levels, we found that GPI biosynthesis is under the control of the ER-associated degradation (ERAD) system. Particularly, cells defective in Hrd1 (a major E3 in the ERAD system), UBE2J1 and UBE2G2 (E2 enzymes working with Hrd1), and Dahlin2 (a component of Hrd1 complex) have high GPI biosynthesis levels (6- to 7-fold increase) (Wang et al. Nat Commun. 2020). We continue working to understand the molecular mechanisms of the upregulation of GPI biosynthesis.

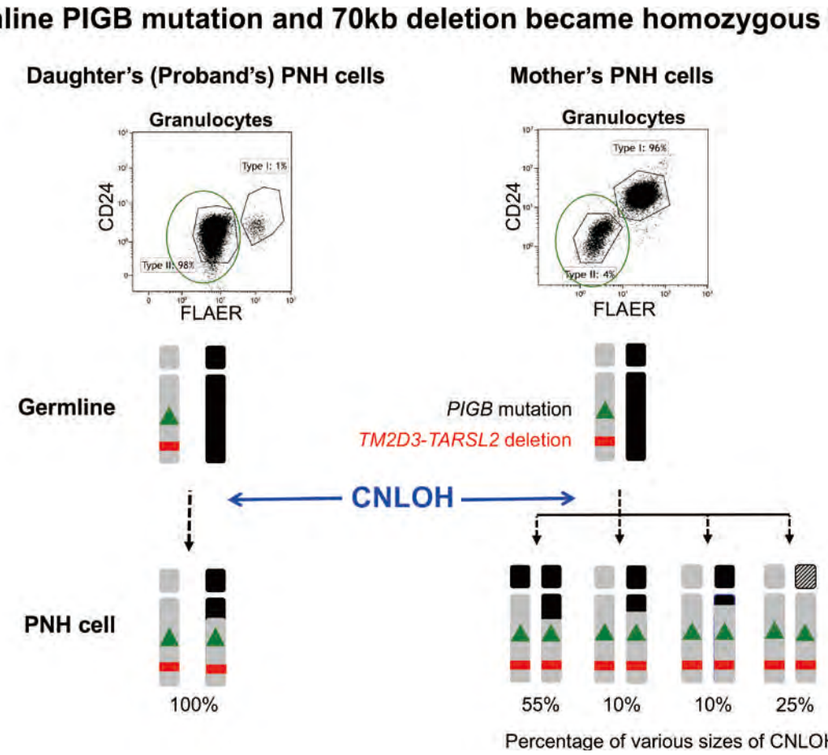


Figure 1. Germline PIGB mutation and 70kb deletion became homozygous by CNLOH.
Upper: Proband's and mother's blood granulocytes contained PNH cells.
Lower: One and four PNH clones with CN-LOH regions of different lengths were found in proband and mother, respectively. (Adopted from Langemeijer, S. et al, Blood Adv., 2020, 4(22): 5755-5761)

Recent Publications

- Langemeijer S, Schaap C, Preijers F, Jansen JH, Blijlevens N, Inoue N, Muus P, Kinoshita T and Murakami Y. Paroxysmal nocturnal hemoglobinuria caused by CN-LOH of constitutional *PIGB* mutation and 70-kb microdeletion on 15q. Blood Adv., 4: 5755-5761 (2020).
- Wang Y, Maeda Y, Liu YS, Takada Y, Ninomiya A, Hirata T, Fujita M, Murakami M & 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, et al. 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 & Kinoshita T. Free, unlinked glycosylphosphatidylinositols on mammalian cell surfaces revisited. J. Biol. Chem. 294: 5038-5049 (2019).
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Immunopathology

Atsushi Kumanogoh, MD/PhD

Professor	Atsushi Kumanogoh
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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 to diagnosis/therapy for human immunological disorders, such as autoimmunity, allergy, immune deficiency, cancer/metastasis, and neurodegenerative diseases. We recently focus on the cross-talk among neuronal, immune and metabolic systems since some of the semaphorins' expression are regulated by a metabolic sensor, mTOR, in which we investigated the biological and pathological significance of Lamtor1/p18, an amino acid sensor localized at the lysosome.

Lysosomes are involved in nutrient sensing via the mechanistic target of rapamycin complex 1 (mTORC1). mTORC1 is tethered to lysosomes by the regulator complex, a heteropentamer in which Lamtor1 wraps around Lamtor. Although the Ragulator complex is required for cell migration, the mechanisms by which it participates in cell motility remain unknown. Here, we show that lysosomes move to the uropod in motile cells, providing a platform where Lamtor1 interacts with the myosin phosphatase Rho-interacting protein (MPRIP) independently of mTORC1 and interferes with the interaction between MPRIP and MYPT1, a subunit of myosin light chain phosphatase (MLCP), thereby increasing myosin II–mediated actomyosin contraction. Additionally, formation of the complete Ragulator complex is required for leukocyte migration and pathophysiological immune responses. Together, our findings demonstrate that the lysosomal Ragulator complex plays an essential role in leukocyte migration by activating myosin II through interacting with MPRIP.

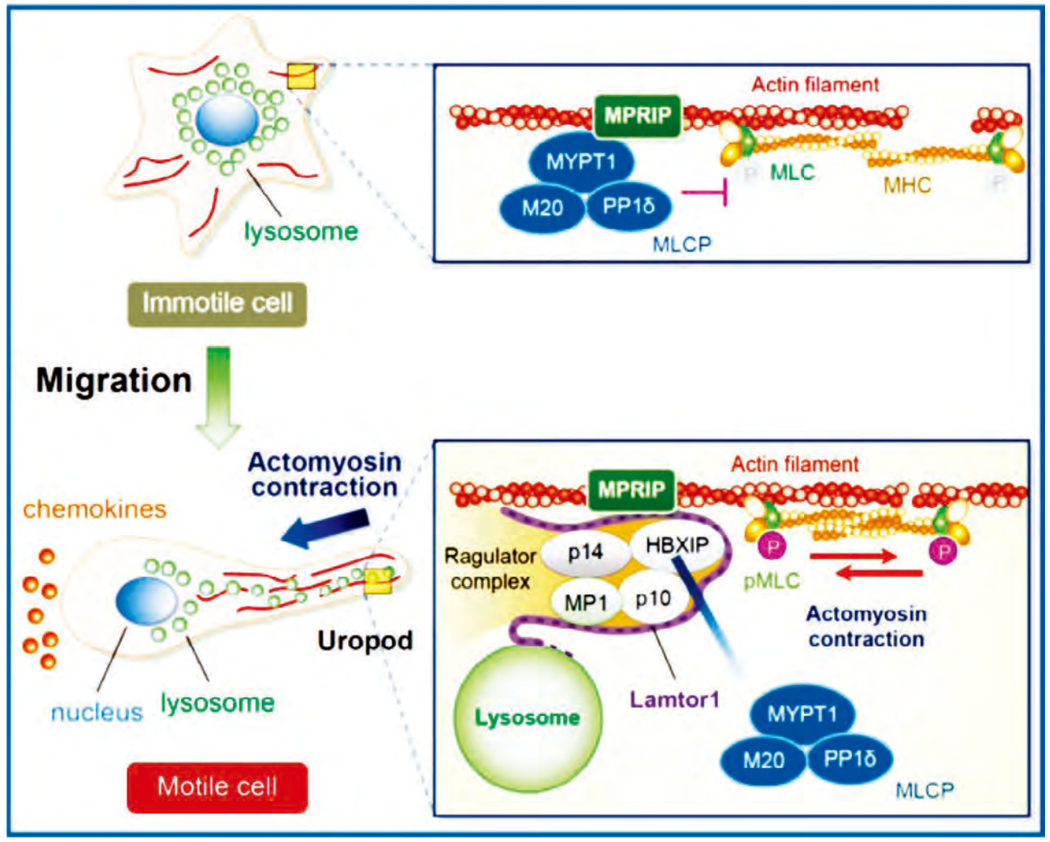

Figure. Schematic of the mechanism of regulation of myosin II activity by the Ragulator complex
In immotile cells (upper), the Ragulator complex localized to lysosomes is preferentially distributed in the perinuclear region (left), and MPRIP anchors MLCP on myosin–actin bundles by binding MYPT1, a subunit of MLCP, resulting in suppression of MLC phosphorylation (right). In motile cells exposed to chemokines (lower), the lysosomes bearing the Ragulator complex moves to the uropod (left), where the Ragulator complex interacts with MPRIP. This interaction interferes with the interaction between MPRIP and MYPT1 and decreases MLCP activity, thereby increasing MLC phosphorylation. Consequently, cell motility is facilitated (right). MLC, myosin regulatory light chain; MLCP, myosin light chain phosphatase; MPRIP, myosin phosphatase Rho-interaction protein; MYPT1, myosin phosphatase-targeting subunit 1; PP1δ, a catalytic subunit of the type I protein serine/threonine phosphatase family; M20, 20-kDa small subunit; p14, Lamtor2; MP1, Lamtor3; p10, Lamtor4; HBXIP, Lamtor5.

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Immunochemistry

Hisashi Arase, MD/PhD

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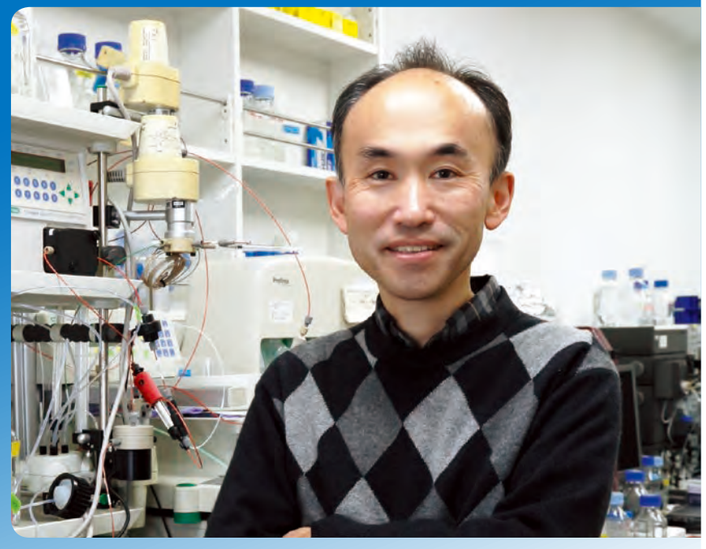
Research Assistants	5
Visiting Scientists	1
Support Staff	5



A) Host pathogen interaction mediated by paired receptor

Paired receptors are composed of activating and inhibitory receptors. We found that PILRα, one of paired receptors, plays an important role in the regulation of immune response (Nat. Immunol. 2012; Int. Immunol. 2015; Eur. J. Immunol. 2016) as well as HSV-1 infection (Cell 2008; J. Virol. 2009). Similarly, Siglec-4 (myelin-associated glycoprotein, MAG), one of paired receptors, is involved in VZV infection (PNAS 2010; J. Biol. Chem. 2015). These findings suggested that paired receptors are involved in immune regulation as well as 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 (Nature Microbiology 2016). On the other hand, we found that RIFINs, products of a multigene family of Plasmodium falciparum, are similar to MHC class I molecules, bind to inhibitory LILRB1 and LILRB2, and downregulate immune response. These findings suggest that binding of RIFIN to LILRB1 plays an important role in immune evasion by Plasmodium falciparum (Figure 1. Nature 2017; Nature 2020; BBRC 2021).

B) 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 (Int. Immunol. 2013). Furthermore, we found that misfolded proteins complexed with MHC class II molecules are targets for autoantibodies in autoimmune diseases such as rheumatoid arthritis, antiphospholipid syndrome, and ANCA-associated vasculitis (PNAS 2014; Blood. 2015; Int, Immunol. 2019; Br. J. Dermatol. 2017; Arthritis. Rheumatol. 2017; Arthritis. Rheumatol. 2021). 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 are involved in the pathogenicity of autoimmune diseases (Figure 2).

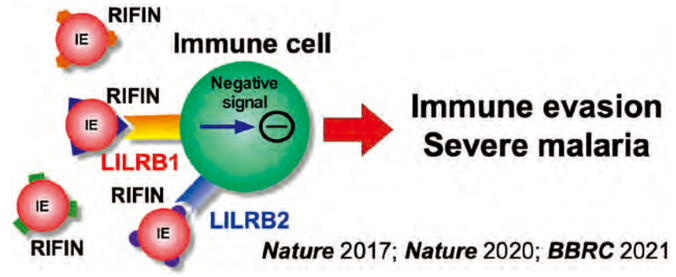


Figure 1. Immune evasion of Plasmodium falciparum by RIFIN via inhibitory receptors
Plasmodium falciparum evades host immune response by engaging inhibitory receptors using RIFINs (Nature 2017; Nature 2020; BBRC 2021).

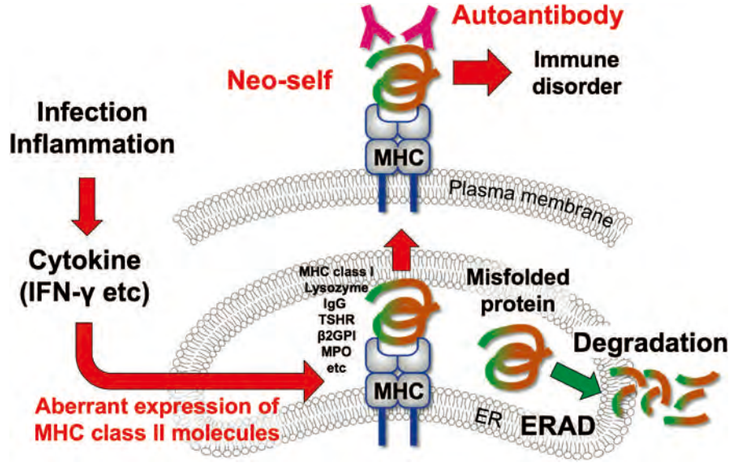


Figure 2. Misfolded proteins transported to the cell surface by MHC class II molecules are targets for autoantibodies.
Cellular misfolded proteins are generally degraded in the cells and are not transported to outside the cells. Therefore, misfolded proteins transported to the cell surface by MHC class II molecules may be recognized as 'neo-self' antigens by the immune system, which might initiate aberrant immune responses to the self-antigens (Int. Immunol. 2013; PNAS 2014; Blood 2015, Br. J. Dermatol. 2017; Arthritis Rheumatol. 2017, Arthritis Rheumatol. 2020).

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- Sakoguchi A, Saito F, Hirayasu K, Shida K, Matsuoka S, Itagaki S, Nakai W, Kohyama M, Suenaga T, Iwanaga S, Horii T, Arase H. Plasmodium falciparum RIFIN is a novel ligand for inhibitory immune receptor LILRB2. Biochem Biophys Res Commun. 2021 548: 167-173 (2021).
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- Jin H, Arase N, Hirayasu K, Kohyama M, Suenaga T, Saito F, et al. 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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Support Staff	2

Role of endothelial homeostasis in cytokine storm and related diseases

Cytokine release syndrome (CRS) is a life-threatening complication induced by systemic inflammatory responses to infections, including bacteria and SARS-CoV-2, and chimeric antigen receptor (CAR) T-cell therapy. There are currently no immunotherapies with proven clinical efficacy and knowledge of the molecular details of CRS pathogenesis is limited. We found that interleukin-6 (IL-6) signaling induced pro-inflammatory cytokine production and activated the coagulation cascade during CRS. Patients diagnosed with CRS, including those with sepsis, acute respiratory distress syndrome, or burns, had common manifestations: strikingly elevated levels of four pro-inflammatory cytokines, IL-6, IL-8, MCP-1, and IL-10, and a coagulation cascade activator, plasminogen activator inhibitor-1 (PAI-1). Endothelial IL-6 trans-signaling formed an inflammation circuit for robust IL-6, IL-8, and MCP-1 production and promoted PAI-1 production, and blockade by a humanized monoclonal antibody, tocilizumab, blunted activation of endothelial cells *in vitro*. Severe SARS-CoV-2 patient plasma also exhibited increased levels of the four cytokines and PAI-1, which were restored by tocilizumab treatment. Thus, a unique pattern of immune dysregulation in CRS was characterized by excessive IL-6-mediated cytokines and PAI-1 production, associated with hyper-inflammation (Kang et al, PNAS, 2020 and Figure 1).

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

Lipopolysaccharide (LPS)–induced toll-like receptor 4 (TLR4) endocytosis has emerged as a key step for the production of interferon (IFN)-β, which activates the transcription of antiviral response genes through Janus kinase (JAK)-activated Tyr^{701} phosphorylation of signal transducer and activator 1 (STAT1) signaling. TLR4 endocytosis also promotes proinflammatory cytokines production, at least in part through mediating a late-phase activation of nuclear factor (NF)-κB. Our research showed that STAT1 serves as a proinflammatory effector downstream of TLR4 endocytosis independently of IFN-β and NF-κB signaling. In human macrophages, lipopolysaccharide LPS-bound TLR4 endocytosis activated noncanonical phosphorylation of STAT1 at Thr^{749}, which altered its DNA target motif. Thr^{749}–phosphorylated STAT1 promoted the expression of the gene encoding AT-rich interactive domain-containing protein 5A (ARID5A), which stabilizes interleukin 6 (*IL6)* mRNA. Moreover, Thr^{749}–phosphorylated STAT1 directly enhanced the transcription of the gene encoding *IL12B* (Metwally et.al., Sci Signal, 2020). In order to understand the biological significance of Thr^{749} (Thr^{748} in mice) phosphorylation of STAT1, we generated mice with a specific non-phospho-mimic (T748A) mutation in STAT1 (KI mice). Intriguingly, STAT1 KI mice showed enhanced survival against LPS-induced sepsis compared to WT littermates indicating a novel fundamental role of Thr^{748} phosphorylation of STAT1 in shaping the host immune response against sepsis. Currently, we are investigating the detailed mechanisms of how $Thr^{748/9}$ phosphorylation of STAT1 regulates the host immune response in the biological contexts of infection and inflammation.

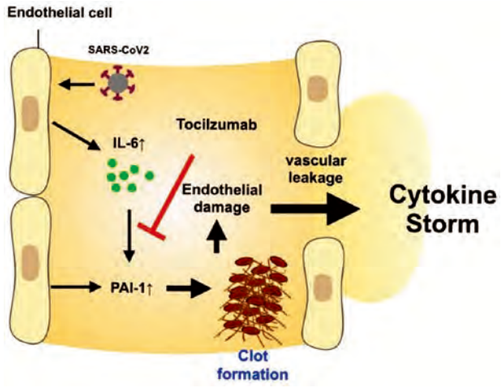

Figure 1. How Actemra© suppresses inflammation; IL-6 in blood promotes thrombus formation via PAI-1. By suppressing IL-6, Actemra prevents reduces the severity of the pneumonia caused by the cytokine storm.

Arid5a regulation of invasion and metastasis of breast cancer through a novel noncoding RNA under IL-6 signaling

Breast cancer (BC) metastasis is the major cause of mortality in women worldwide. Arid5a functions as an RNA-binding protein and a transcription factor, and plays crucial roles in the development of inflammatory and autoimmune diseases via the augmentation of IL-6 signaling. However, the role of Arid5a in hallmark of cancer such as invasion and metastasis remain elusive. We found IL-6-Arid5a axis transcriptionally upregulates a novel long noncoding RNA (lncRNA)-AU021063 which contributes to the promotion of invasion and metastasis of BC cells. Mechanistically, this lnc-AU021063 enhances the levels of a tribble homolog 3 (Trib3) protein in response to IL-6 by protecting the degradation of Trib3 and activates a MAPK pathway which result in exacerbation of invasion and metastasis of BC *in vitro* and *in vivo*. Taken together, our study provides evidence of the existence of an aberrant IL-6/Arid5a/lnc-AU021063 signaling that leads to BC invasion and metastasis through Trib3 induction, which could be novel therapeutic targets in BC malignancies.

Arid5a acts as a dual regulator in mesenchymal tumors to generate an immunosuppressive microenvironment

The acquisition of mesenchymal traits leads to immune evasion in various cancers, but the molecular mechanisms involved remain unclear. We identified Arid5a as a regulator of both immune-evasion and mesenchymal phenotypes of pancreatic ductal adenocarcinoma (PDAC) and colorectal cancer (CRC). Arid5a expression was increased in both cell lines. Arid5a expression was increased upon TGF-β-induced EMT. Arid5a promotes *in vivo* tumor growth in an extrinsic immune cell-dependent manner by disrupting the balance of anti-tumor and suppressive tumor-infiltrating leukocytes in the tumor microenvironment (TME). In Arid5a-deficient cell tumors, gMDSC and Treg infiltration was suppressed, but cytotoxic T-lymphocyte recruitment was promoted. Arid5a augments Ido1 and Ccl2 expression by the post-transcriptional stabilization of their mRNAs by binding to their 3′-UTRs. Our results indicate that Arid5a acts as a dual regulator in mesenchymal malignant tumors. Arid5a induces the suppressive effects of Ido1 on T-cells by reducing intratumoral Trp concentrations, and promotes Treg differentiation/activation. Arid5a induces Ccl2 expression in the TME and recruits immunosuppressive cells (Tregs and gMDSCs) to the TME. Our findings indicates that Arid5a is a promising and possible drug target for tumor immunotherapy.

Recent Publications

- Kishimoto T, Interleukin-6: From arthritis to CAR-T cell therapy and COVID-19. Int Immunol. dxab011. doi: 10.1093/intimm/dxab011 (2021).
- Kang S et al, IL-6 trans-signaling induces plasminogen activator inhibitor-1 from vascular endothelial cells in cytokine release syndrome. Proc Natl Acad Sci U S A. 117(36): 22351-22356 (2020).
- Kang S et al, Historical overview of the interleukin-6 family cytokine. J Exp Med. 217(5): e20190347 (2020).
- Metwally H et al. A Non-canonical STAT1 Phosphorylation Expands its Transcriptional Activity into Promoting LPS–induced IL-6 and IL-12p40 Expression. Sci. Signal. eaay0574 (2020).
- Parajuli G *et al.* Arid5a augments tryptophan metabolism and chemokine expression to promote the immune evasion of mesenchymal subtypes of pancreatic and colorectal cancer. Cancer Immunol Res 2021 (in press).

Immune Regulation

Hitoshi Kikutani, MD/PhD

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Assistant Professor	Chao-Yuan Tsai
Research Assistants	1
Support Staff	1

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

Our previous study has demonstrated that low-affinity anti-ssDNA B cells can acquire high-affinity to both ss- and dsDNA after only one or two mutations (Sakakibara S et al., 2017). 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 the SLE anti-DNA antibody clones (Figure 1). We confirmed that the G9gl BCR-expressing B cells react 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*, which caused their exclusion from GC reactions induced in several experimental settings. When the KI mice were immunized with bacterial DNA-conjugated methylated BSA, G9gl B cells were found in the memory B cell compartment, but not GC B. These G9gl-expressing IgG^+ memory B cells did not acquire somatic mutations to augment their anti-DNA reactivity. Therefore, clonal anergy tightly regulates low-affinity anti-DNA precursor B cells not to evolve into high-affinity anti-dsDNA antibody-producing cells.

The role of enhanced autophagy in memory B cell differentiation

Autophagy is required for a variety of cellular functions including the differentiation of pre-B and B-1a cells and survival of long-lived memory and plasma B cells. Since RUBCN (A RUN domain and cysteine-rich domain containing, Beclin 1-interacting protein), a negative regulator of canonical autophagy, was strongly induced in germinal center B (B_{GC}) cells and activated B cells, B cell-specific RUBCN knockout (RUBCN B-KO) mice were generated to study the function of RUBCN in B cells. The comparative study of RUBCN B-KO and wild-type (WT) mice revealed that, in addition to originally reported 130-kD RUBCN ($RUBCN^{130}$), B cells also expressed a 100-kD isoform ($RUBCN^{100}$) that appeared to be translated from an alternative start-codon of the same messenger RNA. Interestingly, B cells of RUBCN B-KO mice lacked the expression of $RUBCN^{130}$ as expected but still expressed $RUBCN^{100}$. Although selective $RUBCN^{130}$ deficiency did not affect the GC function, the number of antigen-specific $IgG1^+$ memory B (B_{mem}) cells was higher in RUBCN B-KO than WT mice. In contrast, the number of ASCs in the bone marrows of RUBCN B-KO mice was lower than that of WT mice. Such increase of B_{mem} cells also contribute to the increased recall responses in RUBCN B-KO mice. Additionally, the mTOR activity was down-regulated in the B_{GC} cells of RUBCN B-KO mice compared to that of WT mice. Although *atg7* deletion in B cells did not affect B_{GC} and B_{mem} cell differentiations, the increase of B_{mem} cells was cancelled in RUBCN/ATG7 B-DKO mice. These results indicate that selective $RUBCN^{130}$ deficiency enhances autophagy to promote B_{mem} cell differentiation but suppress plasma cell differentiation in GC. Our findings may not only explain the controversy of RUBCN function, particularly the inconsistency in phenotypes of two different RUBCN KO mice, but also posit that focusing vaccine design efforts on enhancing autophagy in B cells.

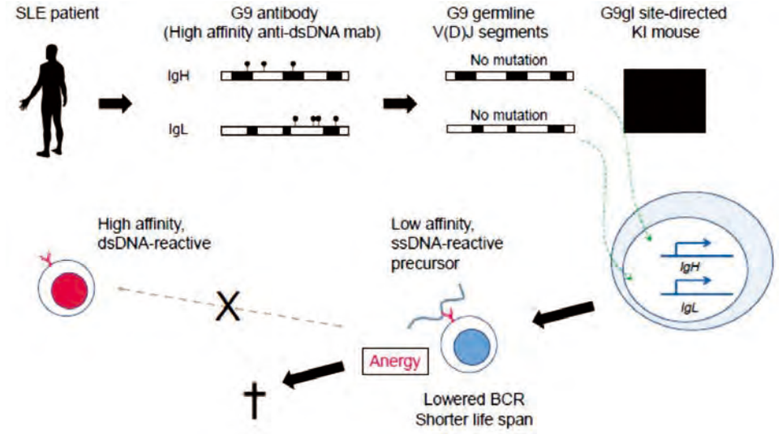

Figure 1. Our mouse model for studying germline precursors of pathogenic anti-dsDNA antibodies derived from SLE patients.

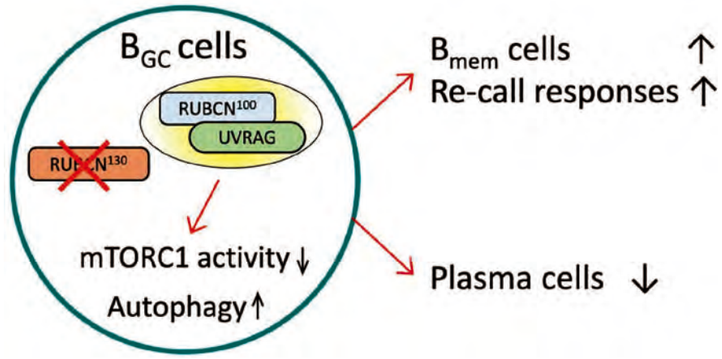

Figure 2. Alteration of B cell fate by RUBCN isoforms through regulation of mTOR activity and autophagy.

Recent Publications

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- 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

Kiyoshi Takeda, MD/PhD

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Postdoctoral Fellows	2
Research Assistants	10
Support Staff	2

We previously demonstrated that extracellular adenosine triphosphate (ATP) released by microbes contributes to induction of Th17 cells by activating $CD70^+$ dendritic cells in the intestinal lamina propria through the P2X and P2Y receptors. To avoid inappropriate immune reactions in the intestine, luminal ATP is strictly controlled by epithelial ATP-hydrolyzing ectoenzymes, such as ectonucleotide pyrophosphatase/phosphodiesterases (E-NPPs) and ectonucleoside triphosphate diphosphohydrolases (E-NTPDases). E-NTPDases convert ATP to ADP as well as ADP to AMP, while E-NPPs hydrolyze ATP to AMP. We have found that E-NTPD7, which highly expressed in small intestinal epithelial cells, inhibits excessive Th17 responses through hydrolysis of luminal ATP. In addition, E-NPP3 on the intestinal epithelial cells depresses the apoptosis of plasmacytoid dendritic cells (DCs) in the small intestine and Peyer's patches.

Although intestinal phagocytes, which express P2X/P2Y receptors, have some protective effects, these cells can also function in pathological conditions. Thus, the number of intestinal phagocytes and their functions are finely tuned to prevent intestinal inflammation.

E-NTPD8 prevents neutrophil-mediated intestinal pathology

In the small intestine, E-NTPD7 and E-NPP3 control luminal ATP concentration, which contributes to the regulation of innate and adaptive immune responses. However, the molecular mechanism underlying the regulation of the luminal ATP concentration in the large intestine remains poorly understood. Among E-NTPD and E-NPP family members, E-NTPD8 was highly expressed in colonic epithelial cells. Therefore, we generated *Entpd8*$^{-/-}$ mice to assess its physiological roles. A higher level of luminal ATP in the colon was observed for *Entpd8*$^{-/-}$ mice than wild-type mice. *Entpd8*$^{-/-}$ mice developed more severe DSS-induced colitis accompanied by increased numbers of IL-17-producing $CD4^+$ T cells and neutrophils in the lamina propria of the colon compared with those in wild-type mice. In this context, the ablation of neutrophils by treatment with anti-Gr-1 antibodies, but not T cells, mitigated the severity of colitis in *Entpd8*$^{-/-}$ mice. In addition, the introduction of *P2rx4* deficiency into *Entpd8*$^{-/-}$ mice ameliorated DSS-induced colitis with lower levels of neutrophils accumulation. Extracellular ATP promoted glycolysis in neutrophils through a P2X4 receptor-dependent Ca^{2+} influx, which is linked to prolonged survival and elevated ROS production in these cells. Thus, E-NTPD8 limits intestinal inflammation by controlling metabolic alteration toward glycolysis via the P2X4 receptor in neutrophils (Figure 1).

Immunomodulatory effects of ATP-hydrolyzing ectoenzymes

A series of studies on ATP-hydrolyzing ectoenzymes expressing in intestinal epithelial cells has shown that control of luminal ATP concentration is essential for the maintenance of intestinal immune homeostasis. Other than epithelial cells, ATP-hydrolyzing ectoenzymes, such as E-NPP3, E-NTPD1, and E-NTPD2, are expressed in innate/adaptive immune cells (mast cells/basophils/regulatory T cells/intraepithelial lymphocytes/macrophages), fibroblasts, and glial cells in the intestine, and mice lacking these molecules are sensitive to allergic or chemically-induced colitis. Thus, regulation of extracellular ATP by mucosal ATP-hydrolyzing ectoenzymes is required for appropriate immune responses in the intestine.

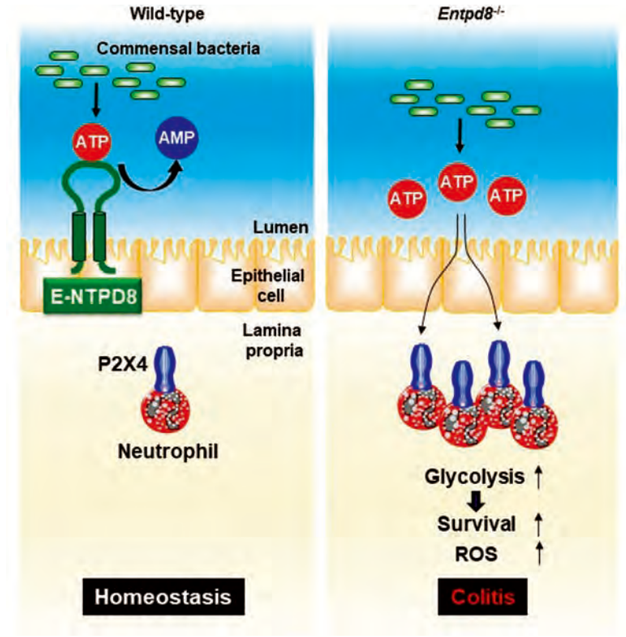

Figure 1. E-NTPD8 prevents neutrophil-mediated colitis by hydrolyzing luminal ATP. E-NTPD8 in colonic epithelial cells maintains gut homeostasis through hydrolysis of luminal ATP produced by commensal bacteria (left). An increased level of luminal ATP caused by lack of E-NTPD8 modulates neutrophil physiology and leads to prolonged survival and enhancement of ROS production, through P2X4-mediated promotion of glycolysis, which links to aggravation of colitis (right).

Recent Publications

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Experimental Immunology

Shimon Sakaguchi, MD/PhD

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Postdoctoral Fellows	4
Research Assistants	2
Visiting Scientists	13
Support Staff	7

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

Treg cells, which specifically express the transcription factor Foxp3, are actively engaged in the maintenance of immunological self-tolerance and homeostasis. Furthermore, the establishment of the Treg-specific epigenetic landscape and the transcription factor network involving FoxP3 coordinate Treg development and function. While FoxP3 functionally acts as a strong repressor of *Il2*, *Ifng*, and other genes upon Treg activation, the Treg epigenome encompassing Treg-specific CpG hypomethylation, chromatin accessibility and histone modifications, reinforces activation of enhancers, especially super-enhancers, associated with Treg signature genes such as *Foxp3*, *Cd25* and *Ctla4*, and stabilizes their expression. Notably, the establishment of such Treg-specific epigenome is FoxP3-independent and a large portion of Treg-specific super-enhancers are concurrently activated at the precursor stage, prior to FoxP3 expression (Kitagawa et al., 2017). These findings suggest that genetic and epigenetic alterations at Treg function-associated genes may be causative of autoimmune and other inflammatory diseases by affecting Treg development and function (reviewed in Sakaguchi 2021).

Mutations of Treg signature genes, such as *FOXP3*, *IL2RA* (*CD25*) and *CTLA4*, indeed impair Treg development and function and consequently produce in humans severe autoimmune diseases reminiscent of murine FoxP3 deficiency. *FOXP3* mutations, in particular, cause profound Treg-specific deficiency/dysfunction, resulting in IPEX (Immune dysregulation, Poly-endocrinopathy, Enteropathy, X-linked) syndrome accompanying a variety of autoimmune diseases (e.g., type 1 diabetes and autoimmune thyroiditis), inflammatory bowel disease, and allergy in infants. In contrast to such monogenic "Tregopathies" with distinct Treg deficiency or dysfunction, functional Treg anomalies found in common polygenic autoimmune diseases (such as type 1 diabetes and rheumatoid arthritis), which afflict ~10% of the population worldwide, have been tantalizingly equivocal and controversial, in particular, over whether a detected anomaly/variation is a "cause" or a "consequence" of autoimmunity. Meanwhile, genome-wide association studies (GWAS) of common autoimmune diseases have revealed that ~60% of autoimmune-causal single nucleotide polymorphisms (SNPs) are mapped to non-coding enhancer regions of immune cells, especially $CD4^+$ T cells. This raises a critical question of whether such autoimmune SNPs, for example, found at the *CD25* or *CTLA4* locus in type 1 diabetes, should affect $CD4^+$ helper T cells or $CD4^+$ Tregs as a gain-of- or loss-of-function variant, respectively.

This year, we addressed the issue by characterizing genome-wide epigenetic profiles of human Tregs and $CD4^+$ Tconvs in naïve and activated states. We found the presence of Treg-specific distinct CpG hypomethylated regions at Treg signature gene loci (e.g., *FOXP3*, *CD25*, and *CTLA4*), which are largely included in Treg-specific super-enhancers and closely associated with Treg-specific gene transcription and other epigenetic changes (Ohkura et al., 2020). Notably, autoimmune SNPs are highly (~9-fold) enriched in Treg-specific DNA demethylated regions present in naïve Tregs, compared with activation-induced demethylated regions. The findings indicate that many causative autoimmune SNPs are present at enhancer regions of Treg function-associated genes such as *CD25* and *CTLA4* (Ohkura et al., 2020), acting as expression quantitative trait loci (eQTLs) controlling endogenous Treg development and function. The results suggest the altered function or development of natural Treg cells holds the key to susceptibility to common autoimmune diseases (Figure 1). Further attempts to link GWAS and Treg immunobiology may elucidate a key contribution of Tregs in common autoimmune diseases.

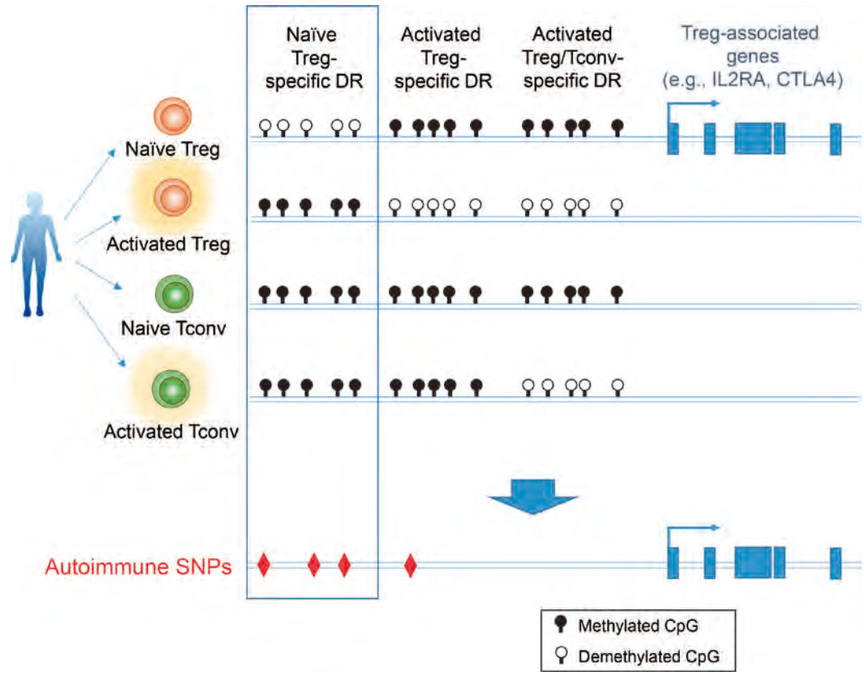

Figure 1. Autoimmune SNPs are enriched in naïve Treg cell-specific CpG hypomethylated regions.
Single-nucleotide polymorphisms (SNPs) associated with common autoimmune diseases were predominantly enriched in CpG demethylated regions (DRs) specifically present in naïve Treg cells but much less enriched in activation-induced DRs common in Tconv and Treg cells.
Adapted from Ohkura, Yasumizu et al., *Immunity*, 2020.

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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. Ultimately, we wish to elucidate the onset of and to modulate T cell function/activation to prevent immune diseases such as autoimmunity and allergic inflammation. For this purpose, we analyzed the regulation of T cell activation/function from a signaling perspective.

1. Regulation of T cell activation through inhibitory checkpoint receptors

Our initial finding that TCR-microclusters (MC) are dynamically generated and initiate T cell activation led us to analyze the dynamics of signaling molecules at the immune synapse.

Similar to our previous studies of CTLA4 and PD-1, we analyzed the dynamic regulation of another inhibitory co-stimulation receptor, LAG3. LAG3 was also colocalized with the TCR-MC to mediate an inhibitory signal for T cell activation. The structural requirement of LAG3 for cluster formation and inhibitory function was analyzed. Various forms of anti-LAG3 together with anti-PD1 Abs were analyzed for the capacity to augment T cell activation for the purpose of checkpoint therapy. Our analyses provide a dynamic view of negative regulation and define inhibitory mechanisms of LAG3.

2. Inhibitory mechanism of T cell activation through phosphatases

We analyzed the negative regulation of T cell activation not only by checkpoint inhibitory receptors but also by receptor-associated phosphatases, particularly by autoimmune-related PTPN22. Its deficiency resulted in enhanced activation and an increase in effector/memory T cells. PTPN22 generates clusters which are associated with TCR-MC. A PTPN22 mutant causing susceptibility to autoimmune diseases was found to be defective in recruitment to the TCR-MC. Analysis of the associated proteins revealed that PTPN22 was recruited to the TCR-MC to comprise an “inhibitory complex” with other inhibitory molecules to inhibit T cell activation. These studies help define the autoimmune susceptibility caused by the mutation.

3. Regulation of T cell function by innate signaling

We also analyzed the modulation of T cell activation and function by innate signals. We previously found that T cells were activated by co-stimulation of TCR and TLRs. TLR2, in particular, activated effector T cells (as Th1) but not naïve T cells in the absence of TCR stimulation. We then analyzed the reason why naïve T cells fail to respond to TLR2. We found that naïve T cells failed to respond to TLR2 stimulation due to the defective expression of TIRAP. TIRAP is expressed upon stimulation through mTORC1 activation via TCR or IL-2 signaling (Figure).

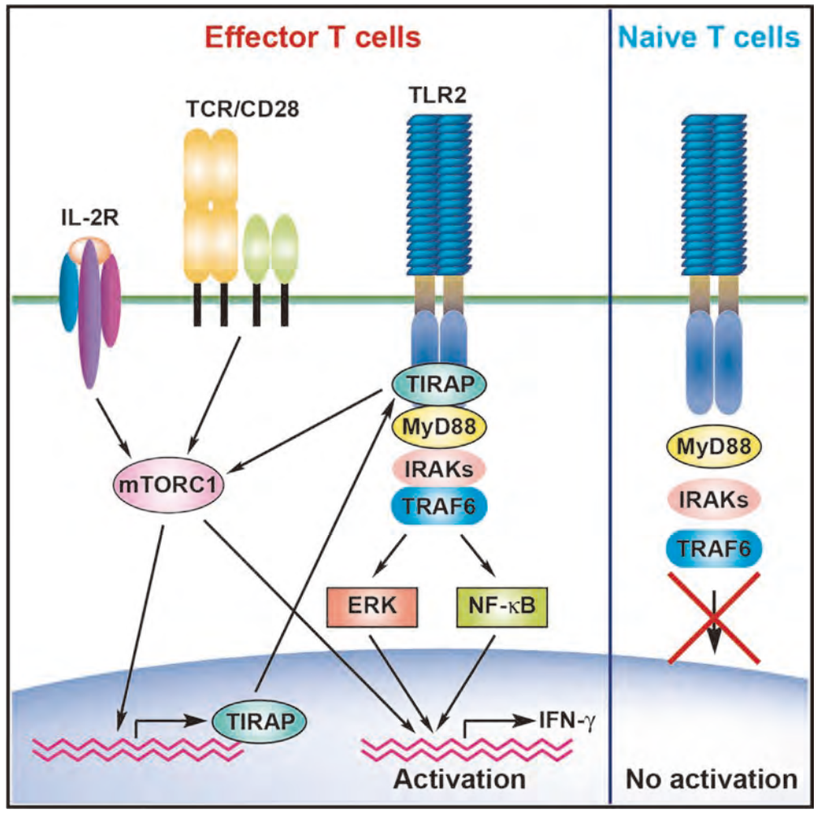

Figure. TLR2-Mediated T-Cell Activation is dependent on TIRAP Expression in T cells and regulated by mTORC1 signaling.
Effector T cells are activated by TLR2 signals in the absence of TCR signals. TLR2-mediated activation is mediated through TIRAP, whose expression is induced by mTOR signals through TCR or IL-2 signals, whereas naïve T cells are not activated by TLR2 due to the lack of TIRAP expression.

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Lymphocyte differentiation

Tomohiro Kurosaki, MD/PhD

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Generation mechanisms of GC-dependent memory B cells

Memory B cells and long-lived plasma cells (LLPCs) 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 secondary antigen challenge, they are primed to elicit rapid antibody responses.

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 weak T cell help are preferentially recruited into the memory B cell compartment. Since GC B cells receiving weak T cell help are generally thought to undergo apoptosis, our model has raised the question of how precursor cells to mature memory B cells are prevented from dying and able to differentiate into memory B cells.

To address this question, after identifying a memory-prone population (pro-memory and pre-memory B cells), we have identified key features for these precursor cells to develop to the mature memory B cells. We found that pro-memory B cells were starting upregulation of Bcl2 and cell-surface BCR, contributing to development of mature memory B cells. Furthermore, we provide evidence that downregulation of Bcl6 in pro-memory B cells could be one of the potential regulatory mechanisms to increase Bcl2 and BCR. Together, we propose a model in which weak help from T cells together with provision of an increased survival signal are key for GC B cells to adopt a memory B cell fate, and that, in regard to the increased survival signal, stepwise decreases in Bcl6 expression (pro-memory, pre-memory, mature memory B cells) play a key role.

Epigenetic regulation on B cell differentiation

Differentiation from B cells into plasma cells is a lineage-switch, thus being accompanied by dramatic changes in gene expression, which extinguish B-cell identity but provide the ability to constantly secrete large amounts of antibodies. The key transcription factors, IRF4, Blimp-1, and Xbp-1, for such lineage-decision are well known and their targets have been intensively characterized. In contrast, epigenetic modification during this transition has been relatively un-characterized. A recent study has revealed that genomic DNA isolated from plasma cells is overall hypo-methylated, compared to that from naïve B cells. However, the precise molecular mechanisms by which DNA methylation regulates plasma cell differentiation are not understood. Hence, we examined the effects of B cell-intrinsic deficiency of DNA demethylases, Tet2 and Tet3. Tet double knockout cells failed to differentiate into plasma cells upon TD and TI-II immunization. In vitro experiments showed that Tet double knockout B cells proliferated normally and were capable of generating cells with IRF4^{int}, but not with IRF4^{hi}. IRF4 overexpression rescued the defects of Tet double knockout B cells in plasma cell differentiation. We also identified CpG islands in the IRF4 gene locus that were demethylated specifically in plasma cells in a Tet2/Tet3-dependent manner. Thus, Tet-dependent demethylation of CpG islands is essential for maintenance of high IRF4 expression, but not for initiation of IRF4 expression.

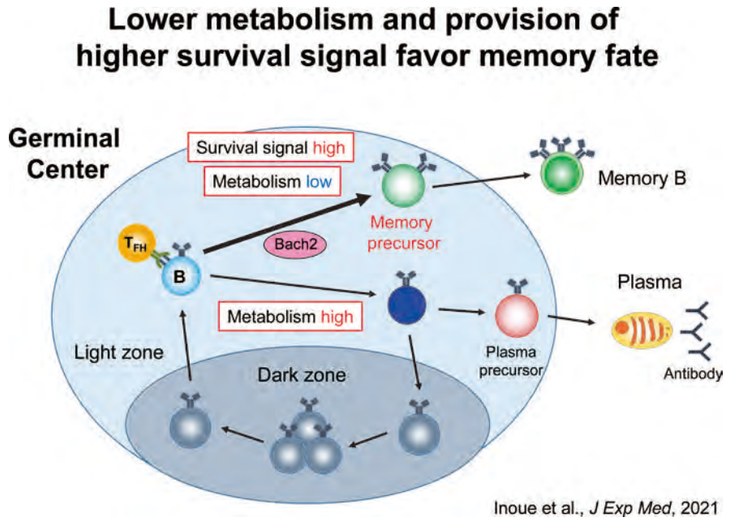

Figure. During germinal center (GC) reaction, light zone B cells are selected to differentiate into memory B cells, plasma cells, or to re-enter the dark zone. We have identified and characterized a small GC population of precursors for memory B cells, and found that the GC B cells with lower mTORC1 activity resulting from receiving weak T cell help and those with a higher survival signal from surface B cell receptors favor a memory B cell fate.

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Malaria Immunology

Cevayir Coban, MD

Professor Cevayir Coban

Malaria, caused by Plasmodium parasites, often leads to severe complications such as cerebral malaria and death. The immune pathology causing these complications is poorly defined. In our lab, we elucidate the mechanisms involved in the host-Plasmodium parasites interactions (*Nature Reviews Immunology, 2018*). Our final goal is to develop vaccines and drugs against malaria and other infectious diseases.

In 2020, the treatment of COVID-19 patients by hydroxychloroquine alone or in combination with other drugs has captured a great deal of attention and triggered considerable debate. Although, the quinoline based-drugs has led to a spectacular reduction in death from malaria in the world, however, scientists have been forced to seek alternative drugs to treat malaria due to the emergence of chloroquine-resistant parasites in the 1960s (Figure). The repurposing of hydroxychloroquine against viral infections, various types of cancer and autoimmune diseases has been ongoing for more than 70 years with no clear understanding of its mechanism of action. We have proposed in our recent review article that further investigation and better understanding of how chloroquine targets the host's cellular and immune responses is needed (*Current Opinion in Immunology, 2020*).

Heparin is a common anticoagulant; however, its biological effects are not limited to coagulation and remain incompletely understood. For example, heparin is used as anti-malarial drug due to its ability to inhibit Plasmodium parasite invasion of erythrocytes. We have demonstrated an unexpected finding that heparin has ability to induce neutrophil extracellular traps (NETs) (*International Immunology, 2020*).

Possible mechanism(s) of action of chloroquine on *Plasmodium*-infected erythrocytes

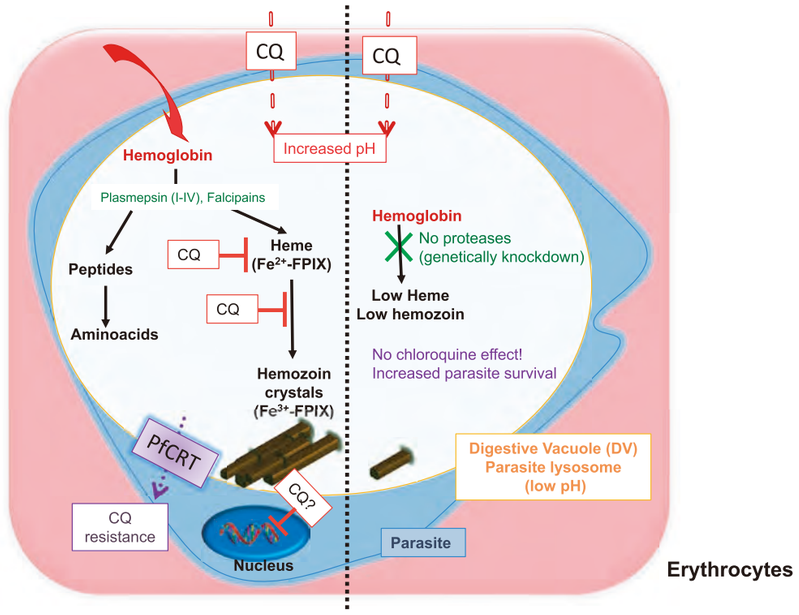

Figure. Possible mechanism (s) of action of chloroquine during blood stage malaria infection. After invasion of erythrocytes, *Plasmodium* parasites form their own digestive vacuole (DV), a lysosome-like acidic compartment important for parasite metabolism and survival. In acidic DVs, the host-hemoglobin is degraded by parasite proteases for the vital needs, such as amino acids and the free-heme is detoxified by converting it into insoluble crystals hemozoin. A weak base chloroquine accumulates in DVs, increases DV pH and binds heme and crystal surfaces, thereby blocks every step of hemozoin formation which eventually leads heme toxicity and parasite death. In the absence of hemoglobin degrading proteases hemoglobin remains undigested and free heme is significantly diminished and the effect of chloroquine on parasites does not occur. Ineffective presence of chloroquine, on the other hand, may create the chloroquine-resistant parasites via a mutation in *P. falciparum* chloroquine resistance transporter (PfCRT) (*modified from Current Opinion in Immunology, 2020*).

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Vaccine Science

Ken J. Ishii, MD/PhD

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Postdoctoral Fellows	1
Research Assistants	3
Visiting Scientists	2
Support Staff	2

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

1) Researches and development of a mRNA vaccine and monoclonal antibodies against SARS-CoV-2

In 2020, the coronavirus disease (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) led to the successful development of two mRNA-based vaccines, encoding the full length of the viral surface spike protein, with high efficacy and reasonable safety. However, reactogenicity, such as fever, caused by innate immune responses to the vaccine formulation remains to be improved. To overcome this potential issue, we developed a lipid nanoparticle (LNP)-based mRNA vaccine, encoding the SARS-CoV-2 spike protein receptor-binding domain (LNP-mRNA-RBD), that improved immunogenicity by removing reactogenic materials from the vaccine formulation in mice and conferred protection against SARS-CoV-2 infection. The vaccine is filed for patent, finished for pre-clinical development, and is underway to first in human clinical trial by the end of March 2021.

In addition to vaccine development, we started to generate monoclonal antibody against SARS-CoV-2 obtained from the volunteer recovered from COVID-19. We have obtained several clones with very high affinity, filed for patent, and now is underway for its efficacy in the animal model.

2) Discovery of Self-Assembling Small Molecules as Vaccine Adjuvants

Immune potentiators, termed adjuvants, trigger early innate immune responses to ensure the generation of robust and long-lasting adaptive immune responses of vaccines. Presented here is a study that takes advantage of a self-assembling small-molecule library for the development of a novel vaccine adjuvant. Cell-based screening of the library and subsequent structural optimization led to the discovery of a simple, chemically tractable deoxycholate derivative (molecule 6, also named cholicamide) whose well-defined nanoassembly potently elicits innate immune responses in macrophages and dendritic cells. Functional and mechanistic analyses indicate that the virus-like assembly enters the cells and stimulates the innate immune response through Toll-like receptor 7 (TLR7), an endosomal TLR that detects single-stranded viral RNA. As an influenza vaccine adjuvant in mice, molecule 6 was as potent as Alum, a clinically used adjuvant. The studies described here pave the way for a new approach to discovering and designing self-assembling small-molecule adjuvants against pathogens, including emerging viruses.

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.

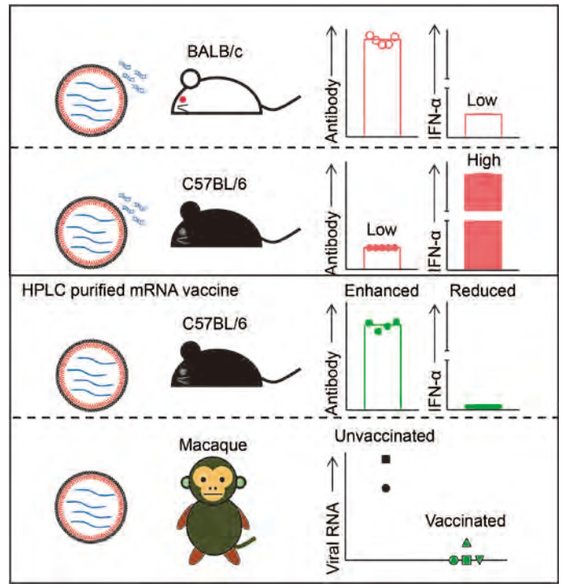

Figure. Summary for the animal experiments.
mRNA vaccine encoding purified LNP-mRNA RBD SARS-CoV2

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Immunoparasitology

Masahiro Yamamoto, PhD

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Visiting Scientists	1
Support Staff	1

Healthy mammalian hosts activate immune responses against pathogenic infections. The innate immune response firstly induces interleukin-12 (IL-12) production by antigen-presenting cells (APCs) such as macrophages and dendritic cells. This is done via recognition of pathogen-derived components by microbe pattern recognition receptors such as Toll-like receptors (TLRs). IL-12 subsequently stimulates the anti-pathogen type I immune response, wherein naïve $CD4^+$ or $CD8^+$ T cells become antigen-specific Th1 cells and cytotoxic T cells, respectively, with the help of APCs. Th1 cells, cytotoxic T lymphocytes, and natural killer cells produce interferon-γ (IFN-γ) to activate the various cell-autonomous programs targeting vacuolar pathogens.

One of the IFN-γ-induced cell-autonomous programs is associated with IFN-inducible GTPases, such as p47 immunity-related GTPases (IRGs) and p65 guanylate binding proteins (GBPs). IRGs and GBPs belong to the dynamin GTPase superfamily, and can target wide ranges of bacterial, fungal, and protozoan vacuolar pathogens. In mice, the IRG protein family consists of three regulator IRG proteins (Irgm1, Irgm2, and Irgm3) and over 20 effector IRG proteins and decoys. There are four effector IRG proteins known to be expressed in mice: Irga6, Irgb6, Irgb10, and Irgd. Regulator IRG proteins harboring GX_4GMS in the first nucleotide-binding motif (G1) are mainly associated with host endomembranes, such as the Golgi apparatus and endoplasmic reticulum (ER). Effector IRG proteins possess a universally conserved GX_4GKS sequence in the G1 motif, enabling binding to both GTP and GDP. GTPase activity has been demonstrated for Irga6 and Irgm3. Regulator IRG proteins can maintain effector IRG proteins in an inactive GDP-bound state, potentially preventing the latter from inappropriate activation on host cell membrane-bounded vesicular systems. In their absence, effector IRG proteins likely form GTP-bound aggregates, and are unable to interact with the *T. gondii* PV. There are 11 members of the mouse GBP family, all of which have the conserved GTP binding motifs. GBP mutants lacking GTPase activity are incapable of accumulating at the *T. gondii* PV membrane (PVM). When these IFN-inducible GTPases are recruited to the PVM, it becomes vesiculated and disrupted, resulting in death of the vacuolar pathogen. Thus, GTPase activity-dependent IRG and GBP accumulation is well established as important for cell-autonomous immunity to vacuolar pathogens.

The mechanism by which IRG proteins access the *T. gondii* PV from the cytosolic compartments can be passive. This process depends on diffusion from the cytoplasmic pools rather than active transport involving TLR-mediated signaling pathways or microtubule networks. Although IRG proteins are localized on the PVM within a few minutes of *T. gondii* infection, little is known about the mechanism by which IRG proteins recognize and destroy the PVM thus far. This process is important for IFN-γ-induced cell-autonomous immunity. Among the effector IRG proteins, Irgb6 and Irgb10 are loaded first and most efficiently onto the *T. gondii* PVM.

Here, we characterized the role of IRG protein Irgb6 in the cell-autonomous response against *T. gondii*, which involves vacuole ubiquitination and breakdown. We show that Irgb6 is capable of binding a specific phospholipid on the PV membrane. Furthermore, the absence of Irgb6 causes reduced targeting of other effector IRG proteins to the PV. This suggests that Irgb6 has a role as a pioneer in the process by which multiple IRG proteins access the PV. Irgb6-deficient mice are highly susceptible to infection by a strain of *T. gondii* avirulent in wild-type mice.

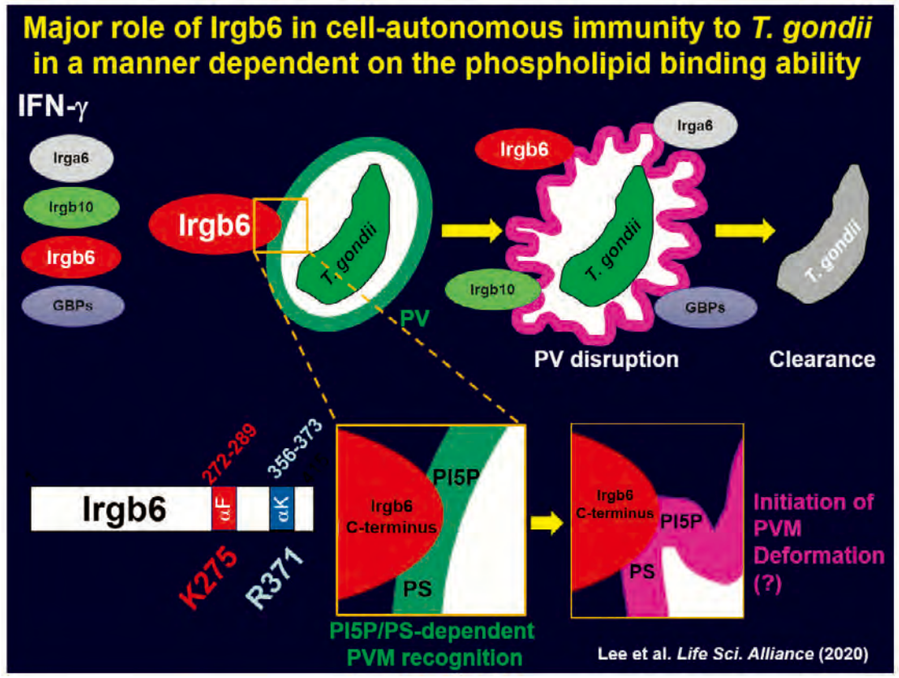

Figure. Initial phospholipid-dependent Irgb6 targeting to *Toxoplasma gondii* vacuoles mediates host defense

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- 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 Sci. Alliance. 3 (2020).
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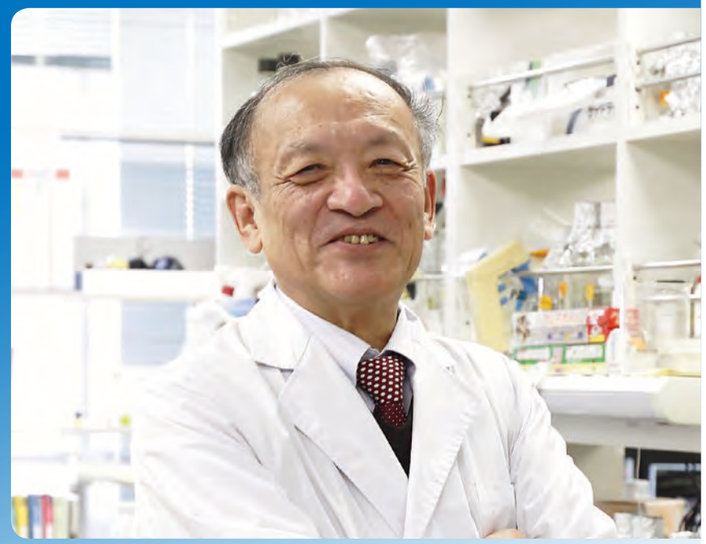
Biochemistry & Immunology



Shigekazu Nagata, PhD

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Support Staff	1

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, which 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 viruses.

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 phospholipids during apoptosis. Xkr8 is expressed ubiquitously, while Xkr4 and Xkr9 are expressed, in a tissue-specific manner, in the brain and 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 and neutrophils express only Xkr8, and a lack of Xkr8 prevents the PtdSer-exposure and engulfment by macrophages. We found that the inefficient engulfment of apoptotic neutrophils and lymphocytes due to the Xkr8 mutation activates 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.

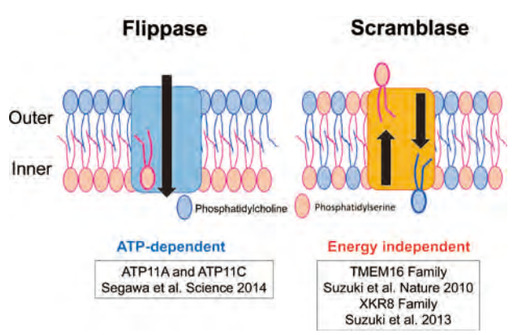

Figure 1. Flippase and Scramblase.
Plasma membranes in eukaryotic cells are comprised of outer and inner leaflets, and phosphatidylserine (PtdSer) and phosphatidylcholine are located exclusively at the inner and outer leaflets, respectively. The asymmetrical distribution of PtdSer is maintained by an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

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

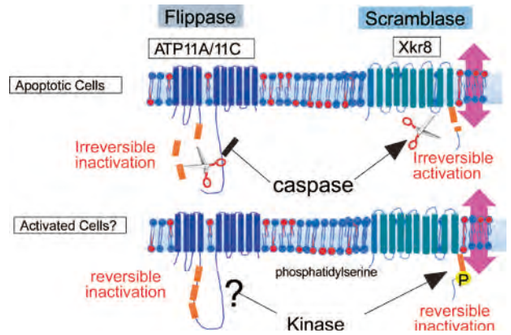

Figure 2. Activation of Xkr8’s scramblase and inactivation of flippases by two independent mechanisms.
In apoptosis, caspase cleaves Xkr8 to activate scramblase while it cleaves flippases (ATP11A and 11C) to inactivate, causing irreversible exposure of PtdSer. Under activated conditions, unidentified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

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- Sakuragi T, Kosako H, and Nagata S Phosphorylation-mediated activation of mouse Xkr8 scramblase for phosphatidylserine exposure. Proc. Nat. Acad. Sci. USA 116: 2907-2912 (2019).
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Molecular Neuroscience

Toshihide Yamashita, MD/PhD

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	Nao Shibuya
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Visiting Scientists	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 (Fig. 1). Further, we have analyzed the mechanism by which the spatiotemporal dynamics in those biological systems control a series of processes (Fig. 2). Particularly, the ultimate goal of this study is to elucidate the manner in which the control mechanism is affected by the associations among the nervous system, immune system, and vascular system. Additionally, we aim to elucidate the processes involved in the functioning 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 observe the central nervous system as a single organ within a biological system. Further, studies from the perspective of how the entire biological system is involved in disorders and recovery of neural networks are scarce. By observing disorders in 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 lead to a new and original trend in Life Sciences.

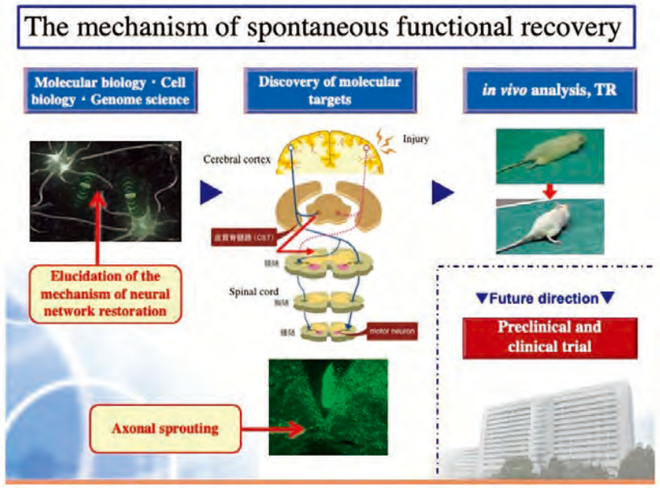

Figure 1. The mechanism of spontaneous functional recovery

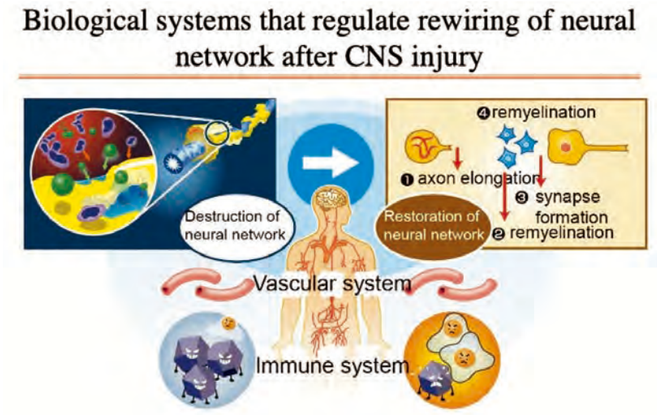

Figure 2. Biological systems that regulate rewiring of neural network after CNS injury

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- Ito M, Muramatsu R, Kato Y, Sharma B, Uyeda A, Tanabe S, Fujimura H, Kidoya H, Takakura N, Kawahara Y, Takao M, Mochizuki H, Fukamizu A & Yamashita T. Age-dependent decline in myelination capacity is mediated by apelin-APJ signaling. Nat. Aging 1: 284-294 (2021).
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Molecular Immunology

Sho Yamasaki, PhD

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Visiting Scientists	4
Support Staff	5

Helicobacter pylori metabolites exacerbate gastritis through C-type lectin receptors

Helicobacter pylori causes gastritis, which has been attributed to the development of *H. pylori*-specific T cells during infection. However, the mechanism underlying innate immune detection leading to the priming of T cells is not fully understood, as *H. pylori* evades TLR detection. Here, we report that *H. pylori* metabolites modified from host cholesterol exacerbate gastritis through the interaction with C-type lectin receptors. Cholesteryl acyl α-glucoside (αCAG) and cholesteryl phosphatidyl α-glucoside (αCPG) were identified as noncanonical ligands for Mincle (*Clec4e*) and DCAR (*Clec4b1*). During chronic infection, *H. pylori*-specific T cell responses and gastritis were ameliorated in Mincle-deficient mice, although bacterial burdens remained unchanged. Furthermore, a mutant H. pylori strain lacking αCAG and αCPG exhibited an impaired ability to cause gastritis. Thus *H. pylori*-specific modification of host cholesterol plays a pathophysiological role that exacerbates gastric inflammation by triggering C-type lectin receptors.

Identification, crystallization and epitope determination of public Tfh TCR shared and expanded in COVID-19 patients

T cells play pivotal roles in protective immunity against SARS-CoV-2 infection. Follicular helper T (Tfh) cells mediate the production of antigen-specific antibodies; however, T cell receptor (TCR) clonotypes used by SARS-CoV-2-specific Tfh cells have not been well characterized. Here, we first identified and crystallized public TCR of Tfh clonotypes that are shared and expanded in unhospitalized COVID-19-recovered patients. These clonotypes preferentially recognized SARS-CoV-2 spike (S) protein epitopes which are conserved among emerging SARS-CoV-2 variants. Among SARS-CoV-2 S epitopes, $S_{864\text{-}882}$, presented by multiple frequent HLA alleles, could activate multiple public Tfh clonotypes in COVID-19-recovered patients. Furthermore, HLA tetramer loaded with this epitope preferentially bound to $CD4^+$ T cells expressing CXCR5. Highly public Tfh clonotypes cross-reacted with symbiotic bacteria, but not common cold human coronaviruses. In this study, we identified and crystallized public TCR for SARS-CoV-2 that may contribute to the prevention of COVID-19 aggravation.

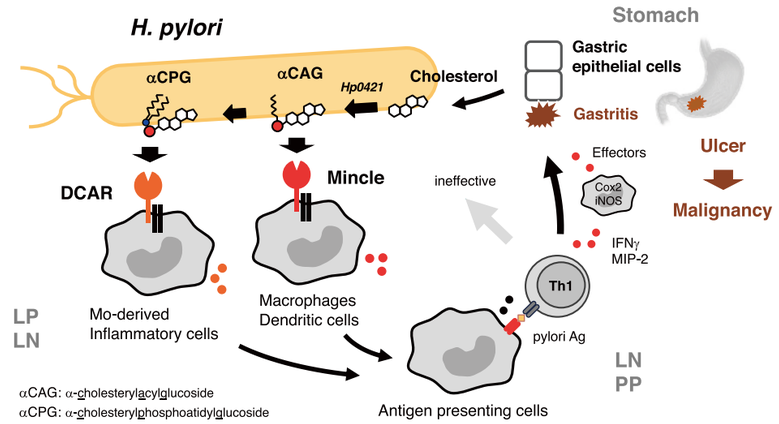

Figure 1. Pathogenic conversion of host lipids by *Helicobacter* exacerbate gastritis through C-type lectin receptors

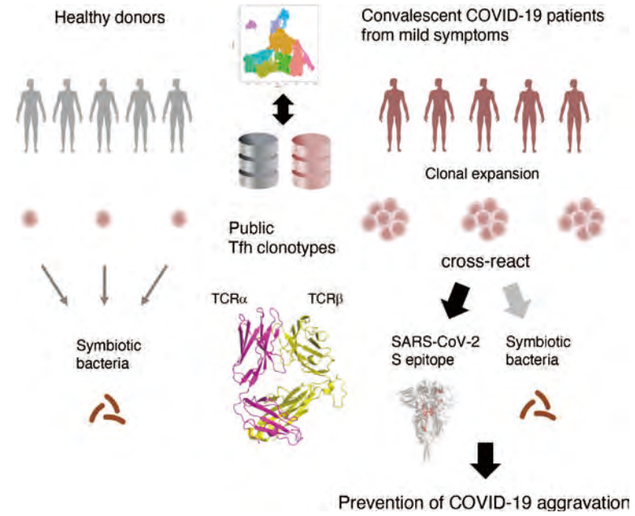

Figure 2. Public Tfh clonotypes cross-reacting with conserved SARS-CoV-2 spike epitopes and commensal bacteria were expanded in recovered COVID-19 patients

Recent Publications

- Nagata M, Toyonaga K, Ishikawa E, Haji S, Okahashi N, Takahashi M, Izumi Y, et al. Helicobacter pylori metabolites exacerbate gastritis through C-type lectin receptors. J Exp Med. 218: e20200815 (2021).
- 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, et al. Lipoteichoic acid anchor triggers Mincle to drive protective immunity against invasive group A Streptococcus infection. Proc Natl Acad Sci U S A. 115: E10662-E10671 (2018).
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- Toyonaga K, Torigoe S, Motomura Y, Kamichi T, Hayashi JM, et al. C-Type Lectin Receptor DCAR Recognizes Mycobacterial Phosphatidyl-Inositol Mannosides to Promote a Th1 Response during Infection. Immunity 45: 1245-57 (2016).

Stem Cell Biology and Developmental Immunology

Takashi Nagasawa, MD/PhD

Professor	Takashi Nagasawa
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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). We 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). Based on these findings, we identified a population of reticular cells expressing CXCL12 at high levels, termed CXCL12-abundant reticular (CAR) cells within murine bone marrow (Tokoyoda et al. *Immunity* 2004; Sugiyama et al. *Immunity* 2006). We found that CAR cells are the major producer of CXCL12 and SCF (Omatsu at al. *Immunity* 2010), and the major cellular components of niches for HSCs and immune cells (Omatsu at al. *Immunity* 2010; Shimoto et al. *Blood* 2017). In addition, we determined the nature of CAR cells, showing that CAR cells are mesenchymal stem cells, which give rise to adipocytes and osteoblasts (Seike et al. *Genes Dev.* 2018) and that transcription factors, Foxc1 and Ebf3, are preferentially expressed in CAR cells and play a critical role in the formation and maintenance of niches for HSCs and immune cells, inhibiting differentiation of CAR cells into adipocytes and osteoblasts, respectively (Omatsu et al. *Nature* 2014; Seike et al. *Genes Dev.* 2018). These studies clarified the nature and functions of CAR cells in murine bone marrow. However, the human counterpart of CAR cells has not been fully described.

To address this issue, we analyzed human bone marrow using a flow cytometry-based *in-situ* technique that enables high-throughput detection of mRNA at single-cell resolution and identified cells expressing much higher CXCL12 than other cells. Like murine CAR cells, $CXCL12^{hi}$ cells had the potential to differentiate into adipocytes and osteoblasts, and most $CXCL12^{hi}$ cells expressed much higher levels of SCF, Foxc1, and Ebf3 than any other bone marrow cells and synovial mesenchymal cells, which had the potential to differentiate into adipocytes and osteoblasts. Histologically, the nuclei of $CXCL12^{hi}$ cells were identified and quantified by Ebf3 expression in fixed marrow sections. $CXCL12^{hi}$ cells sorted from residual bone marrow aspirates of chronic myeloid leukemia (CML) patients expressed reduced levels of HSC niche factors, CXCL12, SCF, Foxc1, and Ebf3 in correlation with increased leukemic burden. Together, we identified the human counterpart of CAR cells and enabled the evaluation of their alterations in various hematological disorders by flow cytometric and histological analyses.

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

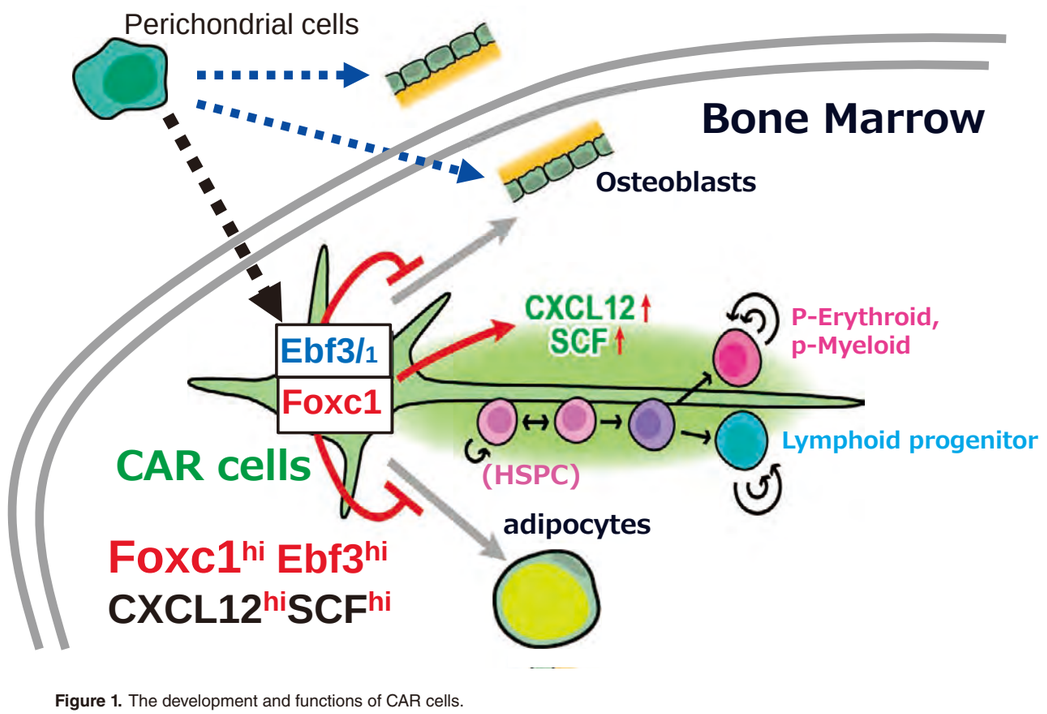

Figure 1. The development and functions of CAR cells.
CAR cells are the major cellular components of non-hematopoietic cells in bone marrow characterized by several salient features in both mice and humans. The transcription factors, Foxc1 and Ebf1/Ebf3, and cytokines, CXCL12 and SCF, are preferentially expressed in CAR cells and critical for the formation and maintenance of niches for HSCs and immune cells, within the bone marrow.

Recent Publications

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- 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: 769-784 (2019).
- Seike M, Omatsu Y, Watanabe H, Kondoh G, Nagasawa T. Stem cell niche-specific Ebf3 maintains the bone marrow cavity. Genes Dev. 32: 359-372 (2018).
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Aging Biology

Eiji Hara, PhD

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Research Assistants	2
Support Staff	2

Cellular senescence is the state of irreversible cell cycle arrest that can be induced by a variety of potentially oncogenic stimuli and has therefore long been considered to suppress tumorigenesis, acting as a guardian of homeostasis. Emerging evidence, however, reveals that senescent cells also promote secretion of various pro-inflammatory and pro-proliferative factors. This newly identified senescence-associated secretory phenotype termed SASP is likely to be associated with homeostatic disorders including cancer. It is therefore quite possible that accumulation of senescent cells during aging *in vivo* may contribute to aging-associated cancers. By conducting the following studies, we aim to clarify the molecular mechanisms underlying aging-associated cancer.

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 a phenomenon 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 of 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 (Figure 1). Indeed, the clearance of $p16^{INK4a}$-positive senescent cells from aged transgenic mice reportedly delays 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 its 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.

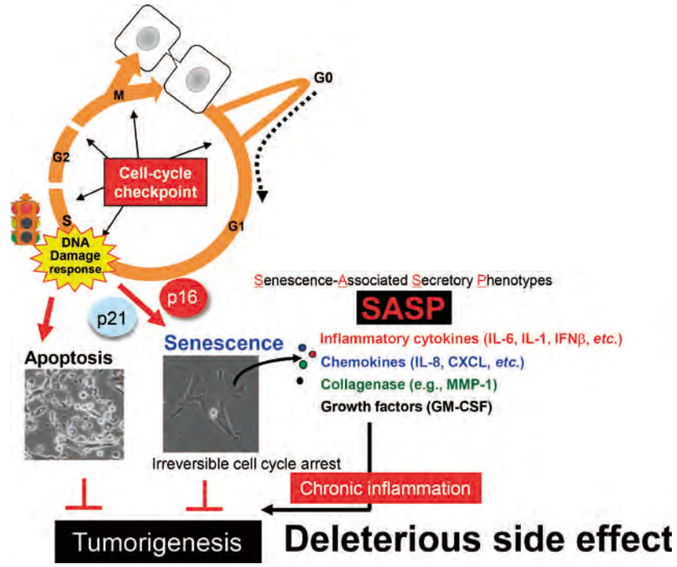

Figure 1. Cellular senescence initially inhibits proliferation of damaged cells, thereby acting as a fail-safe mechanism. However, in the long term, senescent cells eventually promote tumorigenesis via SASPs.

Recent Publications

- Wakita M, Takahashi A, Sano O, Loo TM, Imai Y, Narukawa M, Iwata H, Matsudaira T, Kawamoto S, Ohtani N, Yoshimori T, Hara E. A BET family protein degrader provokes senolysis by targeting NHEJ and autophagy in senescent cells. Nat. Commun. 11: 1935 (2020).
- Takahashi A, Loo TM, Okada R, Kamachi F, et al. Downregulation of cytoplasmic DNases is implicated in cytoplasmic DNA accumulation and SASP in senescent cells. Nat. Commun. 9: 1249 (2018).
- Okuma A, Hanyu A, Watanabe S, Hara E. $p16^{INK4a}$ and $p21^{Cip1/Waf1}$ promote tumour growth by enhancing myeloid-derived suppressor cells chemotaxis. Nat. Commun. 8: 2050 (2017).
- Takasugi M, Okada R, Takahashi A, Chen DV, Watanabe S, Hara E. Small extracellular vesicles secreted from senescent cells promote cancer cell proliferation through EphA2. Nat. Commun. 8: 15728 (2017).
- Takahashi A, Okada R, Nagao K, Kawamata Y, Hanyu A, Yoshimoto S, Takasugi M, Watanabe S, Kanemaki MT, Obuse C, Hara E. Exosomes maintain cellular homeostasis by excreting harmful DNA from cells. Nat. Commun 8: 15287 (2017).

Oncogene Research

Masato Okada, PhD

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Research Assistants	2
Support Staff	1

Role of Src tyrosine kinase in tumor progression

We investigated the role of Src tyrosine kinase in tumor progression. Src is the first oncogenic tyrosine kinase to have been identified and 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-βtreatment directly induces *SRC* gene expression via the Smad pathway coupled with additional 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 (Fig. 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 into lipid rafts of lysosomes. Subsequent analysis revealed that p18 functions by forming a hetero-heptamer complex (Ragulator), consisting of p18, p14, MP1, HBXIP, p10, and RagA/C, and 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 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 previously 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 (Fig. 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.

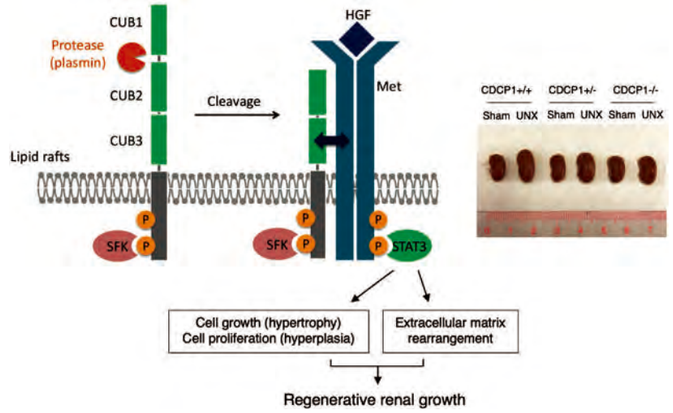

Figure 1. Role of the CDCP1-Met-Src-STAT3 axis in regenerative renal growth.

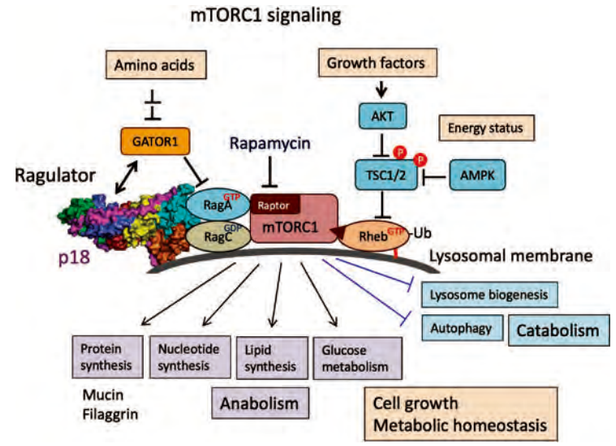

Figure 2. Function and regulation of the mTORC1 signaling on lysosomal membrane.

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- Nada S, Okada M. Genetic dissection of Ragulator structure and function in amino acid-dependent regulation of mTORC1. J Biochem. 168: 621-632 (2020).
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Signal Transduction

Nobuyuki Takakura, MD/PhD

Professor	Nobuyuki Takakura
Associate Professor	Hisamichi Naito Hiroyasu Kidoya
Postdoctoral Fellows	4
Research Assistants	3
Support Staff	2

Our research team is involved in the research of vascular biology and stem cell biology. In 2020, we obtained several achievements and among them, interesting results on self-renewal in hematopoietic stem cells are shown here.

Hematopoietic stem cells (HSCs) give rise to the blood supply and excessive division of HSCs will cause cell exhaustion. Therefore, it is very important to maintain a balance between self-replication and differentiation of HSCs. However, the cellular and molecular mechanisms coordinating the balance between HSC quiescence and differentiation are not fully understood.

As a consequence of analyzing the molecular mechanism for HSC quiescence, we found that galectin-3 (Gal-3) is highly expressed in quiescent HSCs. Gal-3 is a member of the galactose binding lectin family and plays a role in various biological processes such as development, differentiation, cell cycle, and apoptosis. Our research found that the percentage of long-term HSCs (LT-HSC) in the G_0 phase decreased in BM from Gal-3 KO relative to wild-type mice and that the reconstruction ability of LT-HSCs in Gal-3 KO mice was attenuated. These results illustrate that Gal-3 regulates the cell-cycle of HSCs and plays a critical role in maintaining the quiescent state.

To elucidate how Gal-3 maintains HSC quiescence, we analyzed the regulatory mechanism and function of Gal-3 as follows. It is well known that Angiopoietin-1 or Thrombopoietin, secreted from bone marrow niche cells, binds to the receptor Tie2 or Mpl on the surface of HSCs. We found that nuclear translocation of NF-κB mediated by the PI3K/AKT pathway following stimulation by Tie2 or Mpl expressed in HSCs promotes the production of Gal-3. Moreover, we found that Gal-3 upregulated in HSCs binds to Sp1 and induces p21 transcription, resulting in the inhibition of cell cycle progression and maintenance of LT-HSC quiescence.

This research clarified a novel mechanism of bone marrow niche for the maintenance of HSCs and may contribute to stem cell expansion in culture and bone marrow transplantation, as well as promoting leukemia cancer stem cell-targeted therapy and other clinical applications.

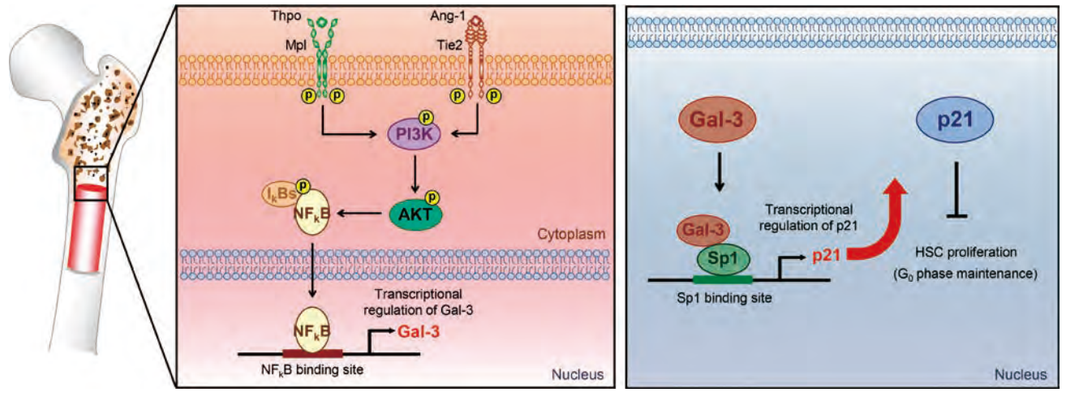

Figure. Proposed model showing the molecular mechanisms essential for maintaining (long term-hematopoietic stem cell) LT-HSC quiescence.
Angiopoietin-1 (Ang-1) or Thrombopoietin (Thpo) secreted from bone marrow niche cells, binds to the receptor Tie2 or Mpl on the surface of LT-HSCs. Nuclear translocation of NF-κB mediated by activated AKT following stimulation by Tie2 or Mpl expressed in LT-HSCs promotes the production of Gal-3, which then binds to Sp1 and induces p21 transcription, resulting in the inhibition of cell cycle progression and maintenance of LT-HSC quiescence.

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- 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).
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Cutaneous Immunology

Manabu Fujimoto, MD/PhD

Professor	Manabu Fujimoto
Associate Professor	Yumi Matsuoka-Nakamura
Research Assistants	1
Visiting Scientists	1
Support Staff	1

Our research team is interested in understanding the pathogenesis of autoimmune diseases, inflammatory skin diseases, and cancer, and translating novel research knowledge into clinical applications.

The local T-cell immunity in the skin is maintained more efficiently in advanced age than circulating T-cell memory

Recent studies have highlighted that human resident memory T cells (TRM) are functionally distinct from circulating T cells. Thus, it can be postulated that skin T cells age differently from blood-circulating T cells. We assessed T-cell density, diversity, and function in skin samples of various ages. No decline in the density of T cells was noted with advancing age, and the frequency of epidermal CD49a$^+$ CD8 TRM was increased in elderly individuals regardless of ethnicity. T-cell diversity and antipathogen responses were maintained in the skin of elderly individuals but declined in the blood. Our findings demonstrate that in elderly individuals, skin T cells maintain their density, diversity, and protective cytokine production despite the reduced T-cell diversity and function in blood.

Ganglioside GD3 suppress the functional activities of TRM in cutaneous T-cell lymphoma

In cutaneous T-cell lymphoma (CTCL), which arises from skin-tropic memory T cells, malignant T cells and benign T cells are confined in the same skin lesions. It is thus difficult to evaluate the phenotypic characteristics and functional activities of benign T cells in CTCL. Disialoganglioside with three glycosyl groups (GD3) is increasingly expressed on the surface of solid malignant tumor cells and takes part in tumor progression and suppression of tumor immunity. However, the role of GD3 in CTCL is not well-understood. We found that the benign T cells included limited TRM in CTCL skin lesions and the expression of GD3 in the malignant T cells inversely correlated with IL-17A production from the benign CD4 T cells. Revealing the role of GD3 in inhibiting the production of IL-17A in CTCL would aid the understanding of the suppressive mechanism of the antitumor activity by malignant tumor cells.

Imbalance of follicular helper CD4+ T cell subsets induces B cell alteration and contributes to the pathogenesis of systemic sclerosis.

Systemic sclerosis (SSc) is a connective tissue disease of unknown aetiology characterized by features of autoimmunity, vasculopathy, inflammation and fibrosis on skin and multiple internal organs, as well as the musculoskeletal system. Until now, the full pathogenesis of SSc remains unknown. We assessed circulating follicular helper CD4$^+$ T cells (cT_{FH}) and B cells, and found that the frequency of T_{FH1} cells was increased and correlated with cytokine concentrations of IL-21 and IL-6 that induce B cell differentiation in SSc. cT_{FH} cells from SSc showed activated phenotype with expressing higher cytokine levels compared with healthy controls. These results suggest that an imbalance of cT_{FH} toward $T_{FH\ 1}$ may induce B cell alteration through IL-21 and IL-6 pathways and promote inflammation, contributing to the pathogenesis of SSc.

Staphylococcus Agr expression is critical for epidermal colonization and associates with atopic dermatitis development.

Atopic dermatitis (AD) is commonly associated with colonization by *Staphylococcus aureus* in the affected skin. Inhibition of *Staphylococcus aureus* Agr–mediated quorum sensing is known to protect against AD. We performed whole-genome sequencing of *S. aureus* strains isolated from the skin of Japanese infants and showed that infants who developed AD early in life were more likely to have cheek skin colonized by *S. aureus*. However, infants harboring *S. aureus* with acquired spontaneous mutations in Agr were more likely to remain healthy, despite the presence of this bacterium on their skin. This work suggests that *S. aureus* and associated functional quorum sensing may play a role in the onset of AD in children.

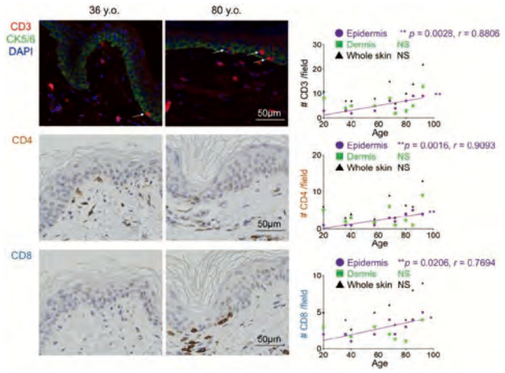

Figure 1. The density of T cells, both CD4 and CD8 fractions, increased in the epidermis of elderly individuals.

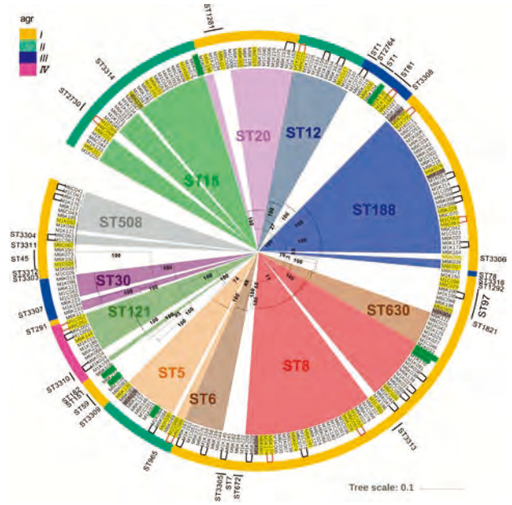

Figure 2. Whole-genome sequencing of *S. aureus* strains isolated from the skin of Japanese infants.

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- Koguchi-Yoshioka H, Hoffer E, Cheuk S, Matsumura Y, Vo S, Kjellman P, Grema L, Ishitsuka Y, Nakamura Y, Okiyama N, Fujisawa Y, Fujimoto M, Eidsmo L, Clark RA, Watanabe R. Skin T cells maintain their diversity and functionality in the elderly. Commun Biol. 4(1): 13 (2021).
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Innate Immune Systems

Kazuyo Moro, PhD

Professor	Kazuyo Moro
Assistant Professor	Eriko Kudo
Postdoctoral Fellows	1
Research Assistants	1
Visiting Scientists	1
Support Staff	1

Our team has been focused on group 2 innate lymphoid cells (ILC2), an innate lymphocyte lineage that we identified in 2010. ILC2 localize in a variety of tissues such as fat, lung, intestine, liver, and skin, and mediate immune responses to helminth and fungal infections via strong type 2 cytokine production, including IL-5, IL-13, IL-9, GM-CSF. Unlike T and B lymphocytes, ILC2 lack antigen-specific receptors and are activated by cytokines produced by epithelial cell-derived cytokines such as IL-33. Because ILC2 are also known to produce type 2 cytokines in many diseases including allergic disorders and pulmonary fibrosis (PF), we wish to establish new therapies for these diseases through regulating ILC2.

IL-4, a key cytokine in allergy, is involved in multiple immune reactions including Th2 cell development, IgG1 and IgE production by B cells and M2 macrophage differentiation. While Th2 cells produce IL-4 together with IL-5 and IL-13 under antigen-induced TCR stimulation, ILC2 fail to produce IL-4 under IL-33 stimulation, which induces a large amount of IL-5 and IL-13. Based on this fact, ILC2 are not thought to contribute to IL-4-mediated immune responses, even though they express high levels of the IL-4 gene. However, we demonstrated the mechanism underlying interleukin (IL)-4 production by ILC2 that depends on IL-2, IL-33 and cysteinyl leukotriene (CysLT)-mediated Ca2+ signaling and induces 'innate immunoglobulin E (IgE)' production by B1 cells. Innate IgE prolonged the survival of FcεR+ cells and accelerated ILC2-mediated allergic responses. The CysLT inhibitor blocked innate IgE-mediated augmentation of asthma symptoms, suggesting that innate IgE-primed FcεR^{+} cells positively regulate the activation of ILC2 via CysLTs. Overall, these data demonstrated that ILC2, B1 cells, and FcεR^{+} cells generate an amplification circuit that defines allergic susceptibility (Fig.1).

Pulmonary fibrosis (PF) is a disease that causes collagen deposition in the lungs. Despite numerous studies on the pathology of PF, lack of animal models has significantly hampered advancement of our understanding of the disease. We found that 100% of *Ifngr1*$^{-/-}$*Rag2*$^{-/-}$ mice lacking suppression mechanism for ILC2 and ILC3 spontaneously developed PF. Through studying of *Ifngr1*$^{-/-}$*Rag2*$^{-/-}$ mice, we have unveiled an indispensable contribution of ILC2 and ILC3 to the fibrosis formation. Single-cell RNA-sequence analysis indicated that ILC3 activation occurred during the disease-onset phase in *Ifngr1*$^{-/-}$*Rag2*$^{-/-}$ mice, which triggered subsequent activation of ILC2 subpopulation. By dissecting pathomechanisms of disease initiation in vitro and in vivo, we have delineated complex networking among ILC2, ILC3 and fibroblasts, which will lead us to develop novel therapeutic options that prevent the progression of the disease (Fig.2).

The development of new drugs is required to suppress ILC2, because ILC2 induces various diseases such as allergies, pulmonary fibrosis, and metabolic diseases. In collaboration with Nagasaki University, we searched for an ILC2 inhibitor from the marine microbe extract library. Among 520 marine microbe extracts, 3 extracts contained a component that suppressed cytokine production by ILC2. We confirmed that these extracts do not affect the survival of ILC2 and only suppress cytokine production. Since these three extracts still contain a variety of components, we are currently trying to further fractionate the components to identify the effective compound.

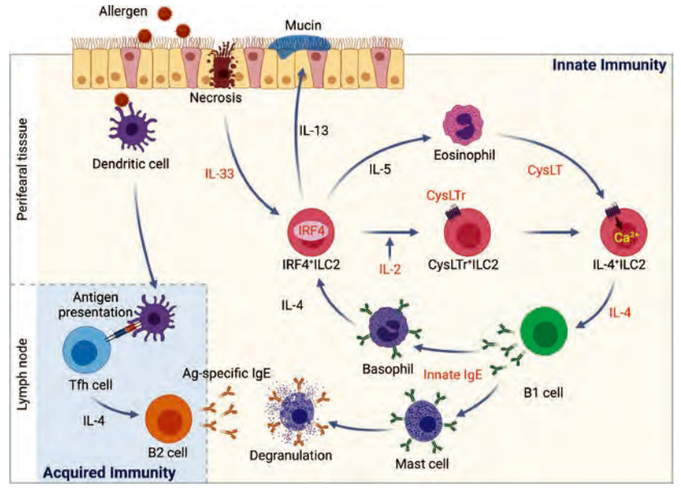

Figure 1. ILC2-derived IL-4 regulates allergic susceptibility through innate IgE production by B1 cells

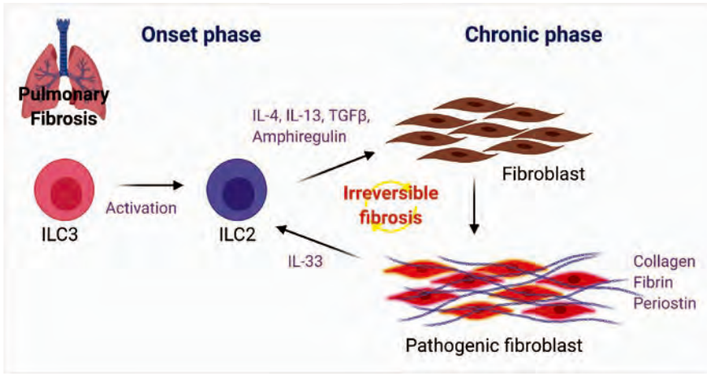

Figure 2. *Ifngr1*$^{-/-}$*Rag2*$^{-/-}$ mouse lacking suppression mechanism for ILC2 and ILC3 spontaneously developed pulmonary fibrosis.

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Human Immunology (Single Cell Immunology)

James Badger Wing, PhD

Associate Professor James Badger Wing

Postdoctoral Fellows 2
Research Assistants 1

The Human Immunology (single cell immunology) focuses on single cell biology and its applications to human immunology. Recent advances in single cell technologies, such as mass cytometry (also known as CyTOF) have revealed high levels of heterogeneity among immune cells. The mass cytometry technique can measure single cell proteome in great details of up to 50 protein markers on millions of single cells (Hartman and Bendall, Nat Rev Rheumatol, 2020). This makes it uniquely well placed to obtain a wide-ranging picture of the human immune system. Primarily we use mass cytometry to explore two areas, autoimmunity and infectious diseases.

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 Tregs present in humans harbor a great deal of heterogeneity in both blood and tumors (Figure 1). 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 allergy. 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 was carried out by us and others in 2017. A clear goal of the field is to determine how to manipulate 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.

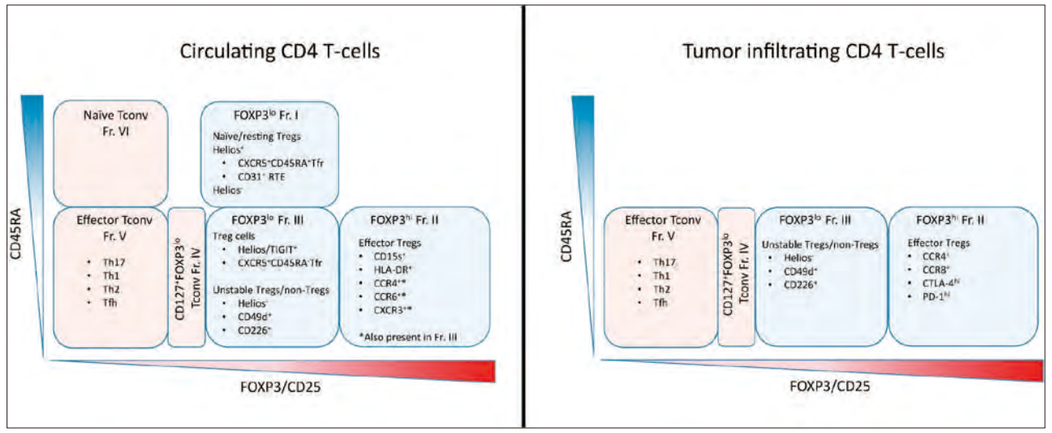

Figure 1. Main Treg Subgroups Present in Human Blood and tumors (Wing et al. Immunity 2019)

Recent Publications

- Wing JB & Sakaguchi S. Using mass cytometry to address Tfh and Tfr heterogeneity. Methods in Molecular Biology. Edition “T follicular helper cells” (2021 in press).
- Mimitou EP et al. Scalable, multimodal profiling of chromatin accessibility and protein levels in single cells. bioRxiv DOI: 10.1101/2020.09.08.286914. Nature Biotechnology (in press).
- Yang et al. CTLA-4 expression by B-1a B cells is essential for immune tolerance. Nature Communications 12: 525 (2021).
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Human Immunology (Single Cell Genomics)

Daisuke Okuzaki, PhD

Associate Professor	Daisuke Okuzaki
Postdoctoral Fellows	3
Visiting Scientists	2
Support Staff	1

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. One of the major research projects we are currently focused on is a genome project in collaboration with the Trauma and Acute Critical Care Center in the Osaka University Graduate School of Medicine. Many of the patients sent to the Trauma and Acute Critical Care Center have acute inflammation and suffer from systemic inflammatory response syndrome (SIRS). The severity of SIRS usually has to be clinically classified within several hours upon patient arrival. Classification is based on clinical criteria such as fever, hypothermia, tachycardia, tachypnea or leucopoenia. Although treatment of the clinical syndrome is a top priority in the Trauma and Acute Critical Care Center, progress in the research of its pathological classification and associated molecular mechanism has not advanced in Japan as it has in Europe and America.

We hereby kick started a collaboration research project involving the measurement and collection of biomolecular data such as DNA, RNA and protein from patented biomaterials, while integrated with cutting edge single cell sequencing technology. As a prospective observational study, we collected ELISA, RNA sequencing, Olink or single cell sequencing data from patients three days and seven days post arrival. The resulting multiomics data, combined with clinical pathology and bioinformatic multi-dimensional analysis, can be utilized to classify SIRS patients based on molecular properties into pathological classifications such as sepsis, ARDS, PCAS, trauma, heat stroke or burn. Through these classifications, proper treatment can potentially be selected in a timely manner upon arrival.

Furthermore, with COVID-19 raging worldwide, it is also classified as a SIRS and the course of treatment for patients brought to Osaka University Hospital should be preferably decided prior to arrival. Our proposal to search for appropriate biomarkers using proteome and transcriptome profiling techniques and to apply them clinically was selected as a project for the AMED " Research Program on Emerging and Re-emerging Infectious Diseases. This project is being conducted in cooperation with the Trauma and Acute Critical Care Center at Osaka University and the Osaka Prefectural Nakakawachi Emergency and Critical Care Center.

Currently there are no conventionally established biomarkers or drug treatments for COVID 19. In order to search for the appropriate biomarkers to reflect the pathological classification for COVID-19, cohorts were divided into the three groups of research, development and verification. Applying the same strategy as the aforementioned SIRS studies, we plan to collect experiment data using RNA sequencing, mass spectrometry, Olink proteomics, and single cell RNA sequencing. Through the integration with panomics data a set of “pathological molecular scores” and “pathological correlated clusters” can be defined. The novel “pathological classification” is determined by the defined clusters and scores, and enables selective therapeutic intervention depending on the COVID-19 symptoms. Especially for the given circumstance, it is urgent to select the proper treatments for prior and post intubation patients.

On the other hand, the importance of evaluating vaccination efficacy is gradually increasing given the current nationwide vaccination efforts. We intend to elucidate the pathological mechanism of the novel coronavirus as well as develop potential treatments through the use of single cell RNA sequencing of peripheral blood samples taken from healthy individuals prior and post vaccination. In connection with the Joint Research Coronavirus Task Force and serving as a member of the All Handai research team, we are currently joining forces with the young researchers of IFReC as well as the Graduate School of Medicine and the Faculty of Medicine, and analyzing the peripheral blood samples taken from the patients sent to Osaka University Hospital during all four waves of COVID-19 in Japan. Currently, we are analyzing multiple aspects of the molecular properties of immune cells from dozens of patients.

We have co-authored two papers in which we apply single cell RNA sequencing. They include a study of PTH-induced bone formation conducted by Morimoto et al., published in Nat Commun. 2021 and a successful observation of hematopoietic recovery supported by innate lymphoid cells under stress conditions performed by Sudo T, et al., published on J Exp Med. 2021.

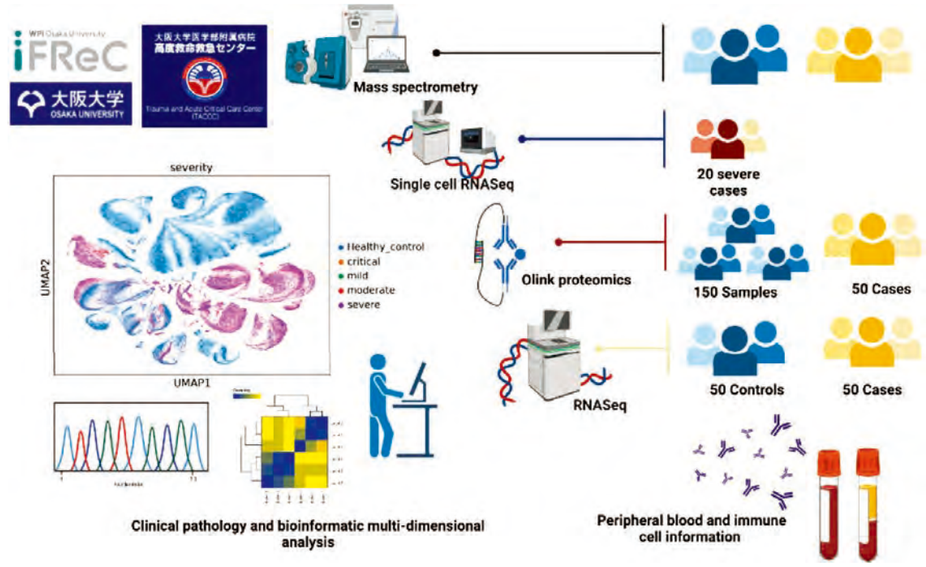

Figure. Overview of the research process.

Recent Publications

- Morimoto A, et al. SLPI is a critical mediator that controls PTH-induced bone formation. Nat Commun. 12(1): 2136 (2021).
- Sudo T, et al. Group 2 innate lymphoid cells support hematopoietic recovery under stress conditions. J Exp Med. 218(5): e20200817 (2021).
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- Okuzaki D, et al. c-Src promotes tumor progression through downregulation of microRNA-129-1-3p. Cancer Sci. 111: 418-428 (2020).
- Hasegawa T, et al. Identification of a novel arthritis-associated osteoclast precursor macrophage regulated by FoxM1. Nat. Immunol. 20: 1631-1643 (2019).

Immune Homeostasis

Yasutaka Okabe, PhD

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Postdoctoral Fellows	1
Research Assistants	2
Support Staff	1

The laboratory of Immune Homeostasis was established at IFReC in September 2020. The focus of the laboratory is to understand tissue homeostasis, which is an essential mechanism for maintaining tissue in its optimal functioning state. The principle of tissue homeostasis can be described as the maintenance of the level of regulated variables (e.g., tissue size, interstitial fluid volume) within an acceptable range. This is regulated by the orchestration of parenchymal cells that perform the primary function of tissues with other supportive cell types under the control of tissue environmental cues.

In this perspective, we study tissue-resident macrophages. Although macrophages have been historically studied in the context of their functions in host defense and the clearance of dead cells, they are increasingly appreciated to serve many important roles in tissue development, normal physiology, and repair. Tissue-resident macrophages are present in virtually every mammalian tissue and they function as essential components for the maintenance of tissue homeostasis. They perform tissue-specific functions that are critical for normal tissue physiology (Figure 1). Accordingly, abnormalities of tissue-resident macrophage functions often link to various pathologies including osteopetrosis, type 2 diabetes, immune deficiency and neurodevelopmental diseases. These evidences suggest that understanding tissue macrophage functions could provide a novel therapeutic approach for these diseases.

The heterogeneity of tissue-resident macrophage phenotypes is considered to be a functional specialization of macrophages as a consequence of their adaptation to local tissue environments (Figure 2). Tissue-resident macrophages, in response to tissue environmental cues, activate the corresponding functional polarization programs which accompany the induction of specific gene-expression programs. We have characterized tissue-specific transcriptional programs of resident macrophages and identified hundreds of genes that are selectively expressed in tissue macrophages. Among these genes, we found transcription factor GATA6 is uniquely expressed in macrophages that reside in the peritoneal cavity. We reported that GATA6 acts as a master transcriptional regulator for functional specialization of peritoneal macrophages, as the deletion of the GATA6 gene in peritoneal macrophages resulted in a defect in the peritoneal-specific gene expression program. Additionally, we and other groups found the GATA6-mediating program controls macrophage positioning within the peritoneal cavity, local proliferation, and peritoneal-specific immune responses.

Although functional specialization of tissue-resident macrophages is thought to be induced under the influence of tissue micro-environments, the identity of tissue-derived signals was largely unknown. We reported that retinoic acid, which is a lipophilic molecule derived from vitamin A, plays an essential role in the induction of the GATA6 gene in peritoneal macrophages. We found nuclear retinoic acid receptors (RARs) bind to the promoter of the GATA6 gene in a retinoic acid-dependent manner. In addition, the treatment of RAR inverse agonist (BMS493) abolished the expression of GATA6 gene in peritoneal macrophages. Lastly, a vitamin A deficiency in mice caused the reduction of GATA6 gene expression. Taken together, these results provide insight into the mechanism of generation of tissue specialization of resident macrophages.

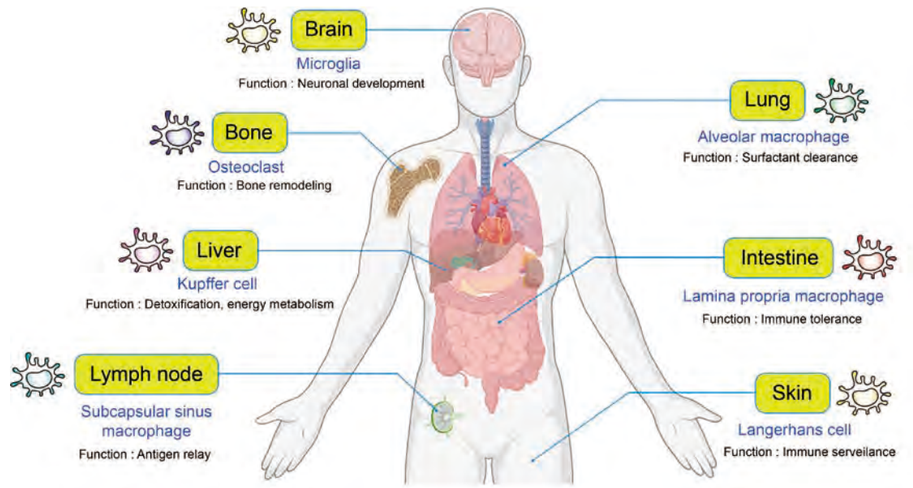

Figure 1. Functional specialization of tissue-resident macrophages is an essential component of tissue homeostasis.

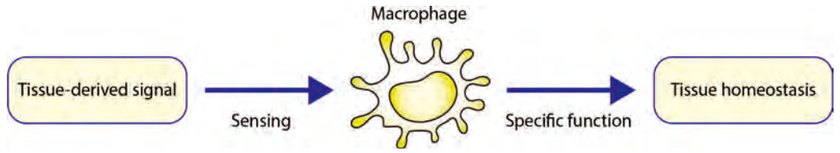

Figure 2. Macrophages are essential components for the maintenance of tissue homeostasis.

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- Okabe Y. Molecular Control of the Identity of Tissue-Resident Macrophages. International Immunology 30: 485-491 (2018).
- Okabe Y, & Medzhitov R. Tissue Biology Perspective on Macrophages. Nature Immunology 17: 9-17 (2016).
- Okabe Y., & Medzhitov R. Tissue-specific signals control reversible program of localization and functional polarization of macrophages. Cell 157: 832-844 (2014).

Cellular Immunotherapy

Naoki Hosen, MD/PhD

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We are focusing on cellular immunotherapy, especially chimeric antigen receptor (CAR)-T cell therapy for cancer. CAR is generated by fusing the antigen recognition domain of a tumor-specific monoclonal antibody (mAb) with CD3ζ and a costimulatory molecule such as CD28 or 4-1BB, and transduced into T cells using retroviruses or lentiviruses to make CAR-T cells. CAR-T cells specifically recognize cancer cells using the cancer-specific mAb-derived antigen-recognition domain, and are activated. Activated CAR-T cells kill tumor cells and also proliferate extensively. CAR-T cells share the advantages of both mAbs and cytotoxic T cells, and have a high affinity and specificity for tumor cells, as well as high cytotoxicity and proliferation potential. CD19 CAR-T cells showed surprisingly high effectiveness against acute lymphocytic leukemia and malignant lymphoma. Currently, many researchers are trying to develop CAR-T cells for various types of cancer. However, cell surface target antigens have to be identified for each kind of cancer to develop CAR-T cells.

Development of a new CAR-T cell therapy for multiple myeloma

Cancer-specific cell surface antigens are ideal targets for CAR-T cell therapy. However, such antigens are not likely to remain unidentified following extensive searching by transcriptome or proteome analysis. We discovered that the active conformer of an integrin could serve as a specific therapeutic target for multiple myeloma (MM), which is an incurable hematological cancer characterized by the accumulation of neoplastic plasma cells in the bone marrow (BM). We first identified MMG49 as an MM-specific mAb after screening more than 10,000 anti-MM mAb clones, and found that it specifically recognized a subset of integrin β7 molecules. The MMG49 epitope is located in the N-terminal region of the β7 chain, which is predicted to be inaccessible in the resting integrin conformer, but exposed in the active conformation. Elevated expression and constitutive activation of integrin β7 conferred high MMG49 reactivity on MM cells, whereas MMG49 binding was barely detectable in other types of cells, including normal integrin β7+ lymphocytes. Furthermore, T cells transduced with MMG49-derived CAR exerted anti-MM effects without damaging normal hematopoietic cells. A clinical trial of MMG49 CAR T-cell for MM is now on-going.

Development of CAR-T cell therapy targeting antigen structures formed as results of post-translational events in various types of cancer

The above finding suggests that cancer immunotherapeutic targets may yet be identified in many cell-surface proteins that undergo post-translational changes, even if the expression of the proteins themselves is not cancer-specific. Thus, we applied the same strategy to various types of cancer. For hematological cancers such as acute myeloid leukemia (AML), the only hurdle for developing CAR-T cell therapy is lack of an appropriate cancer-specific cell surface antigen. We have already established huge numbers of mAbs reacting with AML cells, and are now selecting ones recognizing antigen structures highly specific for AML cells. In the case of solid tumors, while inefficient trafficking of CAR-T cells to tumor sites and immune-suppressive tumor microenvironment hamper development of effective CAR-T cell therapy, the most critical problem is lack of appropriate cancer-specific target antigens. We have started looking for cell surface antigen structures for various types of cancer in collaboration with several departments treating cancer in Osaka University Hospital.

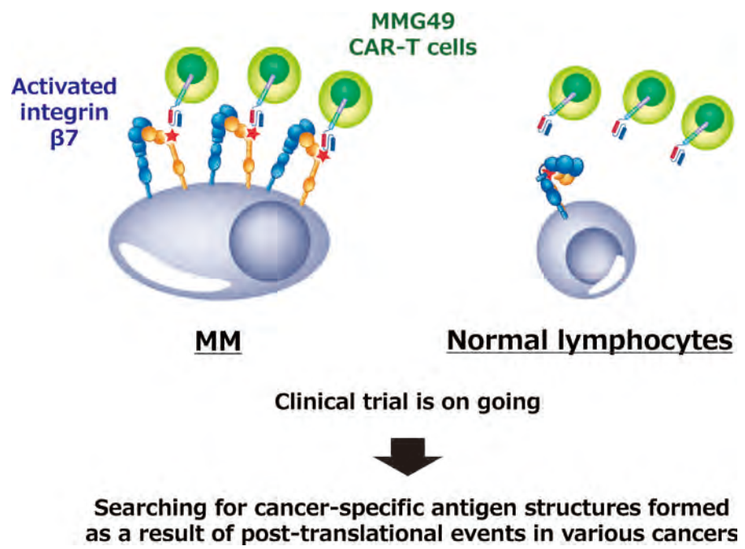

Figure. MMG49 CAR T-cell therapy targeting the active conformation of integrin β7 is promising for MM

Recent Publications

- Shingai Y, Yokota T, Okuzaki D, Sudo T, et al. Autonomous TGFbeta signaling induces phenotypic variation in human acute myeloid leukemia. Stem Cells (2021), in press.
- Toda J, Ichii M, Oritani K, et al. Signal-transducing adapter protein-1 is required for maintenance of leukemic stem cells in CML. Oncogene 39: 5601-5615 (2020).
- Hosen N, Matsunaga Y, Hasegawa K, et al. The activated conformation of integrin beta7 is a novel multiple myeloma-specific target for CAR T cell therapy. Nat Med 23: 1436-1443 (2017).

Single Molecule Imaging

Toshio Yanagida, PhD
Ben Seymour, MD/PhD

Professor — Toshio Yanagida
Ben Seymour

The lab continues to study what happens in the brain during injury and inflammation. We focus on how these elicit a suite of behavioural responses that are appropriate to injury: pain, fatigue, and lowered mood. That is, these behaviours are adaptive in normal circumstances, in that they allow the individual to protect tissue that may be vulnerable as a result of existing damage, conserve energy, and reduce unnecessary reward seeking behaviour that would consume resources not critical for survival. In particular, we have been developing behavioural tasks that allow us estimate and quantify these effects in any individual, which therefore provide a biomarker of the impact of an inflammatory disease on cognitive and affective function. Our analysis is based on sophisticated computational models of behaviour, and yields a set of inferred parameters that capture the underlying mechanistic basis of immune-brain modulation. Practically, this is a panel of results that reflects an immune-behavioural signature in any individual, as shown in Fig 1. We have been validating this methodology in patients with rheumatoid arthritis. In the context of COVID-19, this testing ability has particular relevance to the evaluation of patients with long COVID syndrome. This refers to the persistent symptoms of fatigue and cognitive blunting that effects a high proportion of post-COVID sufferers (up to 87%), and represents a major challenge to healthcare systems in 2021 onwards. We have therefore been developing our methodology to be used in an online setting (as a mobile-phone or tablet application) that would potentially allow us to gather large-scale data in any country.

In parallel, we continue to develop theoretical and computational models of injury-related behaviour, using mathematical modelling and simulation based approaches. In particular, we've been studying how the brain uses pain signals to shape conservative (i.e. non-risky / safe) behaviour in dangerous environments. The broader context for this work is that we want to understand how changes in homeostatic status, such as that which occurs after injury or inflammation, can change our behaviour. Ultimately we want to be identify how and where immune or neural information can signal to the brain that behaviour needs to change. Using our modelling approach, we have proposed algorithms that capture the trade-off between risky and conservative behaviour in the context of dangerous environments, and shown how they predict behaviour in a set of artificial intelligence test-bed scenarios: we do this in simple environments (termed tabular reinforcement learning), and complex environments (termed deep reinforcement learning) – see figure 2. These simulations provide predictions that can now been tested in real human experiments – both in healthy people, and those with injuries or inflammatory disease.

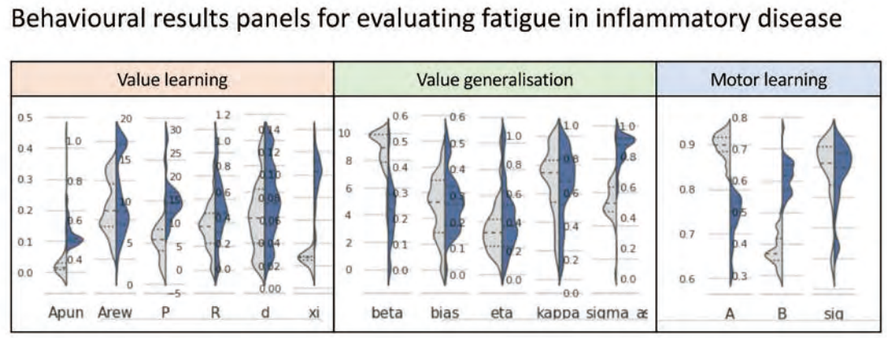

Figure 1. Behavioural data panel based in inferring the underlying computation parameters that may be modulated as a function of inflammatory disease. These can be used as a biomarker of immune-brain interactions.

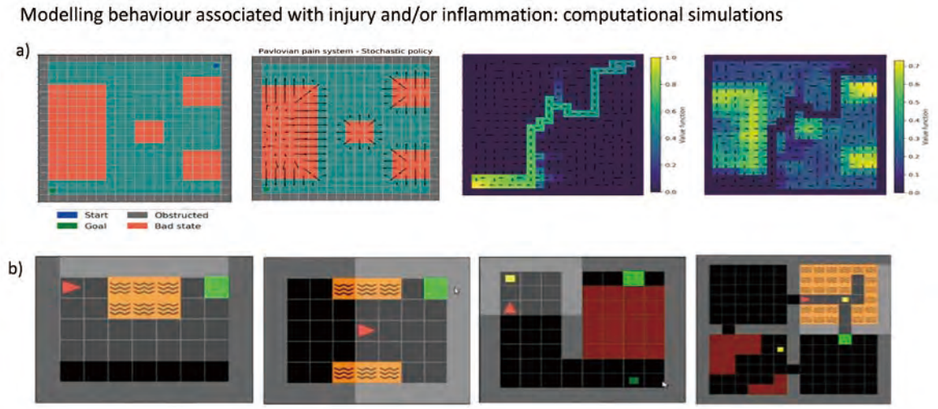

Figure 2. Some examples of simulations of an artificial agent that inhabits a dangerous world, and needs to navigate to avoid states that cause injury, in simple and complex (deep) environments.

Recent Publications

- Zhang S, Yoshida W, Mano H, Yanagisawa T, Mancini F, Shibata K, Kawato M and Seymour B. Pain control by co-adaptive learning in a brain-machine interface. Current Biology 30(20): 3935-3944 (2020).
- Becker S, Loffler M, Seymour B. Reward enhances pain discrimination in humans. Psychological Science 31(11) (2020).

Immunology and Cell Biology

Masaru Ishii, MD/PhD

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Assistant Professor	Takao Sudo
	Yutaka Uchida
Postdoctoral Fellows	3
Research Assistants	2
Support Staff	1

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

We developed a novel imaging system to visualize inside bones using intravital multiphoton microscopy. Using this methodology, we succeeded in visualizing the *in vivo* behaviors of osteoclast and osteoblast as well as the activity of bone-resorbing by osteoclasts. In addition to development of methodology, we are also updating various materials for bone imaging such as mouse models to visualize osteoclast and osteoblast and probes to visualize the bone resorption activity of osteoclasts. Our major contributions to the field of bone biology research are as follows:

- Identifying S1P (Sphingosine-1-phosphate) as a key factor in controlling the migratory behavior of osteoclast precursors *(Nature 2009, J. Exp. Med. 2010)*.
- Showing the substantial contribution of S1P-mediated migration of bone cells by generating S1P transporter deficient mice *(J. Clin. Invest. 2012)*.
- Identifying Vitamin D as significantly suppressing bone destruction by modulating S1P-mediated migration of osteoclast precursor *(Proc. Natl. Acad. Sci. USA 2013)*.
- Proposing two distinct mature osteoclast functional states: bone-resorbing mature osteoclasts and non-resorbing mature osteoclasts *(J. Clin. Invest. 2013)*.
- Showing the importance of epigenetic processes of osteoclast in the regulation of cellular metabolism and differentiation *(Nat. Med. 2015)*.
- Developing a new fluorescence probe for detecting bone surface pH to visualize bone resorption by mature osteoclasts *(Nat. Chem. Biol. 2016)*.
- Applying intravital bone imaging to drug efficacy evaluation and elucidation of mechanism of action *(Ann. Rheum. Dis, 2018; JBMR plus, 2018)*.
- Showing the importance of cell to cell communication between mature osteoblasts and mature osteoclasts for bone homeostasis *(Nat. Commun. 2018)*.

PTH (parathyroid hormone) is an important hormone that controls bone and calcium metabolism and is currently used as a therapeutic agent for osteoporosis. In a previous study *(Nat. Commun. 2018)*, we demonstrate that PTH suppresses bone resorption through promoting interaction of osteoblasts and osteoclasts but could not clarify the detailed molecular mechanism. In the follow-up study *(Nat. Commun. 2021)*, we identify SLPI (secretory leukocyte protease inhibitor) as a key mediator for PTH-induced bone formation. We showed SLPI up-regulation in osteoblasts from PTH-treated mice. Furthermore, *Slpi* mutant mice analysis exhibited that PTH-induced bone mass increase was suppressed in *Slpi* mutant mice. Through this study, we demonstrate that two distinct functions of SLPI for PHT-induced bone formation; 1) intracellular SLPI effects on osteoblasts to promote osteoblast differentiation and proliferation, 2) extracellular SLPI effects on the interaction with osteoblasts and osteoclasts and suppresses bone resorption by osteoclasts. Our results suggest that SLPI regulates both bone formation and bone resorption.

2. Identification of origin and function of abnormal osteoclasts: “AtoM”

Inside live bones, osteoclasts and osteoblasts are constantly generated. Under well-controlled bone destruction and formation processes, bone constantly undergoes destruction and formation to maintain homeostasis. In our previous 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 RA (rheumatoid arthritis), called Arthritis-associated osteoclastogenic Macrophages, or “AtoMs” (Nat. Immunol. 2019). We found that the arthritis-inducing abnormal osteoclasts have distinct properties and origins from normal osteoclasts involved in bone metabolism. By targeting the differentiation and functions of arthritis-inducing abnormal osteoclasts, we will be able to establish innovative therapeutic strategies and next-generation of anti-RA drugs that do not affect normal osteoclasts and normal bone homeostasis.

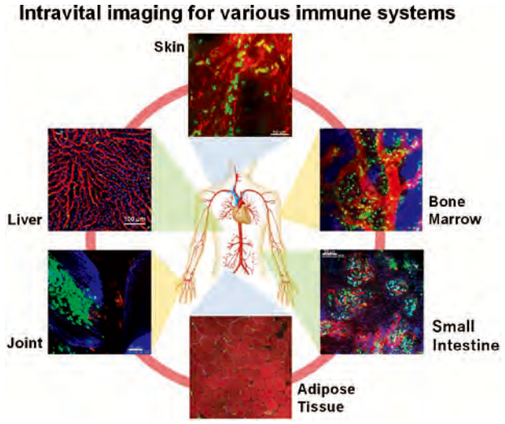

Figure. *In vivo* cellular dynamics in various immune systems. Immune cells are highly dynamic and interconnect various tissues and organs, by forming a ‘soft-wired’ network. We are elucidating the basic principle controlling the dynamic nature of immune cells by visualizing in vivo behaviors using advanced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

3. A new mechanism for bone marrow recovery from chemotherapy treatment: Emergency switch by innate lymphoid cells

We discovered that iLC2 (type 2 innate lymphoid cells) in the bone marrow detect a critical state in bone marrow cells after chemotherapy treatment and promote recovery from blood cell loss through GM-CSF (Granulocyte Macrophage colony-stimulating Factor) secretion (J. Exp. Med 2021). We developed an intravital imaging system for HSPCs (hematopoietic stem/progenitor cells) and found the unusual behavior of HSPCs transplanted into the bone marrow with chemotherapy treatment. We performed gene expression analysis using RNAseq for the HSPCs showing the abnormal behavior and found that the HSPCs proliferated under stimulation of GM-CSF from the bone marrow environment after chemotherapy treatment. Further single-cell RNAseq analysis identified that iLC2 was responsible for GM-CSF secretion in the injured bone marrow. Transplantation of iLC2 into chemotherapy treated mice accelerated the recovery from blood cell loss. We expect GMCSF and iLC2 can be applied in new therapeutic strategies for recovery from blood cell loss after chemotherapy treatment.

Recent Publications

- Morimoto A, et al. SLPI is a critical mediator that controls PTH-induced bone formation. Nat. Commun. 12: 2136 (2021).
- Sudo T, et al. Group 2 innate lymphoid cells support hematopoietic recovery under stress conditions. J. Exp. Med. 218: e20200817 (2021).
- Matsui T, et al. Nonlinear optics with near-infrared excitation enable real-time quantitative diagnosis of human cervical cancers. Cancer Res. 80: 3745-3754 (2020).
- Hasegawa T, et al. Identification of a novel arthritis-associated osteoclast precursor macrophage regulated by FoxM1. Nat. Immunol. 20: 1631-43 (2019).
- Simmons S, et al. High-endothelial cell-derived S1P regulates dendritic cell localization and vascular integrity in the lymph node. eLife. 8: e41239 (2019).

Nuclear Medicine

Jun Hatazawa, MD/PhD

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Postdoctoral Fellows	1
Visiting Scientists	1
Support Staff	1

The Nuclear Medicine Laboratory has been working on a cancer therapy by means of alpha-emitting radioisotopes and on chronic neuro-inflammation imaging by means of positron emission tomography (PET) in patients with multiple sclerosis and in those with child-onset epilepsy.

Targeted Alpha Therapy (TAT) is an application of the radiation emitted from alpha-particles (= ^{4}He nucleus) to treat locally advanced and/or metastatic cancers. Alpha particles have a robust cell-killing effect because of its high radiation quality factor. To develop TAT, the key issues are 1) producing large amounts of alpha particles such as Actinium-225 (^{225}Ac, physical half-life: 9.9 day) and Radium-223 (^{223}Ra, physical half-life: 11.4 day) effective for clinical use, 2) delivering alpha particle-bound molecules to target specific cancer cells, and 3) evaluating damage to normal tissue. In a clinical setting, ^{223}Ra dichloride is used to treat castration-resistant prostate carcinoma patients with bone metastasis. These alpha emitting radio-isotopes are produced in foreign-based nuclear reactors and not in Japan.

The element 85, Astatine-211 (^{211}At, physical half-life: 7.2 hour), is another alpha-emitting radio-isotope and is produced by a particle accelerator in the Research Center for Nuclear Physics at Osaka University. We succeeded in the production and purification of ^{211}At for preclinical studies (Watabe T, et al., J. Nucl. Med. 2019). ^{211}At belongs to the halogen family in the periodic table and its distribution is similar to ^{131}I. After administering 0.1 MBq or 1.0 MBq of ^{211}At-NaAt, elevated amounts accumulated in the thyroid gland, stomach, bladder, heart, lungs, spleen, kidneys, and testis in rats, similar to the results after ^{131}I-NaI administration. The 0.1 MBq group showed no abnormalities while the 1.0 MBq group showed decreased body weight and food intake. Histological analysis showed atrophy and fibrosis in the thyroid gland. The administration of 1.0 MBq of ^{211}At-NaAt resulted in transient toxicity in the white blood cells and testis without severe hematological or renal toxicity. The total cholesterol, total albumin, and total protein increased with no signs of recovery probably due to hypothyroidism (Liu Y, et al., Trans. Oncol. 2020). Based on these results, a phase I/II clinical trial of ^{211}Na-NaAt for the treatment of intractable thyroid carcinoma is planned with support from AMED (Principal Investigator: Watabe T).

We are developing a new TAT molecule, α-Methyl-l-tyrosine (AMT). AMT has a high affinity for LAT1 which is predominantly expressed on the membrane of human major cancers (Kaneda-Nakashima K, et al., Cancer Science 2020). ^{211}At-labeled α-methyl-l-tyrosine (^{211}At-AAMT) is a potential carrier of ^{211}At into tumors. We evaluated the accumulation of ^{211}At-AAMT *in vivo* in mice by means of SPECT (E-cam, Siemens, Munich, Germany). The SPECT image showed an accumulation of ^{211}At-AAMT in the transplanted tumor. In an experiment of tumor growth suppression, the PANC-1 cells tumor-bearing mice were intravenously injected with ^{211}At-AAMT at 0.4 MBq/mouse. This treatment suppressed tumor growth for 40 days without significant loss of body weight. The ^{211}At-AAMT of 0.1MBq/mouse and 1 MBq/mouse reduced metastatic lesions in the lungs of the B16F10 metastasis model in C7BL/65 mice. Our results suggests that ^{211}At-AAMT can be a candidate for the TAT agents to target various entities of human carcinoma.

Another potential TAT agent is fibroblast activation protein inhibitor (FAPI) labeled with ^{225}Ac. FAPI targets cancer stroma cells developed by the Heidelberg University Hospital group (Giesel FL, et al., J. Nucl. Med. 2019). We confirmed a tumor growth suppression effect of ^{225}Ac-FAPI in the tumor xenograft model of mice (Watabe T, et al., J. Nucl. Med. 2020).

Neuro-inflammation is associated with a variety of chronic neurological diseases. We developed methods that use PET to evaluate the severity and extent of neuro-inflammation. In patients with multiple sclerosis, ^{11}C-acetate was utilized to evaluate the metabolism of reactive astrocytes in the brains of patients with multiple sclerosis. Kinetic analysis revealed that the rate constant K2, representing efflux of acetate from glial cells to systemic circulation, was higher than normal in MS patients both in white matter and gray matter (Kato H, et al., J Cerebr. Blood. Flow. Metab. 2020). In patients with child-onset epilepsy, translocator protein targeting PET tracer ^{11}C-DPA713 showed increased accumulation in the epileptogenic foci indicating neuro-inflammation in the epileptic foci (Kagitani-Shimono K, et al., J. Neuroinflam. 2020).

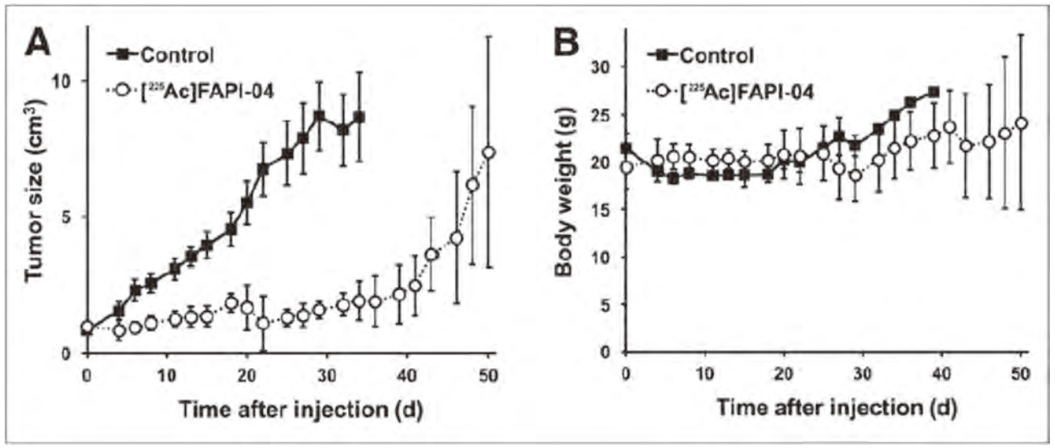

Figure. Treatment effect (A) and change in body weight (B) in PANC-1 xenograft mice model after injection of ^{225}Ac-FAPI-04 (Watabe T, et al. J Nucl Med. 61:563-569, 2020)

Recent Publications

- Liu Y, Watabe T, aneda-Nakashima K, et al. Preclinical evaluation of radiation-induced toxicity in targeted alpha therapy using [^{211}At] NaAt in mice: a revisit. Translational Oncology 13: 100757 (2020).
- Kaneda-Nakashima K, Zhang Z, Manabe Y, et al. K-emitting cancer therapy using ^{211}At-AAMT targeting LAT1. Cancer Science (2020). doi: 10.1111/cas.14761
- Watabe T, et al. Theranostics Targeting Fibroblast Activation Protein in the Tumor Stroma: ^{64}Cu-and ^{225}Ac-Labeled FAPI-04 in Pancreatic Cancer Xenograft Mouse Models. J Nucl Med. 61(4): 563-569 (2020).
- Kagitani-Shimono K, et al. Clinical evaluation of neuroinflammation in child-onset focal epilepsy: a translocator protein PET study J Neuroinflammation. 18: 8 (2021).
- Kato H, et al. Astrocyte metabolism in multiple sclerosis investigated by 1-C-11 acetate PET. J Cerebr Blood Flow Metab. 41(2): 369-379 (2021).

Chemical Imaging Techniques

Kazuya Kikuchi, PhD

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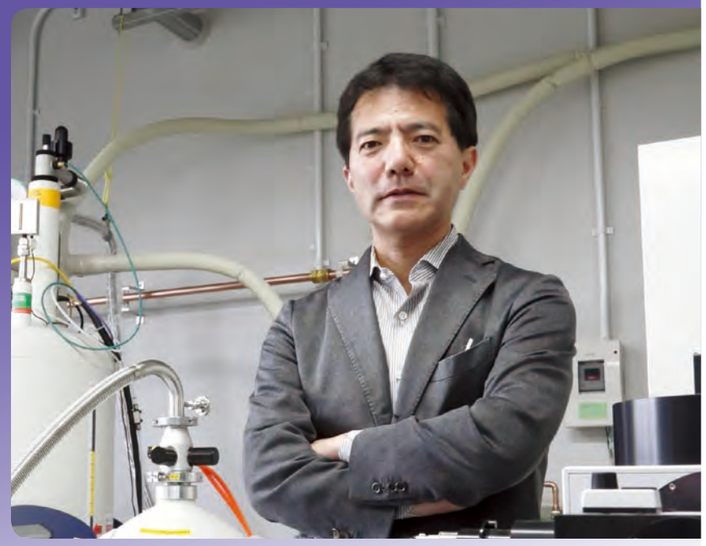

An Acid Activatable Fluorescence Probe for Imaging Osteocytic Bone Resorption Activity in Deep Bone Cavities

Long lived osteocytes constitute around 90-95% of all bone cells and exist within disk-shaped cavities called osteocytic lacunae that are present throughout the mineralized bone matrix. Osteocytes project their long dendrites into these canals, which allows them to detect and modulate shear stress through communication with surface bound osteoblasts and osteoclasts that are responsible for modifying bone structures. Osteocytes are known to directly remodel the bone walls of their lacuna-canalicular systems in response to hormones produced by lactating or hibernating animals, with lacunae bone wall mineralisation producing larger cavities in a process known as osteocytic osteolysis. However, the physiological role of osteocytes in direct bone mineralisation is still open to debate, so the availability of a high-resolution imaging method that would enable osteocytic osteolysis to be imaged in real time would be extremely useful. We have previously developed a reversible OFF/ON pH-sensing probe (pHocas-3) containing bisphosphonate derived alendronate fragments that bind strongly to the bone matrix enabling visualisation of osteoclast mediated bone resorption processes. Unfortunately, attempts to employ pHocas-3 for the intravital imaging of low-pH regions in osteocytic lacunae within the bones of living mice proved unsuccessful.

Thus, we developed a new probe (pHocas-RIS) that could be used to visualize the low pH environments of osteocytic lacunae that contained acid secreting osteocytes (Figure 1, top). Conjugation of the moderate bone binding drug risedronate to a pH-activatable BODIPY fluorophore afforded pHocas-RIS in order to penetrate osteocytic lacunae cavities embedded deep within the bone matrix. The fluorescence quantum yields (QY) of the probes at different pH values were acquired by measuring their absorption and fluorescence emission spectra in a citrate-phosphate buffer. The fluorescence intensity of the pHocas-RIS probe increased approximately 15-fold as the pH decreased from 8.0 to 4.0, with changes in its QY at different pH levels used to calculate the pK_a value of its aniline moiety as 6.8.

Imaging studies were then carried out using transgenic TRAP-tdTomato mice containing osteoclasts expressing a red fluorescent protein. Daily subcutaneous administration of both probes to these genetically modified mice over a 3 day period was followed by their anaesthetisation and two-photon imaging of the medullary cavities of their thin calvaria parietal bones. Processing of the images were carried out using spectroscopic unmixing algorithms that enabled fluorescence signals from the probe (green), tdTomato (red) and second-harmonic generation (SHG) emissions of bone collagen fibres (blue) to be distinguished. Imaging studies revealed the presence of green fluorescence signals from protonated pH-activatable pHocas-RIS produced from the action of stationary acid secreting osteoclasts in direct contact with bone surfaces within the bone marrow cavity.

Further imaging studies revealed the presence of small numbers of ring-shaped green fluorescence signals of the protonated pHocas-RIS that were present in bone obtained from deep cortical regions (Figure 1, bottom). It is proposed that these fluorescent signals originate from probes bound to the walls of osteocytic lacunae containing bone resorptive osteocytes that were actively secreting acid to create a low pH environment. The pH responsiveness of the pHocas-RIS probe within the lacunae of the bone matrix was confirmed by carrying out ex vivo fluorescent imaging of cryogenically prepared deep bone slices at different pH levels. These studies revealed the presence of strong fluorescent signals for osteocytic lacunae in a bone section treated at pH 4.0, reduction in fluorescent intensity of a bone section at pH 7.0, and no fluorescence for a bone section treated at pH 10.0. This clearly demonstrated that the intensity of the pH-activatable fluorescent response of the pHocas-RIS probe can be used to effectively image the pH of osteocytic lacunae in living bone. Our pH responsive probe can be used to visualize the bone mineralizing activities of acid producing osteocytes in real time, thus providing a valuable imaging method to explore their central role in remodeling bone-matrix in health and disease.

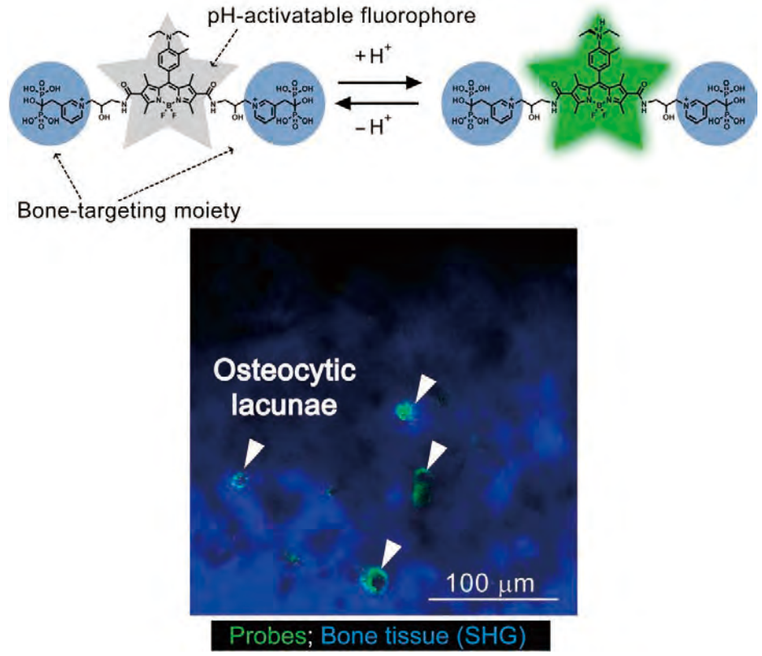

Figure 1. (top) Chemical structure of risedronate containing pH-activatable pHocas-RIS probe. (bottom) Two-photon excitation imaging of calvaria parietal bones after administration of pHocas-RIS. White arrows indicate protonated pHocas-RIS bound to the walls of acidic osteocytic lacunae.

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- Kowada T, Arai K, Yoshimura A, Matsui T, Kikuchi K & Mizukami S. Optical Manipulation of Subcellular Protein Translocation Using a Photoactivatable Covalent Labeling System. Angew. Chem. Int. Ed. in press (2021).
- Hori Y, Nishiura M, Tao T, Baba R, Bull SD & Kikuchi K. Fluorogenic Probes for Detecting Deacylase and Demethylase Activity towards Post-translationally-modified Lysine Residues. Chem. Sci. 12: 2948-2503 (2021).
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- Torii K, Hori Y, Watabe K, & Kikuchi K. Development of Photoswitchable Fluorescent Molecules Using Arylazopyrazole. Bull. Chem. Soc. Jpn. 93: 821-824 (2020).

Immune Response Dynamics

Kazuhiro Suzuki, MD/PhD

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Research Assistants	2
Support Staff	2

Our laboratory has been studying the interactions between the nervous and immune systems with a special focus on the roles of adrenergic nerves, which constitute the efferent arc of the sympathetic nervous system, 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 phase 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), of which the functions had been totally unclear (Fig. A).

We first identified COMMD8 as a protein that binds to the C-terminal tail of a representative chemokine receptor CXCR4. Additional proteomic 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 mediated by the receptors to which the COMMD3/8 complex was recruited. Thus, the COMMD3/8 complex is a positive regulator of chemoattractant receptor signaling (Nakai et al., J. Exp. Med. 2019).

Agonist binding to GPCRs activates trimeric G proteins to induce 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 of the action of the COMMD3/8 complex demonstrated that this protein complex functions as an adaptor that selectively recruits GRK6 to chemoattractant receptors, which promotes MAPK activation and consequently lymphocyte chemotaxis (Fig. 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 (Nakai et al., J. Exp. Med. 2019).

Consistent with the reduced chemotactic responses of COMMD3- and COMMD8-deficient B cells, the mutant B cells showed multiple defects in their migration in vivo (Fig. C). Additionally, deficiency of COMMD3 or COMMD8 in B cells severely impaired production of antigen-specific antibodies (Fig. D), which was accompanied with reduced formation of germinal centers (Fig. E). Therefore, the COMMD3/8 complex plays essential roles in the control of B cell migration and induction of humoral immune responses (Nakai et al., J. Exp. Med. 2019). To test the involvement of the COMMD3/8 complex in autoimmune diseases, we established an experimental system that allowed inducible deletion of the COMMD3/8 complex in moue models of autoimmunity. Notably, deletion of the COMMD3/8 complex in the course of the diseases blocked their clinical and pathological progression with suppressed production of autoantibodies. These findings suggest that the COMMD3/8 complex is a potential therapeutic target for autoimmune diseases.

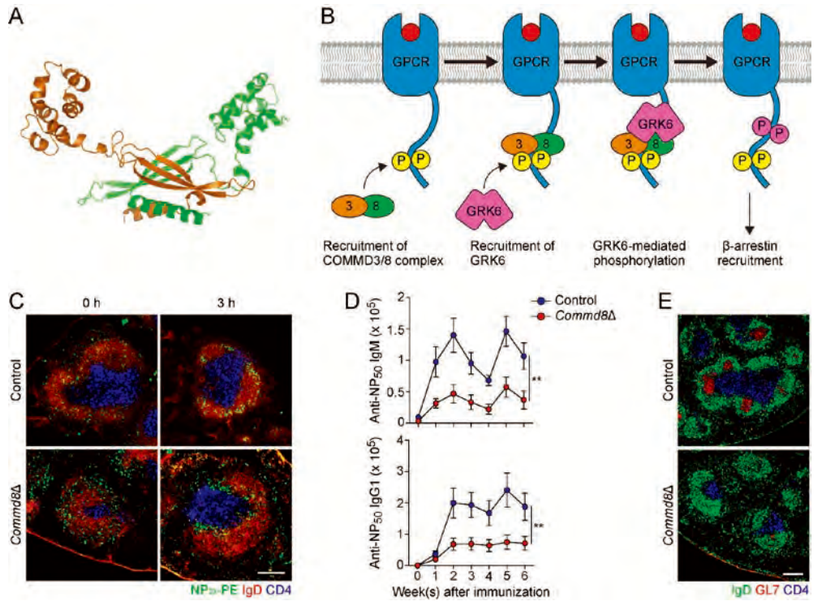


Figure. Role of the COMMD3/8 complex in GPCR signaling and immune responses.
(A) ***In silico*** modeling for the structure of the COMMD3/8 complex. COMMD3, orange; COMMD8, green.
(B) Proposed role of the COMMD3/8 complex in GRK6 recruitment to GPCRs.
(C) Defective outer follicular migration of COMMD8-deficient (***Commd8***Δ) B cells at an early time point (3 h) after immuniza tion.
(D and E) Impaired antibody production (D) and germinal center formation (E) in B cell-specific COMMD8-deficient mice. Scale bars, 200 μm.

Recent Publications

- Nakai A, Leach S and Suzuki K. Control of immune cell trafficking through inter-organ communication. Int. Immunol. 2021 *in press*.
- 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).
- 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).

Biophotonics

Nicholas Isaac Smith, PhD

Associate Professor	Nicholas Isaac Smith
Associate Professor (Lecturer)	Nicolas Pavillon
Assistant Professor	Alison Hobro
Postdoctoral Fellows	1
Support Staff	1

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

We develop both fundamental techniques for label-free analysis, and in collaboration with other groups in IFReC, also develop applications in immunological cellular imaging and analysis. We use a variety of label-free approaches, including Raman scattering, which provides molecular signatures from a target cell, as well as quantitative phase imaging, which provides a complete quantitative map of the cell morphology. It is fortunate that both of these sets of information can be linked to cellular functions of interest, and can be used as classifiers of cell type or cell behavior. When looking at a culture dish, the morphology of cells can be seen to differ dramatically from cell to cell. Similarly, the biomolecular composition of each cell varies, though it is less trivial to observe. It may seem obvious that the composition of a cell reflects important aspects of its biological role, such as the protein expression in response to a stimulus, but each cell's composition also varies due to inherent and somewhat random biological variability. This is one reason why a multimodal approach can be ideal for teasing out subtle cellular biomolecular signals that are related to biological questions, which can otherwise be hidden by cell-to-cell differences. It also that to ensure the cellular information we measure is significant, a large number of single cell measurements is required.

We then continued to push towards higher throughput single-cell analysis. With larger numbers of samples, and subtle but important variations between classes of cells, we evaluated how well different machine-learning tools could assist the data analysis. There are many types of machine learning tools available. Some of the more "deep-learning" style of tools can classify aspects of samples, but are less helpful in terms of interpreting the differences in a biological sense. We therefore choose the method based on the problem we are trying to solve. Our recent study published this year (Pavillon et. al. Analyst, in press, 2021) showed that if we use linear methods and suppress any features that do not contribute to classification, the ability to discriminate between subtle features across single cells can become very robust. The remaining features can also be easier to interpret than similar information in more traditional methods. We demonstrated this in a well-defined case where cellular protein synthesis was inhibited, measurements were made in inhibited and control cells, and the resulting discrimination model showed us features consistent with mRNA accumulation in the cell.

In applications more directly related to human disease, we completed a study of fatty-acid induced changes in macrophage cells. Non-alcoholic fatty liver disease (NAFLD) is related to lipid-induced transitions of cells. There are very few ways to label and track the uptake and downstream changes associated different fatty acids in live cells. We therefore used Raman imaging which can non-invasively track these changes, finding that the uptake, processing and recovery from exposure to fatty acids varies depending on the chain length of the fatty acid and the degree of saturation. Toxicity was also clarified at higher levels of lipid exposure (Sugiyama et. al. Analyst, 2020). Images of the process are shown in Figure 1.

We continued our ongoing 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. NETS are important both in pathogen defense and also in numerous disease pathology, for example, vasculitis, sepsis, and others. It is thought that different types of NETs form in different circumstances, however, there are very limited methods for easily evaluating the NETs as they form around the neutrophils. We are now combining quantitative phase imaging, Raman analysis, and deep-learning to evaluate this specific question.

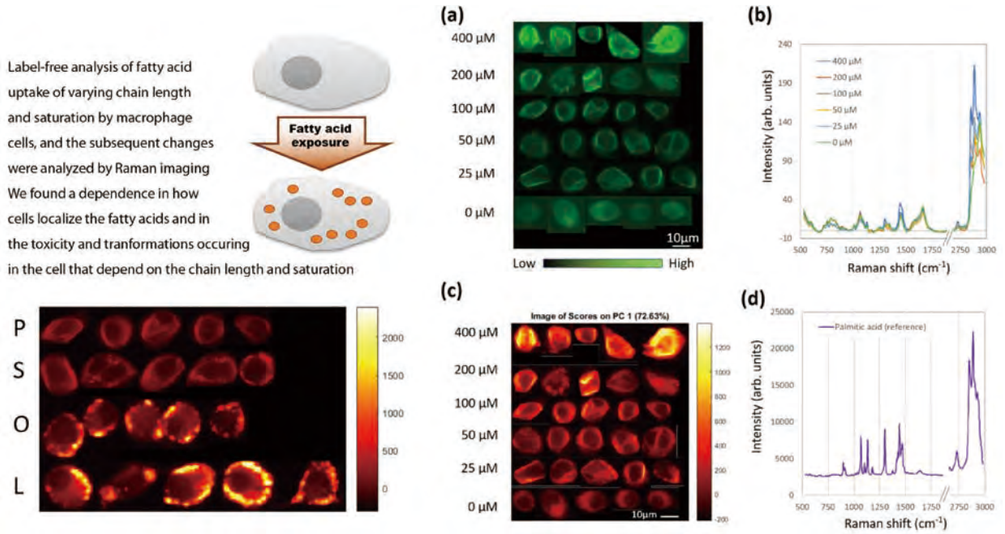


Figure 1. Uptake of palmitic acid observed in Raw264.7 macrophage cells. We compared the uptake of four different fatty acids (Palmitic, Steric, Oleic and Linoleic), and evaluate the transitions occurring in the macrophage. Changing the type of fatty acid, by varying the chain length and degree of saturation, affects the amount of uptake, the uptake timeline and the later recovery from exposure. Raman images were acquired at different concentrations, fatty acid type, and different time points. Panel (a) shows the Raman intensity (area under the curve between 2876 - 2891 cm-1) of five macrophage cells exposed to different concentrations of palmitic acid for 24 hours, (b) shows the average Raman spectra extracted from a 10 x 10 pixel block from all cells for each concentration of palmitic acid used and a reference Raman spectrum of palmitic acid, (c) PCA scores images for PC1 and PC2 for cells exposed to palmitic acid and (d) the reference spectra for purified palmitic acid aids the interpretation of the signals found in the macrophage cell. The five cells shown here are representative of the larger datasets collected for all cells per condition. From: Sugiyama et al. Analyst, 2020.

Recent Publications

- Pavillon N and Smith NI. Deriving accurate molecular indicators of protein synthesis through Raman-based sparse classification. Analyst. in press (2021).
- Sugiyama T, Hobro AJ, Pavillon N, Umakoshi T, Verma P, and Smith N. Label-free Raman mapping of saturated and unsaturated fatty acid uptake, storage, and return toward baseline levels in macrophages. Analyst 146 (4): 1268-1280 (2020).
- Lelliott PM, Momota M, Shibahara T, Lee MSJ, Smith NI, Ishii KJ, and 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): 2676-2685 (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).

Systems Immunology

Daron Standley, PhD

Professor	Daron Standley
Associate Professor	Kazutaka Katoh Shunsuke Teraguchi
Assistant Professor	Li Songling Llamas Covarrubias Mara Anais
Postdoctoral Fellows	2
Research Assistants	4
Support Staff	1

A. Software for sequence and structural analysis of Abs and TCRs

MAFFT is a very widely-used multiple sequence alignment (MSA) package. Aside from the obvious importance of MSAs in evolutionary biology, MSAs are becoming more and more important in structural bioinformatics as well. We utilize MAFFT MSAs for antibody and TCR structure prediction using Repertoire Builder. Repertoire Builder, in turn, is used by our antibody docking package, AbAdapt. Repertoire Builder is also used in our TCR-peptide-MHC modeling package, ImmuneScape, as well as for our BCR/TCR clustering software, InterClone. Moreover, protein structures can be used to improve protein MSA accuracy. To this end, we have developed MAFFT-DASH for structure-informed multiple alignment (Rosewicki, J. et al. 2019).

B. Structural modeling SARS-CoV-2 infection-enhancing Abs

During the COVID-19 pandemic, we analyzed betacoronaviruses that have infected non-human hosts, and identified a group of potentially important residues for virus evolution (Saputri, D. S. et al 2020), a subset of which turned out to characterize SARS-CoV-2 variants with enhanced infectivity (Katoh, K. and Standley, D.M. 2021). We also worked closely with Prof. Arase and several other labs to understand the structure and mechanism of a subset of antibodies that enhance SARS-CoV-2 infection (Liu, Y. et al, 2021). A key finding is that these antibodies appear to bridge neighboring spikes, and, by ding so, alter the conformation of the host receptor binding domain. We are continuing to study these antibodies using molecular dynamics and repertoire analysis in order to clarify both their role in disease progression and their prevalance in human pupulations.

C. Structural modeling of CAR T cell receptors

One of the applications of protein structural modeling is to develop more effective chimeric antigen receptor T cells (CAR T cells), most of which utilize a single-chain antibody heavy and light chain (scFv) linked by a short peptide fragment. In collaboration with a U. Penn team using anti-CD22 CAR T cells to treat acute lymphoblastic leukemia (ALL), we predicted the oligomerization state of the scFV and helped show that dimeric scFVs were significantly more effective than monomeric scFVs at tumor killing (Singh, N. *et al.* 2021). This research, in turn, has renewed efforts in our lab to understand the mechanistic role of TCR oligomerization in T cell activation and inactivation.

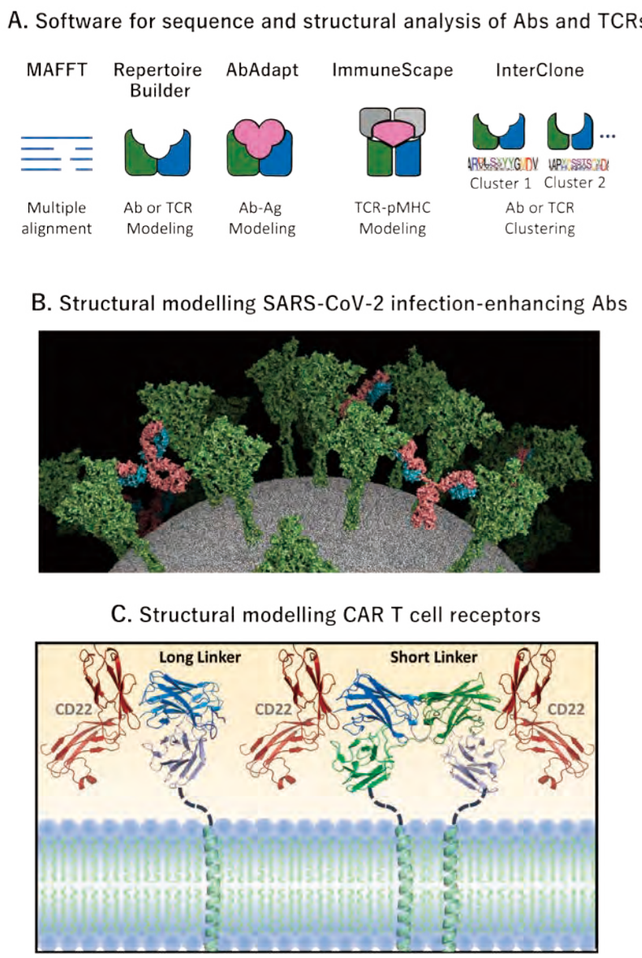


Figure 1. A, in-house tools that can be used to model antibody structure and function; B, enhancing antibodies (pink, cyan) bridge SARS-CoV-2 spike trimers (green) on the virus surface (grey); C, the length of the scFv linker affects oligomerization, which, in turn, has significant effects on CAR T cell efficacy.

Recent Publications

- Singh N et al. Antigen-independent activation enhances the efficacy of 41BB co-stimulated CD22 CAR T cells. Nat. Med. in press (2021).
- Liu Y et al. An infectivity-enhancing site on the SARS-CoV-2 spike protein is targeted by COVID-19 antibodies. Cell in press (2021).
- Xu Z et al. Functional clustering of B cell receptors using sequence and structural features. Mol. Syst. Des. Eng. 4: 769-778 (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 (2019).

Statistical Immunology

Yukinori Okada, MD/PhD

Professor	Yukinori Okada
Research Assistants	4
Support Staff	2

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 of millions of the 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 to the latest large-scale disease genome and multi-layer omics data.

Metagenome-wide association study of gut microbiome revealed disease-specific feature of multiple sclerosis

Microbiomes play substantial roles in biology of a variety of human immune-related diseases. We conducted metagenome-wide association studies (MWAS) of gut microbiome of multiple sclerosis (MS) utilizing whole-genome shotgun sequencing, a promising tool to elucidate microbiome etiology. Phylogenetic MS case-control association tests showed discrepancies of clades, including those implicated in immune system (e.g., *Erysipelatoclostridium_sp.* and *Gemella morbillorum*). Gene association tests found an increased abundance of one putative dehydrogenase gene (Clo1100_2356) and one ABC transporter related gene (Mahau_1952) in the MS metagenome. We further demonstrated interaction between the MS metagenome and host genome pathways (i.e., MWAS and genome-wide association study (GWAS) interaction; Kishikawa T et al. *Front Cell Infect Microbiol* 2020).

Application of machine-learning technologies to human genomics

Dynamic application of machine learning (ML), especially deep learning (DL), is warranted in the field of human immunology. As an initial introductory application of DL to the large-scale population genomes, we developed a new HLA imputation method named DEEP*HLA (Figure 1). By converting population genomes into digital images, DEEP*HLA can computationally estimate HLA gene variants from the surrounding SNPs. DEEP*HLA imputation is less biased from distant-dependent linkage disequilibrium (LD) decay, which enabled more accurate imputation of rare HLA alleles when compared with previous ML methods for HLA imputation (Naito T et al. *Nat Commun* 2021).

Novel drug discovery by in silico screening utilizing human genome and gene expression databases

Consideration of biological dynamics in disease status provides directional dosage effect on drug targets, which facilitates efficient high-throughput screening of chemical compounds from library. To this end, we have developed a novel in silico drug discovery pipeline named Trans-Phar (Figure 2). Trans-Phar screens potential chemical compounds by assessing negative correlation between genetically-regulated case-control gene expression profiles and compound-regulated gene expression profiles. Application of Trans-Phar successfully identified the drugs currently indicated (or under clinical trial) for the input GWAS diseases themselves. Trans-Phar is computationally simple and only requests publicly available data, such as GWAS summary statistics and compound-perturbed gene expression data (Konuma T et al. *Hum Mol Genet* 2021).

Genetic studies of human immune-related phenotypes

We conducted GWAS of human immune-related diseases and clinical subtypes, to identify genetic risk loci and responsible genes. An GWAS of rheumatoid arthritis-interstitial lung disease (RA-ILD) in the Japanese population identified one novel RA-ILD risk locus of *RPA3-UMAD1* at 7p21. The RA-ILD risk of the identified variant was relatively high in the CT image patterns related to fibrosis. Our study should contribute to elucidation of the complicated aetiology of RA-ILD. A GWAS of autoimmune pulmonary alveolar proteinosis (aPAP) of Japanese, which identified that one common HLA class II allele, HLA-DRB1*08:03, strongly drove risk of aPAP. The HLA-DRB1*08:03 allele was also associated with an increased level of anti-GM-CSF antibody, a key driver of the disease (Shirai Y et al. *Ann Rheum Dis* 2020, Sakaue S et al. *Nat Commun* 2021).

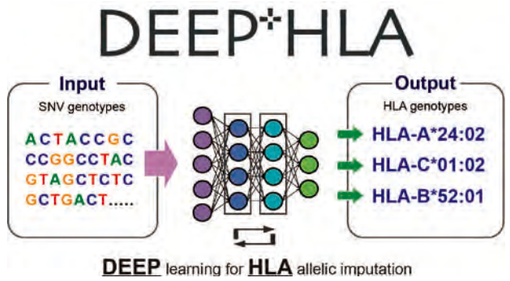


Figure 1. DEEP*HLA, a convolutional deep learning method for imputing HLA genotypes.

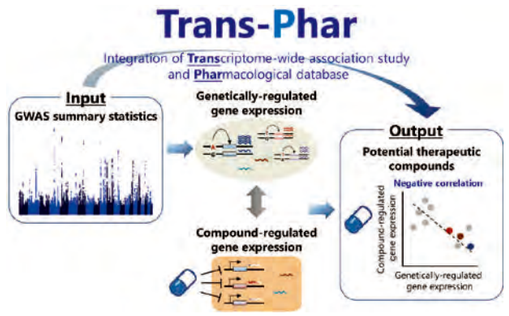


Figure 2. Trans-Phar, integration of Transcriptome-wide association study and pharmacological database for *in silico* drug screening.

Recent Publications

- Naito T. et al. A deep learning method for HLA imputation and trans-ethnic MHC fine-mapping of type 1 diabetes. Nat Commun 12: 1639 (2020).
- Konuma T. et al. Integration of genetically regulated gene expression and pharmacological library provides therapeutic drug candidates. Hum Mol Genet 30: 294-304 (2021).
- Sakaue S. et al. Genetic determinants of risk in autoimmune pulmonary alveolar proteinosis. Nat Commun 12: 1032 (2021).
- Kishikawa T. et al. A Metagenome-Wide Association Study of Gut Microbiome in Patients with Multiple Sclerosis Revealed Novel Disease Pathology. Front Cell Infect Microbiol 10: 585973 (2021).
- Shirai Y. et al. Association of the RPA3-UMAD1 locus with interstitial lung diseases complicated with rheumatoid arthritis in Japanese. Ann Rheum Dis 79: 1305-1309 (2021).

Quantitative Immunology

Associate Professor Diego Diez

Postdoctoral Fellows 1

Our group integrates computational and single cell genomics techniques to understand the immune system. We develop computational methods to analyze single cell data. We integrate experimental data (including transcriptome, chromatin accessibility, protein expression, immune repertoire and spatial transcriptomics) with publicly available information into network models of immune regulation. We apply this framework to study gene regulatory networks controlling immune cell development and function.

Development of computational methods

An important problem in single cell genomics is how to combine different datasets while correcting for batch effects. A key focus is on preserving the original cell population structure while not introducing bias. We have developed Canek, a method that leverages a fuzzy logic framework that enables efficient batch correction without bias. Another problem is the identification of marker genes. In collaboration with Alexis Vandenbon at Kyoto University, we have developed *singleCellHaystack*, a method to identify differentially expressed genes from multi-dimensional representations of single cell genomics data.

Mathematical modeling

The large number of cells obtained in single cell genomics experiments opens the door to approaches that study the immune system using mathematical modeling and machine learning. Transcriptional regulatory networks are critical determinants of cell identity and function. We use linear regression and machine learning to model immune transcriptional regulatory networks. Using the expression level of the regulators as a proxy for their activities we apply these methods to study how these networks change during immune cell differentiation and under different experimental conditions.

Applications to immunology

We study the development of NKT cells. SKG mice have a mutation in ZAP70 molecule that causes weakened TCR signaling resulting in a bias towards development of NKT1 compared to NKT2 in the wild type. Using single cell transcriptomics, protein expression, immune repertoire and chromatin accessibility we study the differentiation of NKT cells in the thymus and spleen of SKG and WT mice. In a more clinical setting, we apply single cell genomics to get insight into IgA nephritis onset and therapies. We analyze the transcriptome and protein expression of T and B cells in the PBMC from IgAN patients before and after treatment with e.g., tonsillectomy and/or steroid immunosuppression. We combine these datasets with spatial transcriptomics from tonsils to identify factors that lead to successful therapy.

We collaborate with other groups at IFReC to study diverse aspects of immune responses in a basic and clinical setting. With the Experimental Immunology laboratory and the Systems Immunology laboratories, we study how TCR signaling impacts T cell repertoire in SKG mice. With the Immune Regulation laboratory, we study the role of CD4 T cells in human Eosinophilic Chronic Rhinosinusitis. With the Host Defense laboratory, we study the role of the RNase Reg-1 in immune cell development.

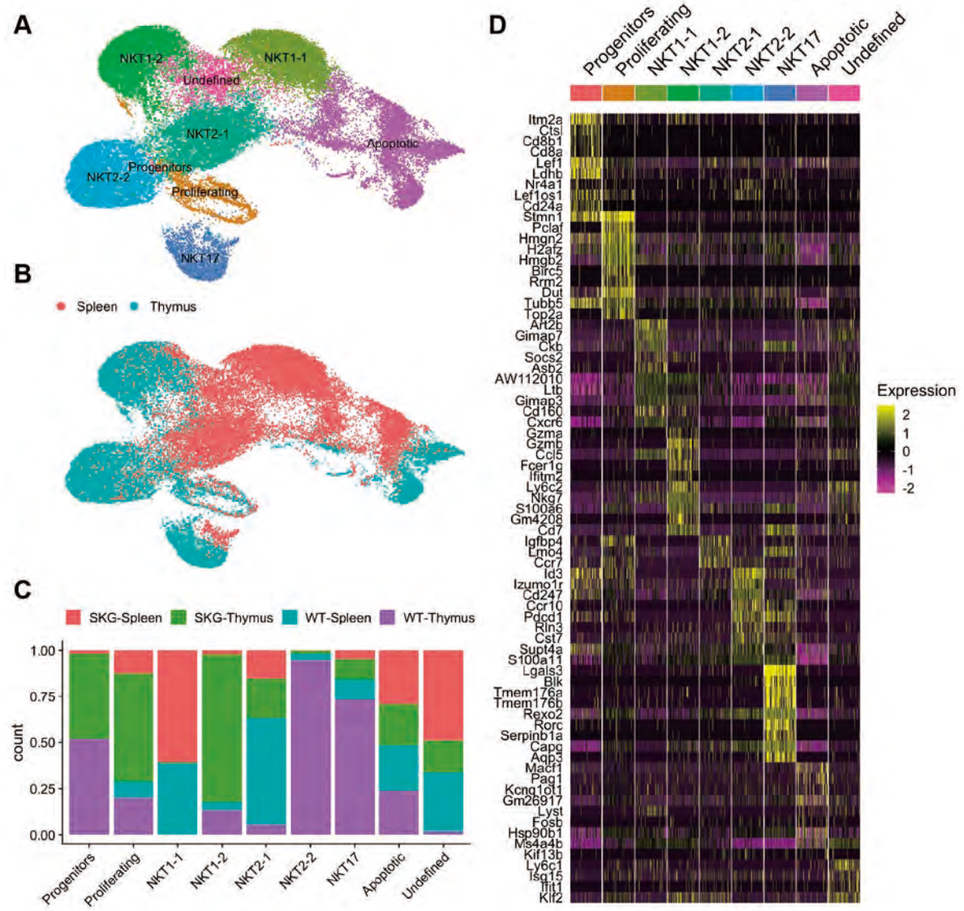


Figure 1. Single cell transcriptomics defines signatures of NKT cells in the thymus and spleen of WT and SKG mice.

Recent Publications

- Vandenbon A and Diez D. A clustering-independent method for finding differentially expressed genes in single-cell transcriptome data. Nat Commun 11(1): 4318 (2020).
- Teraguchi S, Saputri DS, Llamas-Covarrubias MA, Davila A, Diez D, et al. Methods for sequence and structural analysis of B and T cell receptor repertoires. Comput Struct Biotechnol J 18: 2000-2011 (2020).
- Nakai W, Yoshida T, Diez D, et al. A novel affinity-based method for the isolation of highly purified extracellular vesicles. *Scientific Reports* 6(1): 33935 (2016).
- Bahrini I, Song JH, Diez D & Hanayama R. Neuronal exosomes facilitate synaptic pruning by up-regulating complement factors in microglia. *Scientific reports* 5: 7989 (2015).
- Diez D, Agusti A & Wheelock CE. Network analysis in the investigation of chronic respiratory diseases. From basics to application. *American journal of respiratory and critical care medicine* 190: 981-988 (2014).

Events & Outreach Activities

Online Academic Events at IFReC

Due to the COVID-19 pandemic, travel by researchers has been restricted for the past year, and IFReC is no longer able to invite researchers as speakers to its academic events. As a result, the IFReC Colloquium, an important event held every two months allowing researchers of IFReC to gather together, had to be postponed several times to prevent the spread of COVID-19. Fortunately, the use of online tools has now made it possible to hold various events, although in a different format.

IFReC Colloquia in FY 2020

No.	Date	Title	Speakers
46	Sept. 30, 2020	Vaccine and Immunotherapy against viruses and cancer.	Ken J. Ishii (Vaccine Science, Ishii Lab)
		All-optical single cell phenotyping and pathway-specific activation of immune cells.	Nicolas Pavillon (Biophotonics, Smith Lab)
47	Nov. 25, 2020	Differential roles of RUBCN isoforms in memory B and plasma cells differentiation.	Chao-Yuan Tsai (Immune Regulation, Kikutani Group)
		Applications of single cell genomic approaches to immune cell development and differentiation processes.	Diego Diez (Quantitative Immunology Research Unit)
48	Jan. 27, 2021	Identification of human CAR cells and another specific transcription factor essential for maintenance of the HSC cellular niche.	Yoshiki Omatsu, and Kazunari Aoki (Stem Cell Biology & Developmental Immunology, Nagasawa lab)
		Characterization of the COMMD3/8 complex as a therapeutic target for inflammatory diseases.	Taiichiro Shirai (Immune Response Dynamics, Suzuki Lab)
49	Mar. 10, 2021	Metagenome and metaviorme-wide association studies of autoimmune diseases.	Yukinori Okada (Statistical Immunology, Okada Lab)
		A sublethal mutation in phospholipid flippase that causes aberrant phosphatidylcholine-translocation in the plasma membrane.	Katsumori Segawa (Biochemistry & Immunology, Nagata Lab)

"ImmunoSeminar" by online

IFReC started a series of online seminars called the "ImmunoSeminar Series." As the first speaker of this series, Prof. Jason Cyster (UCSF) was chosen, and the seminar was held in real time across several continents. This first seminar of the series was well received and the series is planned to be continued by inviting world-class immunologists to speak online at the seminars.

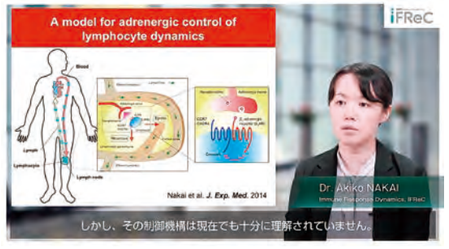
The Memorial Lecture by Prof. Kurosaki

In a first for IFReC, a special lecture in celebration of Prof. Tomohiro Kurosaki's 65th birthday was held on March 18, 2021 in a hybrid format connecting the online audience with the venue. As the demand for events in this type of format is expected to increase in the future, IFReC intends to continually upgrade the hosting environment for an improved and more productive online experience.

Online Outreach Activities

As with IFReC colloquia and seminars, in-person outreach activities such as science cafes have been largely curtailed due to COVID-19. As a consequence, IFReC began implementing a new approach to holding its outreach activities by making use of the web and online tools. The experience gained from holding these virtual events is expected to be highly useful even after the COVID-19 restrictions on activities end.

Enhancement of IFReC's Official YouTube Channel



Science Agora - Ten days of connecting science and society

Date Nov. 13-22, 2020
Organizer IFReC & Research Institute for Microbial Diseases (RIMD), Osaka University
Speakers Masayuki Miyasaka (IFReC) & Tokiko Watanabe (RIMD)

Research Frontline Lecture for Educators "Let's get to know the latest research at WPI!"

Date Nov. 28 & Dec. 5, 2020
Organizer IFReC (Osaka Univ), IRCN (Univ Tokyo), ITbM (Nagoya Univ), and MANA (NIMS)
Speakers Toshihide Yamashita (IFReC) & Takamitsu Watabe (IRCN), and others

Let's learn about Life Science! "World-class researchers from WPI!"

Date Dec. 26, 2020
Organizer JSPS and 13 WPI institutes
Speakers Mari Murakami (IFReC) and others

The 9th WPI Science Symposium "Life Science Research in the 21st Century"

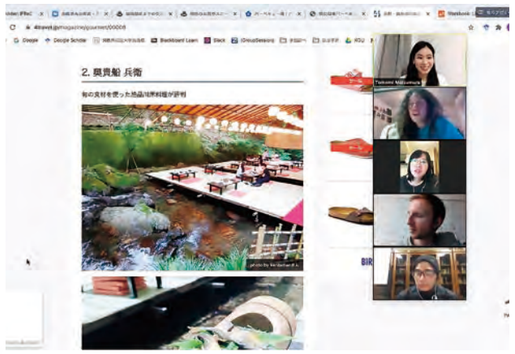
Date Feb. 7 & 8, 2021
Organizer ELSI (Tokyo Tech), WPI institutes and JSPS

Japanese Language Class

These 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. Totally, over 300 IFReC members have joined the classes since 2012. 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. In FY2020, all the classes were organized by the use of an online tool.

Message from Ms. Tomomi Tomomune, a Japanese language teacher

I would like to support all of my students by providing effective lessons to make your life more enjoyable. During class, there are many activities which ask you to share your experiences and opinions. By interacting with others and hearing their stories, you can gain an insight into unique, intriguing cultures and also widen your perspective. Let's expand our horizons through learning Japanese together!



Information

Major Awards

Shimon SAKAGUCHI

- Robert Koch Award 2020

“The regulatory T-cells (Tregs) discovered by Prof. Sakaguchi are regarded as peacemakers of the immune system, because they prevent defense cells patrolling the body from attacking the body’s own tissue. The clinical application of Tregs are already being pursued very actively.”

Tadamitsu KISHIMOTO

- The Tang Prize 2020

“Prof. Kishimoto discovered interleukin 6 (IL-6), which plays important roles in various inflammatory responses, and led to the development of Tocilizumab (commercial name: ActemraR), which may be effective in treating the pneumonia caused by COVID-19.”

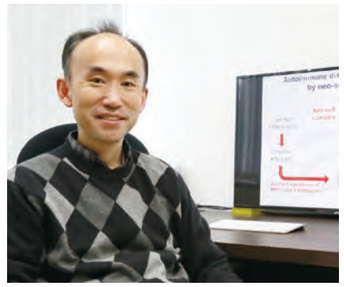
Shigekazu NAGATA

- American Association for Cancer Research (AACR) Academy Fellow

“Prof. Nagata identified the Fas membrane protein as a member of the tumor necrosis factor receptor family, and defined the role of the Fas ligand as an inducer of apoptotic signaling.”

Toshio YANAGIDA

- Member of the Japan Academy



Hisashi ARASE

- Hideyo Noguchi Memorial Prize 2020

Sujin KANG

- The Young Scientists’ Award (MEXT) 2020
- Osaka University Prize 2020

Yukinori OKADA

- JSPS Prize 2020
- President award of AMED 2020

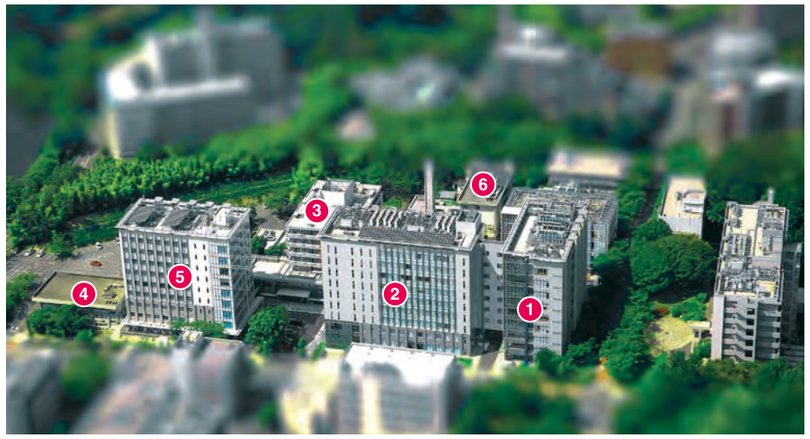
Masaru ISHII

- The 38th Osaka Science Prize

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.

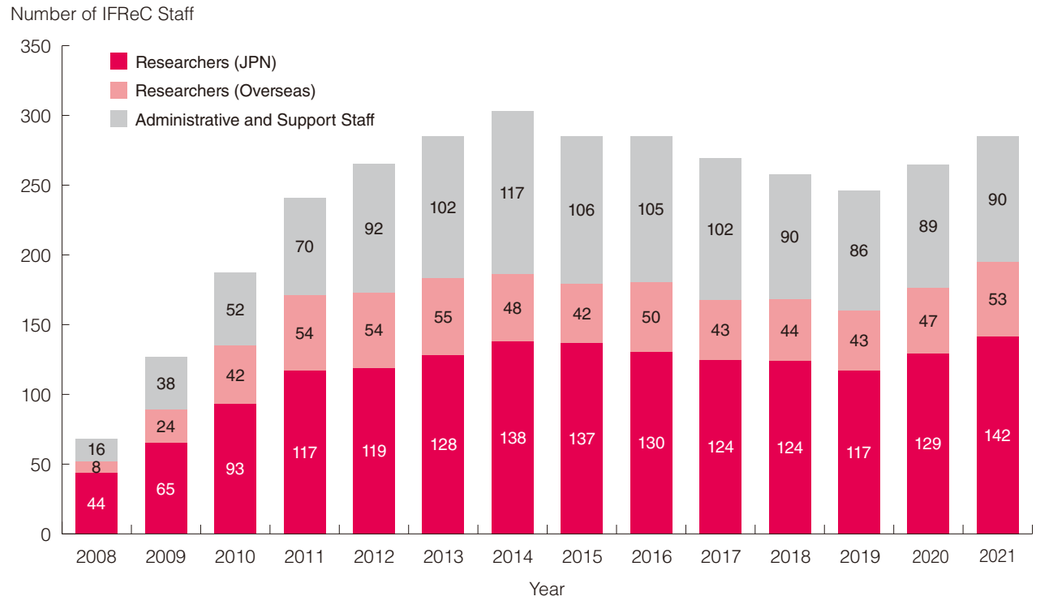
Core Instrumentation Facility

- 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

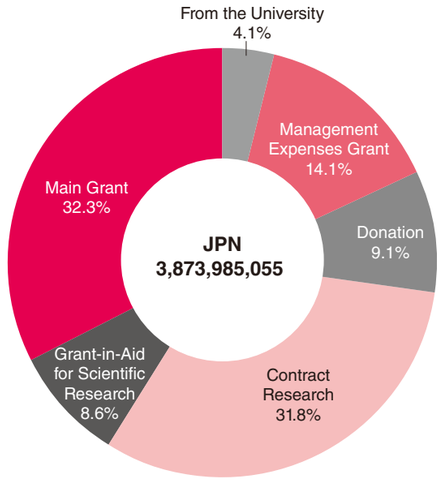
Composition

As of Feb, 1st

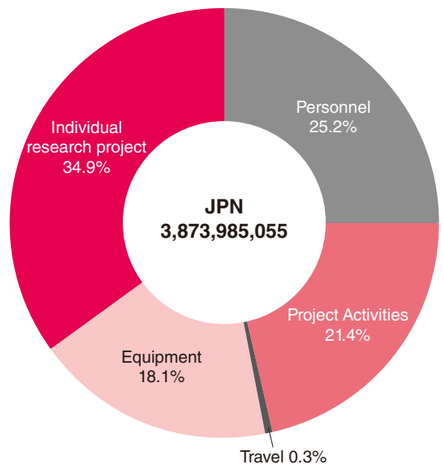


Finance

Sources

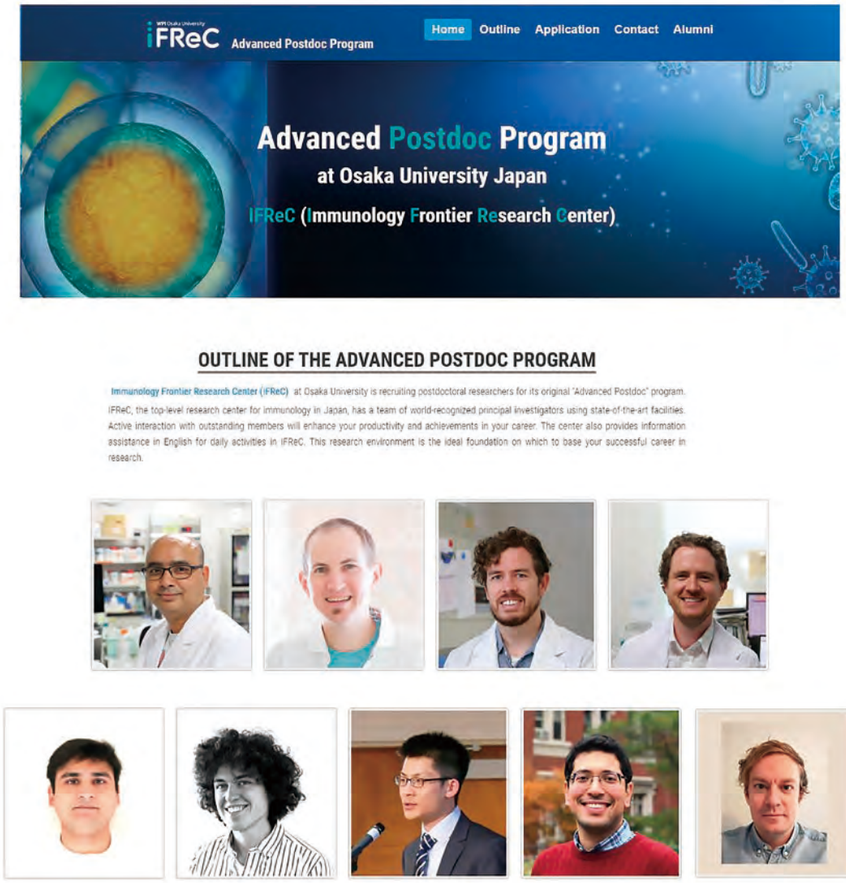


Expenditure



Advanced Postdoc Program at IFReC

IFReC has established the Advanced Postdoc system, which offers outstanding young researchers opportunities to work with diverse researchers in IFReC as well as to conduct their own research under their own merit. Under this system, IFReC has employed excellent postdoctoral fellows from 2018. They were assigned to laboratories in IFReC with an internationally competitive salary and research funds (three million JPY per year) to conduct original research.



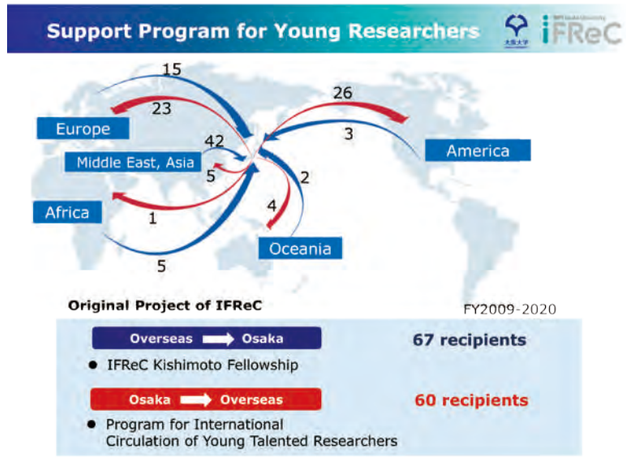
Website of the Advance Postdoc Program including the members as of March, 2021

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 in FY2018, two in FY2019, and three in FY2020 with the continuous research funding for three fiscal years. The grant is expected to generate excellent research achievements, raise the international recognition of these PIs, and contribute to the acquisition of external research funding.

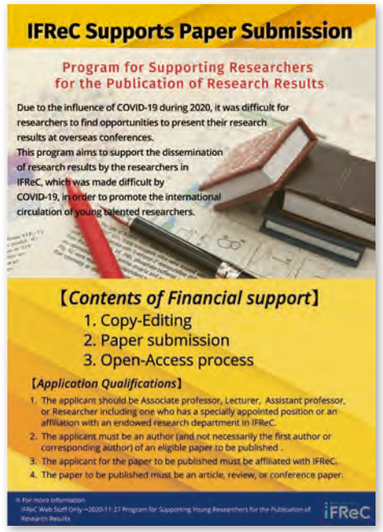
Original Support Programs for Young Researchers

To strengthen our international research network and our basis for international collaborative research, IFReC has established two kinds of financial support programs for researchers. 1) “IFReC Kishimoto Foundation Fellowship,” which has been used to invite international researchers to Osaka. 2) “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, 127 researchers have received these grants.



Support for Paper Submission

Due to the influence of COVID-19 during 2020, it was difficult for researchers to find opportunities to present their research results at overseas conferences. This program aims to support the dissemination of research results by the researchers in IFReC.



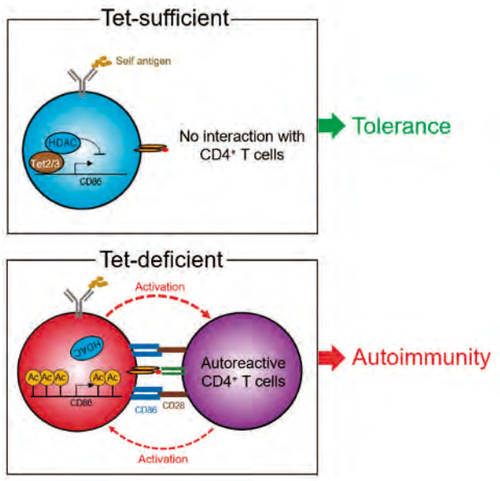
Selected Articles

Tet2 and Tet3 in B cells are required to repress CD86 and prevent autoimmunity.

Tanaka S, Ise W, Inoue T, et al.

***Nature Immunology* 21: 950-961 (2020)**

Wataru Ise, Tomohiro Kurosaki and their research group reported that deficiency of ten-eleven translocation (Tet) DNA demethylase family members, Tet2 and Tet3, in B cells led to hyperactivation of B and T cells, autoantibody production and lupus-like disease. Mechanistically, in the absence of Tet2/Tet3, down-regulation of CD86, which normally occurs following chronic exposure of self-reactive B cells to self-antigen, did not take place. The importance of the dysregulated CD86 expression in Tet2/Tet3-deficient B cells was further demonstrated by restraining, albeit not completely, aberrant T and B cell activation by anti-CD86 blocking. Tet2/Tet3-deficient B cells had a decreased accumulation of histone deacetylase 1 (HDAC1) and HDAC2 at the Cd86 locus. Their findings suggest that Tet2/Tet3-mediated chromatin modification participates in repression of CD86 on chronically stimulated self-reactive B cells, which, at least partly, contributes to preventing autoimmunity.

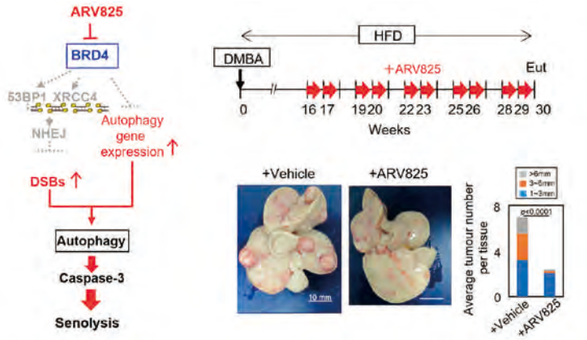


A BET family protein degrader provokes senolysis by targeting NHEJ and autophagy in senescent cells.

Wakita M, Takahashi A, Sano O, et al.

***Nature* Communications 11: 1935 (2020)**

Although cellular senescence acts primarily as a tumour suppression mechanism, the accumulation of senescent cells in vivo eventually exerts deleterious side effects through inflammatory/tumour-promoting factor secretion. Thus, the development of new drugs that cause the specific elimination of senescent cells, termed senolysis, is anticipated. Here, by an unbiased high-throughput screening of chemical compounds and a bio-functional analysis, Eiji Hara group identified BET family protein degrader (BETd) as a promising senolytic drug. BETd provokes senolysis through two independent but integrated pathways: (i) attenuation of non-homologous end joining (NHEJ), and (ii) up-regulation of autophagic gene expression. Notably, BETd treatment eliminates senescent hepatic stellate cells in obese mouse livers, accompanied by the reduction of liver cancer development. Furthermore, the elimination of chemotherapy-induced senescent cells by BETd substantially increases the efficacy of chemotherapy against xenograft tumors in immunocompromised mice. These results reveal the vulnerability of senescent cells and open up the possibilities for its control.



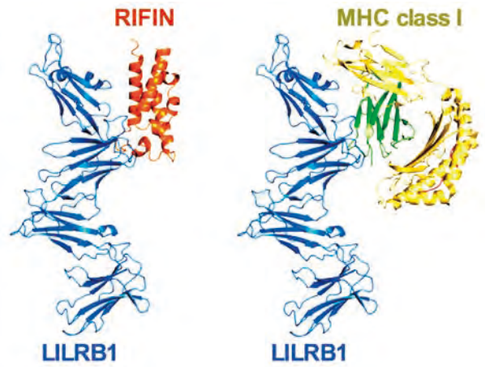
Structural basis for RIFIN-mediated activation of LILRB1 in malaria.

Harrison TE, Mørch AM, Felce JH, Sakoguchi A, et al.

***Nature* 587: 309-312 (2020)**

The Plasmodium species that cause malaria are obligate intracellular parasites, and disease symptoms occur as they replicate within human blood. Despite risking immune detection, the parasite delivers proteins that bind host receptors to infected erythrocyte surfaces. In the causative agent of the most deadly human malaria, Plasmodium falciparum, RIFINs form the largest erythrocyte surface protein family (Saito et al. *Nature* 2017). Some RIFINs can bind inhibitory immune receptors, acting as targets for unusual antibodies containing a LAIR1 ectodomain, or as ligands for LILRB1. RIFINs potentially dampen human immune responses against malaria.

The research group of University of Oxford and Osaka University led by Hisahi Arase determined a structure of a RIFIN bound to LILRB1. They showed that the RIFIN mimics the natural activating ligand of LILRB1, MHC class I, in its LILRB1-binding mode. Therefore, LILRB1-binding RIFINs suppress NK cell function.



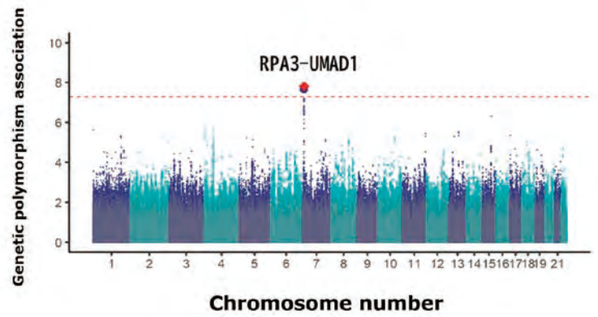
Association of the RPA3-UMAD1 locus with interstitial lung diseases complicated with rheumatoid arthritis in Japanese.

Shirai Y, Honda S, Ikari K, et al.

***Ann Rheum Dis* 79 (10): 1305-1309 (2020)**

It is known that the mortality rate of rheumatoid arthritis patients increases due to complications from interstitial pneumonia.

Using genome-wide association analysis (GWAS) for 5,000 rheumatoid arthritis patients in Japanese, the research group led by Yukinori Okada revealed that a gene region RPA3-UMAD1 to be related to interstitial pneumonia associated with rheumatoid arthritis. The RPA3-UMAD1 is strongly associated with the chest CT image pattern of pulmonary fibrosis. Their research is expected to contribute to elucidating the pathology of interstitial pneumonia associated with rheumatoid arthritis.

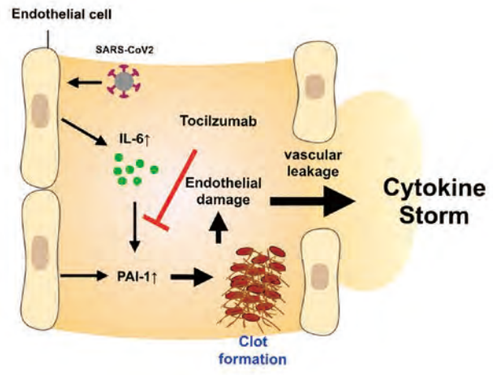


IL-6 trans-signaling induces plasminogen activator inhibitor-1 in cytokine release syndrome.

Kang S, Tanaka T, Inoue H, et al.

***Proc Natl Acad Sci USA* 117 (36): 22351-22356 (2020)**

Cytokine release syndrome (CRS) is a life-threatening complication induced by hyperinflammatory responses. However, no specific immunotherapies are available for its treatment. Sujin Kang, Tadamitsu Kishimoto and the research group found that interleukin (IL)-6 signaling plays a crucial role in endothelial cell dysfunction during bacterial and viral CRS. Specifically, they identified that the pathogenesis of CRS in patients with sepsis, acute respiratory distress syndrome, and burns involved the IL-6–mediated production of hyperinflammatory cytokines and plasminogen activator inhibitor-1 (PAI-1), which indicates that IL-6 signaling blockade has potential as a therapy for CRS. The group also found that the inhibition of IL-6 signaling by Tocilizumab treatment decreased PAI-1 production and alleviated clinical manifestations in severe COVID-19 patients.



Helicobacter pylori metabolites exacerbate gastritis through C-type lectin receptors.

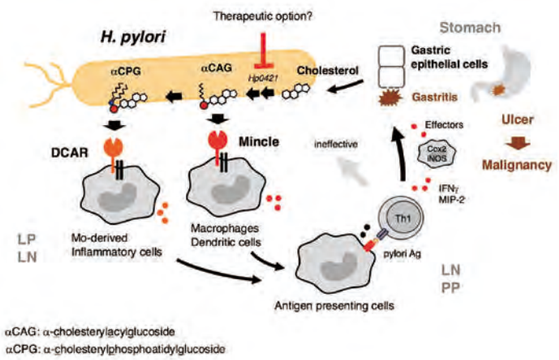
Nagata M, Toyonaga K, Ishikawa E, et al.

***J Exp Med* 218 (1): e20200815 (2021)**

Helicobacter pylori (*H.pylori*) causes gastritis, which has been attributed to the development of *H. pylori*-specific T cells during infection. However, the mechanism underlying innate immune detection leading to the priming of T cells is not fully understood, as *H. pylori* evades TLR detection.

Sho Yamasaki and his research group reported that *H. pylori* metabolites modified from host cholesterol exacerbate gastritis through the interaction with C-type lectin receptors.

Cholesteryl acyl α-glucoside (αCAG) and cholesteryl phosphatidyl α-glucoside (αCPG) were identified as noncanonical ligands for Mincle (*Clec4e*) and DCAR (*Clec4b1*). During chronic infection, *H. pylori*-specific T cell responses and gastritis were ameliorated in Mincle-deficient mice, although bacterial burdens remained unchanged. Furthermore, a mutant *H. pylori* strain lacking αCAG and αCPG exhibited an impaired ability to cause gastritis. Thus *H. pylori*-specific modification of host cholesterol plays a pathophysiological role that exacerbates gastric inflammation by triggering C-type lectin receptors.



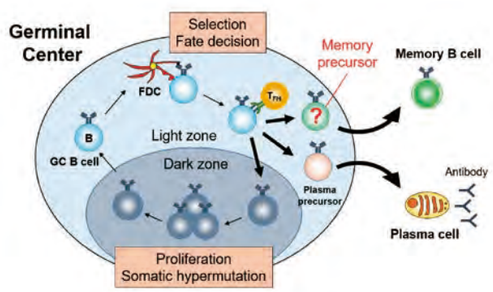
Mechanism for the establishment of immunological memory.

Inoue T, Shinnakasu R, Kawai C, et al.

***J Exp Med* 218 (1): e20200866 (2021)**

When exposed to antigens, such as viruses or bacteria, “germinal center (GC)” is generated in the secondary lymphoid organs including spleens and lymph nodes. B cell fate decision occurs during GC reaction, but it is still unclear how they are selected to differentiate into plasma cells, memory B cells, or to remain in the GC.

A group of researchers with Takeshi Inoue and Tomohiro Kurosaki identified and characterized a small GC population of precursors for memory B cells. They found that the GC B cells with lower mTORC1 activity and increased survival signal from surface B cell receptors favor a memory B cell fate. This group previously reported that the transcription factor Bach2 is required for memory B cell generation (Shinnakasu et al., Nat Immunol, 2016), and in this study, they clarified that Bach2 has an important role for the regulation of metabolism during GC responses. This group’s achievement provides the underlying mechanism for the establishment of immunological memory, which will help to develop new vaccine strategies.

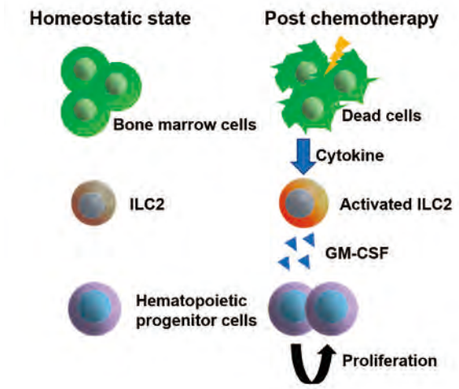


Group 2 innate lymphoid cells support hematopoietic recovery under stress conditions.

Sudo T, Motomura Y, Okuzaki D, et al.

***J Exp Med* 218 (5): e20200817 (2021)**

The cell-cycle status of hematopoietic stem and progenitor cells (HSPCs) becomes activated following chemotherapy-induced stress, promoting bone marrow (BM) regeneration; however, the underlying molecular mechanism remains elusive. Masaru Ishii and the research group showed that BM-resident group 2 innate lymphoid cells (ILC2s) support the recovery of HSPCs from 5-fluorouracil (5-FU)–induced stress by secreting granulocyte-macrophage colony-stimulating factor (GM-CSF). Mechanistically, IL-33 released from chemo-sensitive B cell progenitors activates MyD88-mediated secretion of GM-CSF in ILC2, suggesting the existence of a B cell–ILC2 axis for maintaining hematopoietic homeostasis. GM-CSF knockout mice treated with 5-FU showed severe loss of myeloid lineage cells, causing lethality, which was rescued by transferring BM ILC2s from wild-type mice. Further, the adoptive transfer of ILC2s to 5-FU–treated mice accelerates hematopoietic recovery, while the reduction of ILC2s results in the opposite effect. Thus, ILC2s may function by “sensing” the damaged BM spaces and subsequently support hematopoietic recovery under stress conditions.



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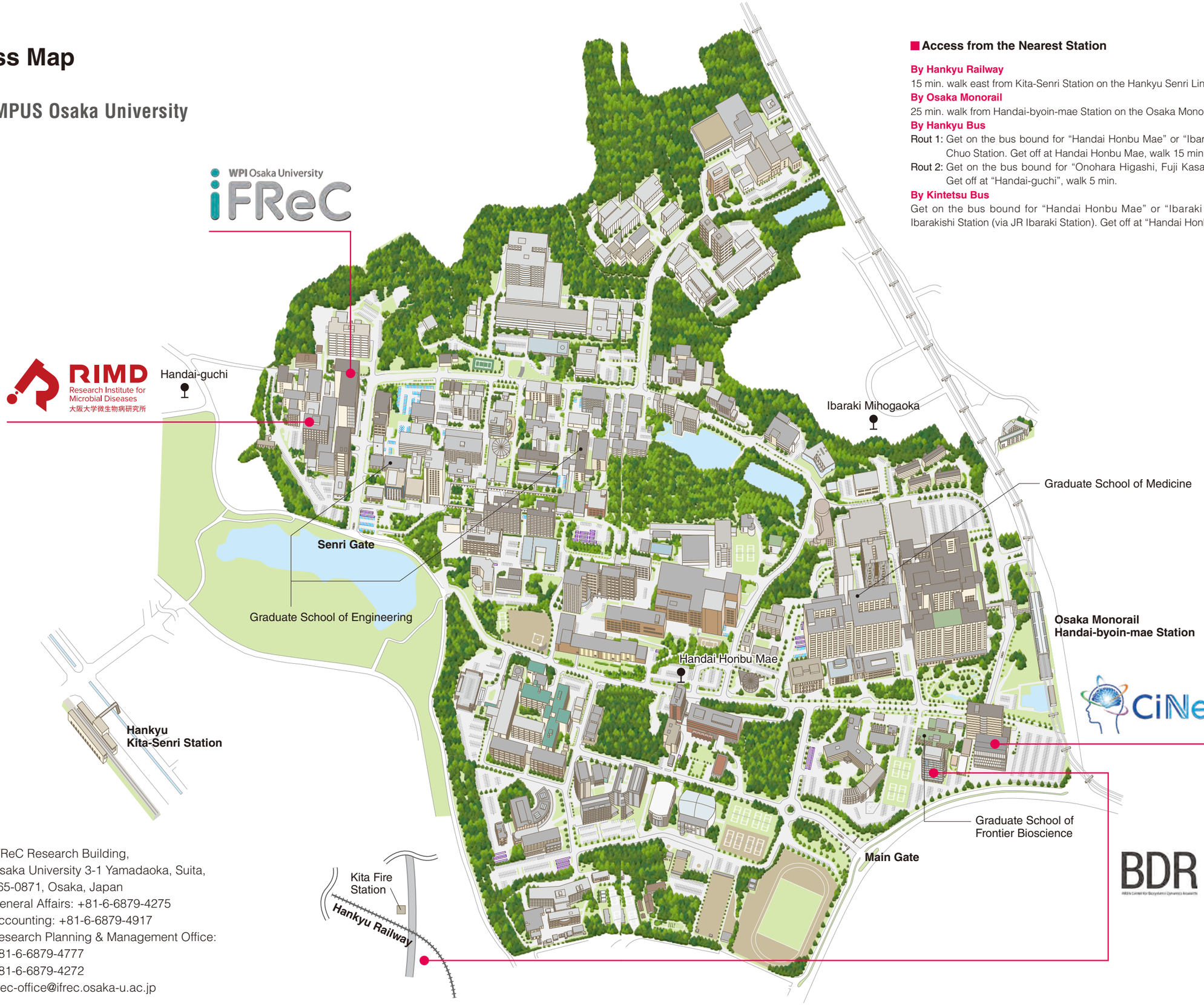

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