

PROGRESS REPORT

Division of Integrated Research

Atsushi Ochiai, M.D., Ph.D.

Members

Faculty members

Professor (concurrent appointment)

Atsushi Ochiai, M.D., Ph.D.

Visiting Researcher

Shinichiro Takeda

(Kavli IPMU Tokyo University)

Atsushi Yagisita

(Kavli IPMU Tokyo University)

Miho Katsuragawa

(Kavli IPMU Tokyo University)

Research Collaborators

Professors

Shin Aoki, Ph. D.

(Faculty of Pharmaceutical Sciences)

Collaborate company

AGRI SMILE

Shuhei Ogawa, Ph.D.

Members

Faculty members

Junior Associate Professor

Shuhei Ogawa, Ph.D.

Guest researchers

Shiho Watanabe, Ph.D



Division of Integrated Research

Chairman: Atsushi Ochiai, M.D., Ph.D.

The Research Institute for Biomedical Sciences (RIBS) plays a central role as a hub for promoting interdisciplinary collaborative research among life science, medical science, and engineering. In particular, researchers in our division collaborate closely with other faculties on campus, external research institutes, and various industries.

To achieve our mission, we maintain a high-quality animal facility and provide technical support for the generation of genetically modified mice. We also offer a wide range of advanced, well-maintained equipment and research space within our institute.

Promotion of Joint Research and Operation of Joint Laboratories

Our institute renovated and has maintained a joint laboratory space on the first floor (approximately 400 m²) since 2021. Since then, we have accepted research proposals for joint collaborative research involving faculty members of our institute, the Faculty of Pharmaceutical Sciences, and companies collaborating with the Tokyo University of Science (TUS). To promote these collaborative research projects, we established an operational committee for joint research and made effective use of conference rooms and common spaces to facilitate active discussions.

Animal Facility and Research Support for Developmental Engineering

Our animal facility maintains SPF conditions through stringent animal care and breeding practices. Microbiological quality and health monitoring are conducted six times per year. We currently maintain over 8,000 mice in the SPF area and approximately 200 mice in an infection experiment area.

We have also provided technical assistance for the generation of genetically modified mice (knockout, knock-in, and transgenic), producing eight mouse lines. In addition, we introduced 15 mouse lines into the SPF area via in vitro fertilization (IVF) and cryopreserved five lines as genetic resources (frozen embryos). Furthermore, we successfully recovered 12 mouse lines from frozen embryos.

To carry out these activities, we employed one guest researcher.

In recent years, the number of universities and research institutes where radioisotopes (RI) can be used has decreased due to increasingly strict radiation control regulations. Meanwhile, there is a growing demand for the development of imaging reagents for human medical applications, such as drug discovery using alpha-emitting nuclides (e.g., At-211 and Ac-225) and positron emission tomography (PET).

To meet this demand, RIBS applied for approval of its RI laboratory as a facility capable of handling new radioactive nuclides and immediately began developing alpha-ray imaging equipment in collaboration with the Faculty of Science and Technology at the University of Tokyo.



Division of Integrated Research

Shuhei Ogawa, Ph.D.

A major goal of our group is to understand how the immune system develops and is regulated and how inappropriate immune responses produce local and systemic disease. I believe that the outcome of these efforts will give rise to better tools and strategies to overcome immunological disorders such as autoimmune disease, graft-versus-host disease, and allergy, and lead to the development of efficient immune therapies to treat cancer and infectious disease.

My laboratory has been working on the analysis of the major T cell costimulatory signal transmitted through the CD28 receptor family (CD28, ICOS, CTLA-4, and PD-1) with respect to its role in signal transduction as well as in normal physiological and immunological functions. It has been shown that CD28-mediated costimulation contributes to metabolic reprogramming, thereby regulating functional differentiation of T cells, such as effector helper T cells and memory T cells. We try to investigate the role of CD28 receptor family in T cell activation, effector T cell development, and memory formation.

We are also committed to the maintenance of a high-quality animal facility in RIBS and the services for developmental engineering research, such as clean up and cryopreservation of mice. We also generated genetically modified mice for requests from both inside and outside the University.

The molecular mechanisms of CD28-mediated costimulatory signaling.

CD28-mediated costimulation is important

for full activation of T cells. Crosslinking of CD28 leads to activation of various signaling pathways, such as, PI3K/Akt, Grb2/Gads/MAPK, mTOR, Ca²⁺/NFAT, and PKCθ/NF-κB pathways. CD28-mediated costimulation also contributes to metabolic reprogramming, and consequently regulates functional differentiation of T cells, such as effector helper T cells and memory T cells. Tyrosine phosphorylation of CD28 is thought to be one of the key events to transduce CD28 specific signal. Previously, we showed that the Y189 but not PYAP motif is critical for tyrosine phosphorylation of CD28. The last decades, we have investigated the interactions between PI3K, Grb2, and Gads to CD28 Y189MNM motif by structural analyses. This year, we found that several compounds (inhibiting CD28 pYMNM – PI3K p85 binding and enhancing CD28 pYMNM – Grb2) showed stimulatory function on T cell activation depending on concentration of compound. We think that these signaling pathways are potential therapeutic targets for cancer immunotherapies, effective vaccines, autoimmune disease, and graft survival by manipulating T cell responses. We have analyzed the effects of compounds on the interactions between CD28 phosphopeptides and SH2 domains using a surface plasmon resonance (SPR) biosensor, Biacore. Several compounds were found to decrease CD28 binding to PI3K cSH2 and increase binding to Grb2 SH2. We also analyzed the effects of trisubstituted carboranes on the function of T cells obtained from C57BL/6 mice and found that they efficiently increased proliferation. In this year, we continued to improve the compounds to exhibit similar effects at lower concentrations than previous compounds,

and actually identified several compounds with stronger effects. We will attempt to evaluate the in vivo function of these compounds using animal models, particularly preclinical mouse tumor models.

Collaborators:

Watanabe, S., Oda, M (Kyoto Prefectural University), Nakamura, H. (Institute of SCIENCE TOKYO)

Several signaling inhibitors of metabolic pathway not only inhibit but also augment CD28-mediated proliferation of T cells.

CD28-mediated costimulation is critical for the activation of T cells. CD28 has no enzymatic activity in the intracellular domain but contains four tyrosine residues and several functional motifs, such as a YNM motif and two PxxP motifs, which recruit several adaptor proteins and activate PI3K, MAPK, NF- κ B, and mTOR signaling pathways. Most inhibitors of signaling pathway inhibit T cell activation and proliferation. However, we found that several signaling inhibitors of metabolic pathway, such as mTOR or mitochondrial Carrier, not only inhibit but also augment CD28-mediated proliferation of T cells depending on the concentration of the inhibitor in the PMA plus anti-CD28 mAb stimulatory condition. In this year, we examined the phenotype of T cells when a signaling inhibitor is added to the above T cell stimulatory conditions. We are now attempting to investigate how one inhibitor both inhibits and enhances T cell proliferation depending on the concentration.

Collaborators:

Watanabe, S.

Development of methods and devices for efficiently recovering CTCs from blood.

Circulating tumor cells (CTCs) are cancer cells that have detached from a primary tumor and entered the bloodstream. The ability to detect and analyze CTCs is important for cancer research and clinical oncology, as these cells can provide valuable information about the status of a patient's cancer, treatment response, and potential for cancer progression.

We have been attempting to capture circulating tumor cells (CTCs) by selectively isolating larger cells from blood using microfluidic devices. However, this method inevitably leads to the inclusion of white blood cells. To address this issue and remove white blood cells, they have explored the use of biological affinity. But capturing white blood cells required stopping the flow and waiting for adsorption. We think that surface irregularities on the cells may be a potential reason for the difficulty in capturing cells. Therefore, we are testing to the hypothesis using microfluidic channels with narrow slits. The ultimate goal is to develop a high-throughput white blood cell capture device that incorporates a mechanism to push cells against the channel walls.

Collaborators:

Hayase, M. (Tokyo University of Science, Faculty of Science and Technology, Department of Mechanical and Aerospace Engineering)

Developmental Engineering Research Support Program at Tokyo University of Science

Since 2015, we have been conducting a developmental engineering research support program at Center for Animal Disease Models, established under the Ministry of Education,

Culture, Sports, Science and Technology's (MEXT) Strategic Research Foundation Grant-aided Project for Private Universities. This research support project has been taken over as a project of RIBS from 2021. In this support project, we support generation of genetically modified mice, cleaning mice, freezing mice embryos, and recovery of mice from frozen embryos.

In this year, we generated 6 strains of genetically modified mice. Additionally, we conducted cleaning of 6 strains, embryo freezing for 25 strains, and restoration from frozen embryos for 17 strains. In recent years, requests for the generation of Knock-in mice, such as reporter mice or floxed mice have been subject to increasing demand compared to the generation of simple Knock-out mice.

Since August 2021, we have employed one technician with support from the Tokyo University of Science to assist in improving our technical capabilities. Moving forward, we aim to further enhance both our technical expertise

and reliability to strongly support not only our institute but also the broader research conducted at our university at a higher level.

Collaborators:

Watanabe, S.

Publications

Fujisaki K, Okazaki S, **Ogawa S**, Takeda M, Sugihara E, Imai K, Mizuno S, Takahashi S, Goitsuka R. B Cells of Early-life Origin Defined by RAG2-based Lymphoid Cell Tracking under Native Hematopoietic Conditions. *J. Immunol.* 2024, 213: 296-305

Takada S, **Ogawa S**, Mizuta R. Role of DNase I in DNA degradation and cell-free DNA generation after acetaminophen-induced hepatic injury. *J. Vet. Med. Sci.* 2024, 86(11): 1124-1128.