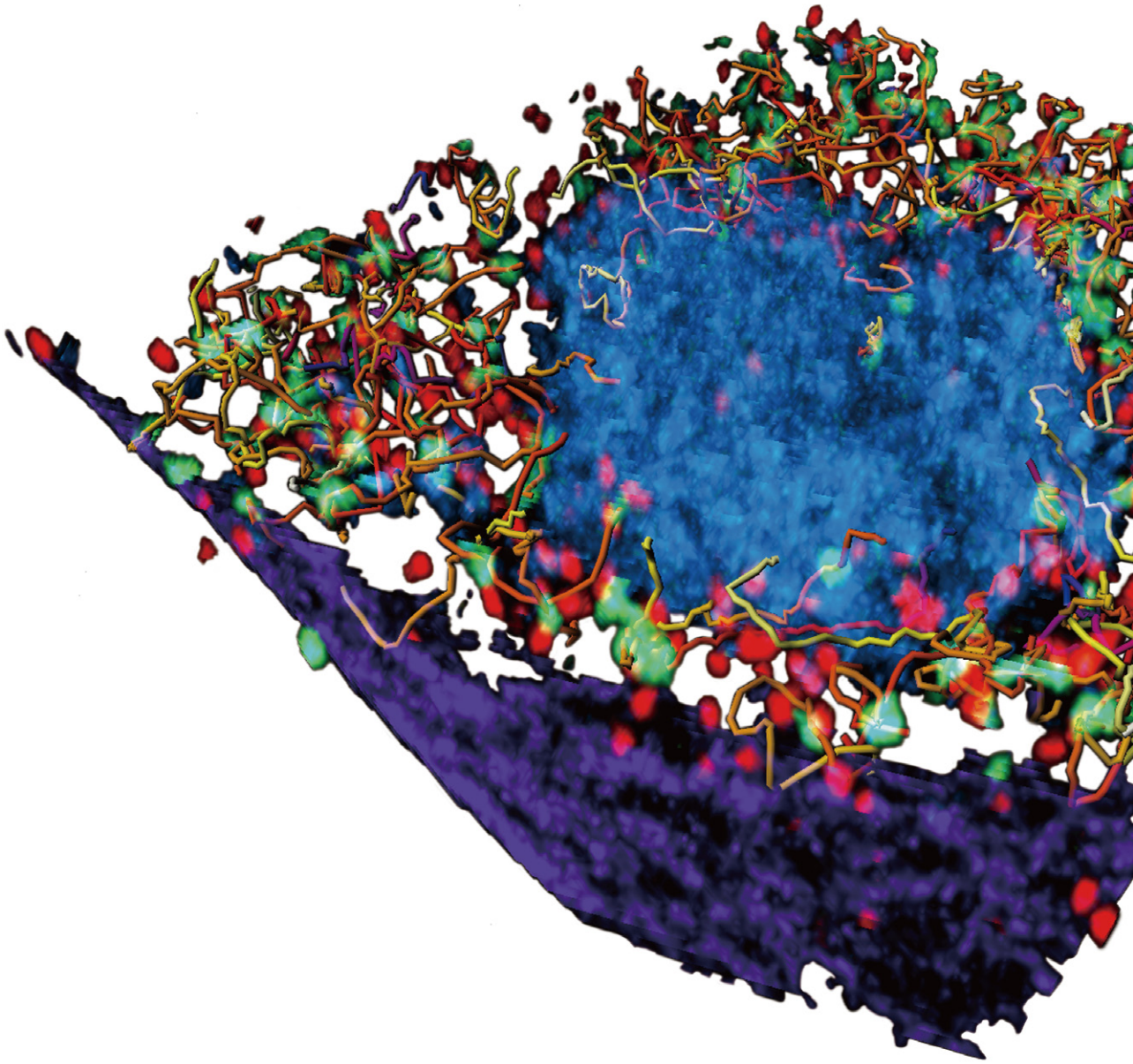
RIKEN RCAI Annual Report 2011

RIKEN Research Center for Allergy and Immunology



RCAI Events

2001

RCAI

Establishment of RCAI

2004

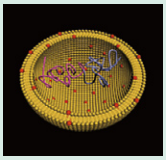
Opening of RCAI in RIKEN Yokohama Institute

2005

1st RCAI-JSI International Symposium on Immunology

2006

1st RCAI International Summer Program (RISP)



REGImmune, a spin-off from RCAI

2007

PIDJ

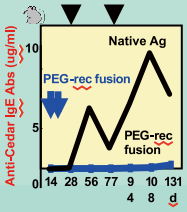
Launch of PIDJ, Primary Immunodeficiency Diseases Network in Japan

2008

Establishment of RIKEN Jeffrey Modell Diagnostic and Research Center for Primary Immunodeficiencies

1st Harvard Summer School at RCAI

2009



Establishment of the RIKEN-Torii Joint Research Team for allergy vaccine development

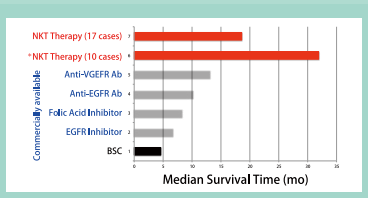
2010

Establishment of the RIKEN Young Chief Investigator Program

2011

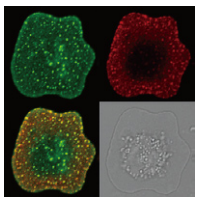
MIWI

Establishment of Medical I mmunology World Initiative

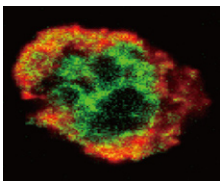


Approval of NKT cell therapy for cancer by The Advanced Medical Care Assessment System

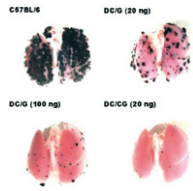
Major Discoveries from RCAI



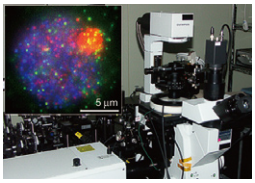
TCR microclusters



The zinc signal

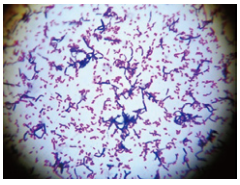


α-C-GalCer inhibits tumor metastasis in mice

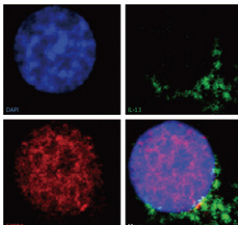


Single molecule imaging microscopy

Generation of atopic mouse model by ENU mutagenesis

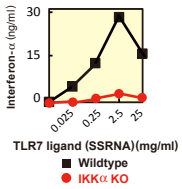


Bifidobacterium protects from *E. coli* infection

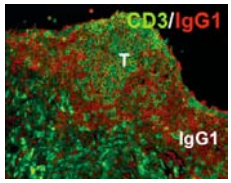


E4BP4 is responsible for IL-10 expression

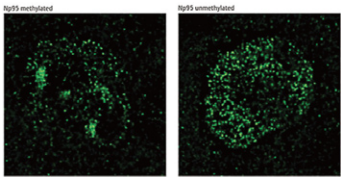
10 years history of RCAI



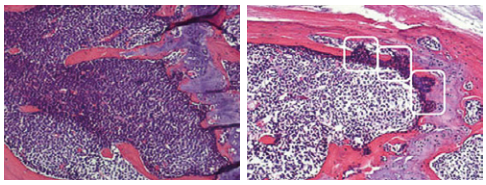
IKKα is the key player in IFN induction of innate immunity



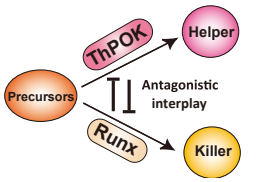
Development of an artificial lymph node



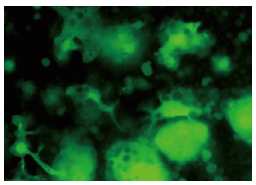
Np95 restores DNA methylation



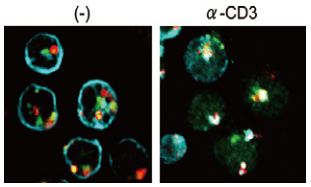
Leukemic stem cells in bone marrow



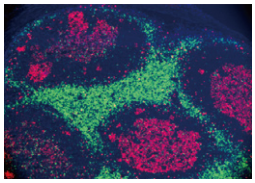
Antagonistic interplay between ThPOK and Runx in the helper/killer lineage choice



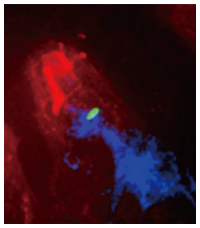
Myeloid-based model of T cell differentiation



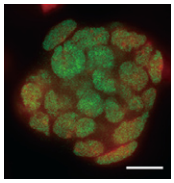
LAPTM5 negatively regulates TCR signaling



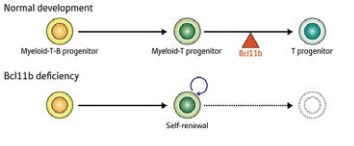
Foxp3⁺ T cells differentiate into follicular B helper T cells



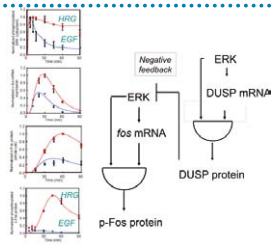
GP-2 is a receptor for mucosal antigens



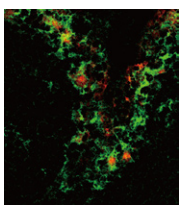
Generation of iPSC-derived NKT cells



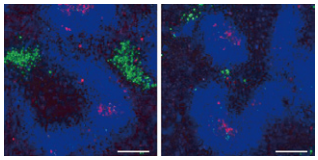
Bcl11b regulates the formation of T cells from progenitors



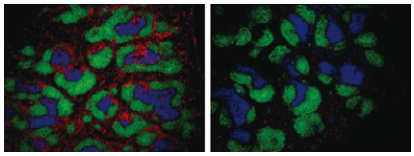
Modeling of ERK signaling networks that determine cell division or differentiation



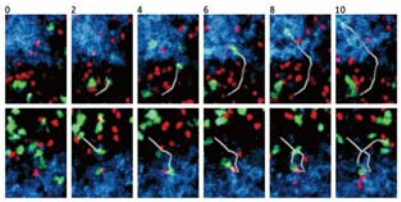
CD169 macrophages consume dead tumor cells



ERK controls B cell development and antigen production



Siglec-H controls cytokine production by plasmacytoid DCs



In vivo imaging of B cell migration by two-photon microscopy

RIKEN
Research Center for
Allergy and Immunology (RCAI)

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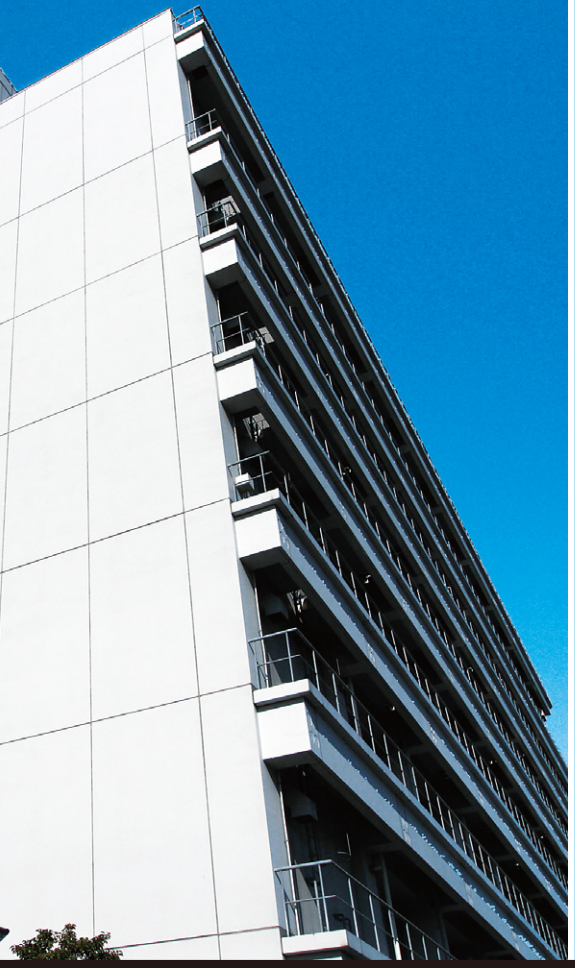

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Unit for Immunodynamics **Takaharu Okada, Ph.D.**

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Unit for Immunoepigenetics **Miguel Vidal, Ph.D.**
Unit for Immue Crosstalk **Hilde Cheroutre, Ph.D.**

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Part 5 Outreach Activities

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Director's Report

Aftermath of the Great East Japan Earthquake

After the devastating Great East Japan Earthquake on Mar. 11, 2011, RIKEN Research Center for Allergy and Immunology (RCAI) launched a support program for immunology researchers, hoping to be of assistance in rebuilding the educational and research environment in the severely damaged Northeastern region. In collaboration with the Japanese Society for Immunology (JSI), we provided support to 11 affected laboratories. Because their individual circumstances varied so much, we provided need-based assistance with research supplies and biological samples (research equipment, cell lines, antibodies, mice, etc.), derivation of mice to SPF status, and emergent support for research funding. We appreciate many offers from immunology institutions abroad that kindly cooperated in these efforts: Max Planck Institute (Germany), the National Institutes of Health (U.S.), INSERM (France), Jackson Laboratory (U.S.) and the La Jolla Institute for Allergy and Immunology (U.S.).

Following the accident at the Fukushima Nuclear Power Plant, saving electricity became one of the biggest challenges for the summer of 2011. The Ministry of Economy, Trade and Industry (METI) ordered a 15% cut in the electricity usage compared to the last year with a penalty of up to 1 million yen / hour (on weekdays 9:00 a.m. - 8:00 p.m.). The consumption in RIKEN Yokohama Institute was monitored and reported every hour. At RCAI, air conditioners were set at 28 ℃, and people used stairs instead of elevators. Some freezers and refrigerators were switched off, and many people shifted their working time, starting earlier in the day. The summer was unusually quiet because of the cancellation of several events including the Harvard Summer School at RCAI, the RCAI International Summer Program, RCAI-JSI International Symposium on Immunology, Briefing of Joint Graduate School Programs, and the RCAI Retreat. Thanks to the cooperation of every staff, we never exceeded the imposed limit.

Ralph Steinman's Vision Inspired Us to Create a New Program in Human Immunology, the Medical Immunology World Initiative (MIWI)

Ralph Steinman, Professor of The Rockefeller University and a member of the RCAI Advisory Council, received the Nobel Prize for Physiology and Medicine on October 3, 2011. However, just soon after this

wonderful news, and to my great regret, we were informed that he had passed away on September 30. We mourned his death and expressed our deep condolences to his family.

Ralph, who was famous as the discoverer of the dendritic cell, visited RCAI every year for the RCAI Advisory Council Meeting. He always respected the ideas of the young scientists, offering positive and constructive advice and suggestions with a very broad perspective. He repeatedly proposed a plan to promote internal collaborations to nurture young researchers by constructing a support system within the center. He was also a strong proponent of human immunology as a future direction for RCAI. Although he was planning to visit us to attend the meeting in August 2011, the meeting had to be replaced with an email review because of the cancellation caused by the earthquake. Instead of inviting the Advisory Committee members to Yokohama, in June we asked them all to submit comments and an evaluation of the RCAI Future Plan. Even though the document sent to the committee members consisted of more than 100 pages, Ralph sent back his report within one week. He made valuable suggestions about how RCAI should approach human immunology in order to step into the next new phase and also to establish a system to nurture young scientists.

Seriously taking his advice into consideration, in 2011 RCAI launched the Medical Immunology World Initiative (MIWI) as a new human immunology consortium using humanized mice. Eight institutes are affiliated with MIWI: Immunology Fronteer Research Center (iFReC) Osaka University, NIH, IMSUT, two departments of Zurich University, INSERM/Necker Hospital, Pasteur Institute, Imperial College London, and RCAI. MIWI is an international humanized mouse users' consortium and, as well, an integrative network for human immunology research in the next generation.

We also established the Young Chief Investigator Program as a tenure track system at RCAI. In this program, we appoint researchers younger than 40 years old to positions guaranteed for 7 years, and provide mentoring support by 3-4 senior PIs inside or outside RIKEN. In the 5th year, YCIs are reviewed by the Program Committee for possible promotion and also for the relevance and significance of their research projects to the future core research programs at RCAI. In 2011, we selected three researchers, whose previous research fields were mathematics, neuroscience and epigenetics. The YCI program is also linked to MIWI, with programs designed to nurture young scientists in interdisciplinary areas of research.

I strongly wish to establish this new and unique current of human immunology and the young investigator program through MIWI and to develop it to realize Ralph Steinman's vision.

Research Achievements

Although we had many difficulties in 2011, RCAI researchers continued to make important research discoveries. I would like to mention some of them.

The transcription factor Bcl6 was known to be important for germinal center B cells. Dr. Takaharu Okada and colleagues used two photon imaging and reporter mice and found that the protein levels are very important in dictating B cell behavior and also that Bcl6 is important for a special type of T helper cell, the T follicular helper (Kitano, et al. "Bcl6 Protein Expression Shapes Pre-Germinal Center B Cell Dynamics and Follicular Helper T Cell Heterogeneity", *Immunity*, Vol. 34, pp. 961–972, 2011).

IL-10 is an important anti-inflammatory cytokine and Dr. Masato Kubo and the team found that the E4 promoter-binding protein (E4BP4) transcription factor is responsible for driving the expression of IL-10 in multiple types of immune cells (Motomura, et al. "The transcription factor E4BP4 regulates the production of IL-10 and IL-13 in $CD4^+$ T cells", *Nature Immunology*, Vol. 12, pp. 450-459, 2011).

The question of whether regulatory T cells can be “reprogrammed” to become CD4 T helper cells has been very controversial in the field. Dr. Hori and colleagues showed that the real Tregs are not reprogrammable. (Miyao, et al. "Plasticity of $Foxp3^+$ T Cells Reflects Promiscuous Foxp3 Expression in Conventional T Cells but Not Reprogramming of Regulatory T Cells", *Immunity*, Vol. 36, pp. 262-275, 2012).

Dr. Sato and the team used a clever combination of gene and cellular knockouts to reveal an important role for plasmacytoid dendritic cells in the regulation of inflammation and T cell immunity *in vivo. (*Takagi, et al. "Plasmacytoid Dendritic Cells Are Crucial for the Initiation of Inflammation and T Cell Immunity *In Vivo*", *Immunity*, Vol. 35, pp. 958–971, 2011).

The minimal T cell receptor signaling unit is called a TCR microcluster, and these structures must move and eventually coalesce by an unknown mechanism. Dr. Saito and colleagues discovered that this movement is mediated by dynein, a ‘motor protein’ that shuttles cargos along the cytoskeleton (Hashimoto-Tane, et al. "Dynein-Driven Transport of T Cell Receptor Microclusters Regulates Immune Synapse Formation and T Cell Activation", *Immunity*, Vol. 34, pp. 919–931, 2011).

Dr. Kubo and his team identified a *cis*-acting gene regulatory element in the *Il-4* locus that is critical for IL-4 production by T follicular helper cells, which are important in stimulating B cell antibody production and clearing viral and bacterial infections (Harada, et al. "The 3' enhancer CNS2 is a crucial regulator in follicular helper T cells of interleukin-4 mediated humoral immunity", *Immunity*, Vol. 36, pp. 188-200, 2012).

T_H17 cells can induce a massive inflammatory response and also have essential roles in eliminating microbial pathogens. Dr. Takashi Tanaka and collaborators discovered that the ubiquitin ligase PDLIM2 inhibits T_H17 cell differentiation by degradation of the STAT3 transcription factor (Tanaka, et al. "PDLIM2 Inhibits T Helper 17 Cell Development and Granulomatous Inflammation Through Degradation of STAT3", *Science Signaling*, Vol 4, ra85, 2011).

Dr. Hase and Dr. Ohno's team investigated the role of AP-1B–mediated protein sorting in the maintenance of gastrointestinal immune homeostasis. AP-1B-deficient mice developed spontaneous chronic colitis and moreover, AP-1B expression was reduced significantly in colonic epithelium samples from patients with Crohn's disease (Takahashi, et al. "The Epithelia-Specific Membrane Trafficking Factor AP-1B Controls Gut Immune Homeostasis in Mice", *Gastroenterology*, Vol. 141, pp. 621-632, 2011).

Dr. Kurosaki and his team discovered the importance of the CIN85 adaptor protein in BCR-mediated survival, proliferation and antibody production due to its association with BLNK and participation in NF-κB signaling (Kometani, et al. "CIN85 drives B cell responses by linking BCR signals to the canonical NF-κB pathway", *The Journal of Experimental Medicine*, Vol. 208, pp.1447-1457, 2011).

Dr. Watarai and my team demonstrated that an important subset of *i*NKT cells, the IL-17RB$^+$*i*NKT subset, develops distinct from classical *i*NKT cell developmental stages in the thymus and play important roles in the pathogenesis of airway diseases (Watarai, et al "Development and Function of Invariant Natural Killer T Cells Producing TH2- and TH17-Cytokines", *PLoS Biology*, Vol. 10, e1001255, 2012).

Dr. Ishikawa and his team created an improved humanized mouse that has significantly improved engraftment of human hematopoietic cells (Takagi, et al. "Membrane-bound human SCF/KL promotes *in vivo* human hematopoietic engraftment and myeloid differentiation", *Blood*, Vol. 12, pp. 2768-2777, 2012).

I would also like to emphasize here that several RCAI researchers have become professors, a fact that underscores the vibrant and vital nature of RCAI; Dr. Kaisho became a professor of Osaka University, Dr. Udono became a professor of Okayama University, Dr. Masato Tanaka became a professor of Tokyo University of Pharmacy and Life Sciences, and, recently, Dr. Kawamoto became a professor of Kyoto University, Dr. Ishido became a professor of Showa Pharmaceutical University, Dr. Hoshino became a professor of Kagawa University, and Dr. Hase became a professor of The Institute of Medical Science, the University of Tokyo.

Towards human immunology

Although fundamental research in immunology has always been a focal point and strength of our center, over the past years we have developed several programs with a translational component, for example a very large effort in primary immunodeficiency diseases. In March 2012, building on the work of the RCAI, RIKEN decided to establish a new research center for integrative medical science, starting in April, 2013. Since its establishment in 2001, RCAI has continued to make new discoveries in immunology research and has pioneered the creation of new paradigms. After these initial 12 years of research endeavor, RCAI will open the door for the new direction to human immunology. I believe that our effort will continue to lead the world's immunology.

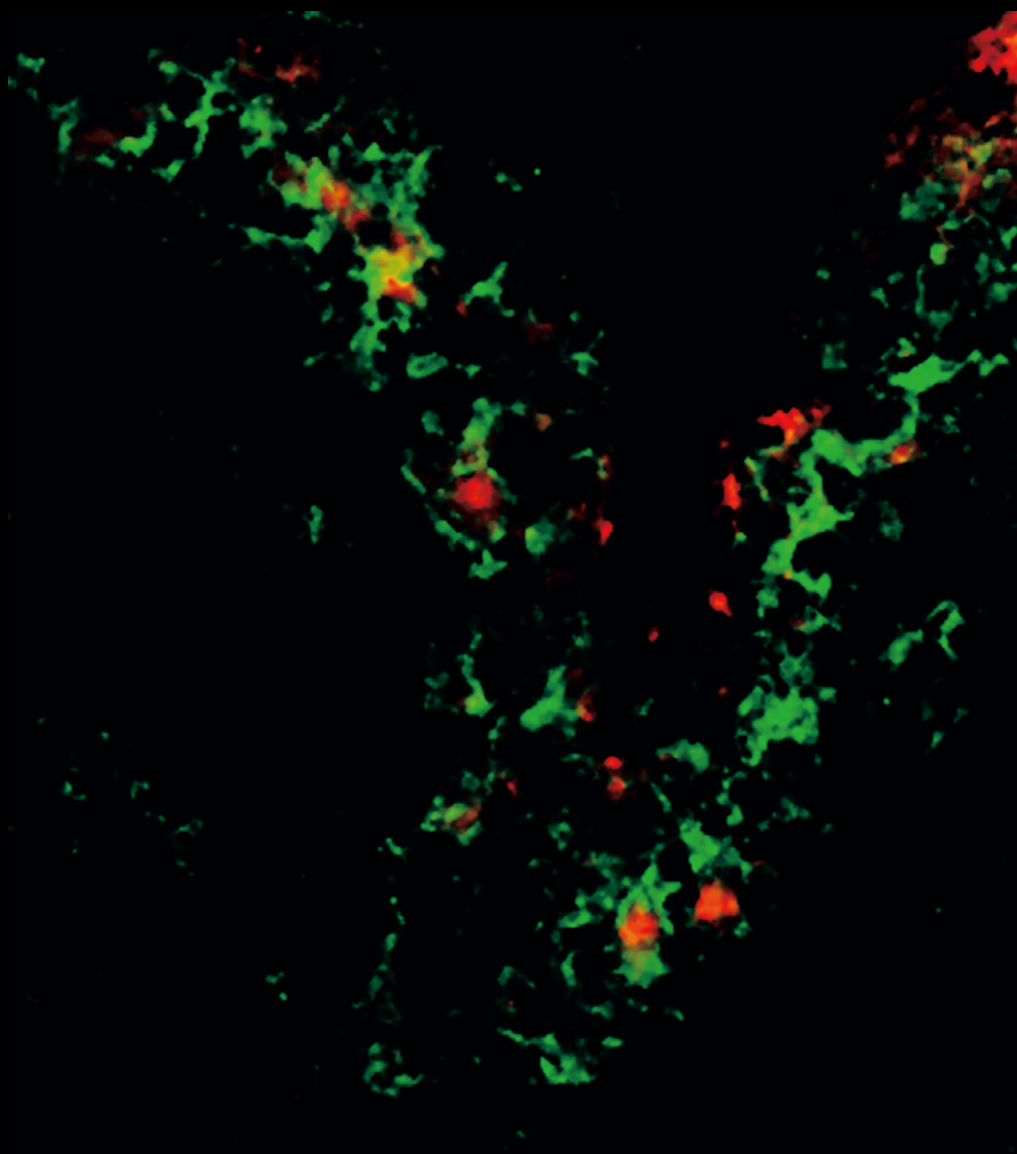
Mar. 31, 2012

Masaru Taniguchi

2011

Part 1

Creation of New Paradigms



MIWI

Medical Immunology World Initiative is launched

RCAI launched the Medical Immunology World Initiative (MIWI) as a new human immunology program. MIWI is an international humanized mouse users' consortium and, as well, an integrative network for human immunology research in the next generation. Eight institutions are currently affiliated with MIWI: iFReC, NIH, IMSUT, two departments of Zurich University, INSERM/Necker Hospital, Pasteur Institute, Imperial College London, and RCAI.

The immune system is a critical component for maintaining homeostasis of our body and also a key target for development of therapeutics and prevention of numerous diseases. Understanding the principles of the human immune system is therefore of central importance to discover clinically useful biomarkers as well as molecular and cellular targets for therapies. However, our knowledge of the human immune system is still quite limited compared to what we know about the mouse and other model organisms. This is mainly because of the shortage of quantitative molecular data obtained from representative and well-defined human materials, and the lack of suitable experimental strategies for functional dissection of the human immune system.

Recent advances in bioinformatics and in technologies for genome and RNA sequencing, quantitative identification of proteins, metabolites and other small compounds have enabled us to collect an enormous amount of digitalized data derived from human materials. In fact, advances in human genomics have allowed us to identify many genes that potentially may contribute to the pathogenesis of human diseases. However, experimental strategies to mechanistically interpret these data are not sufficiently coordinated or robust to understand the human immune system during pathogenic processes.

To overcome these problems, it is necessary to establish an interdisciplinary biomedical research platform and an international consortium of multiple research groups that possess different experimental expertise but share a central interest. For establishment and maintenance of such a consortium, it is thus critical to share comprehensive goals directed toward elucidating the role of the human immune system in disease onset and progression.

The goal of MIWI is to use integrative immunological approaches to obtain fundamental knowledge about the human immune system and mechanisms of disease development, and to discover the principles for diagnosis and therapy of human diseases, particularly allergy, primary immunodeficiencies, influenza and other viral infections, human cancer, and so on.

To accomplish this goal RCAI proposed the following 5 aims:

1. Generate humanized mice with greater levels of humanization of their immune and hematopoietic microenvironments than are currently available and use these mice to study the diseases listed above.
2. Accumulate tools to manipulate the reconstituted human immune system by depleting distinct immune compartments with specific antibodies or modification of their functions by transduction of human hematopoietic progenitor or other cells with regulatory molecules, e.g. shRNA.
3. Establish a database that integrates the accumulated data from conventional studies on the human immune system and humanized mouse studies with the data collected from clinical samples.
4. Utilize this integrated information to identify pivotal molecules in human immune cells as therapeutic targets and biomarkers in the different disease conditions.
5. Establish a system to nurture young researchers.

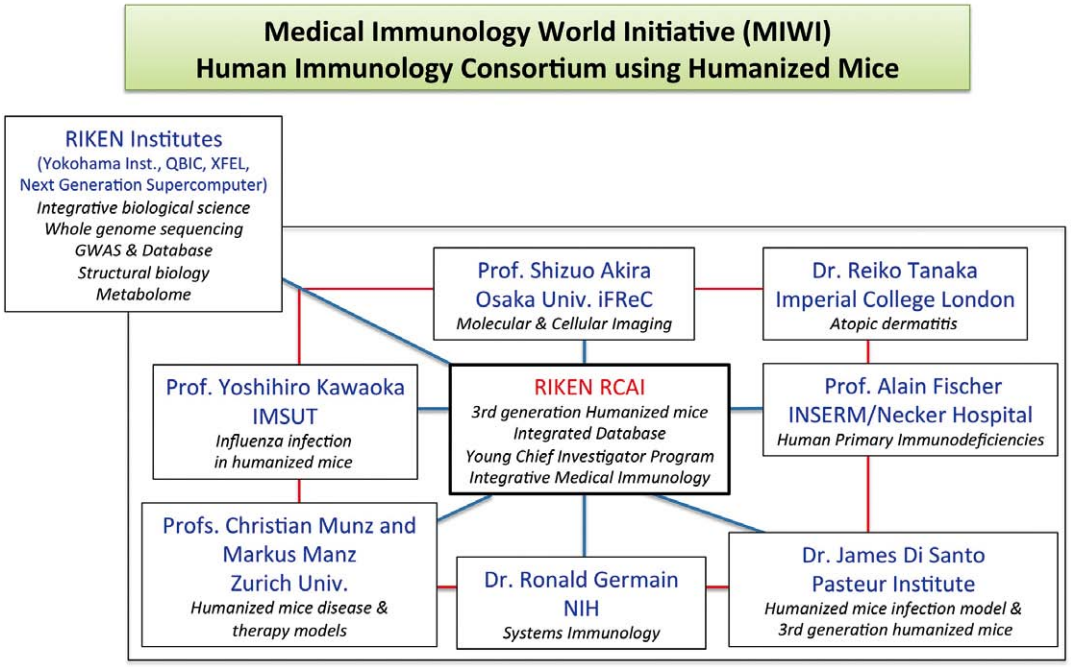


Figure: **Scheme of MIWI**
A new platform for human immunology research is required. For this purpose, the Medical Immunology World Initiative (MIWI) as the Human Immunology Consortium using Humanized Mice is being established. MIWI focuses on creation of the 3rd generation of humanized mice, integrative medical immunology, and an integrated database, as well as a mentoring system for nurturing young investigators. The initiative involves RIKEN RCAI together with eight institutes; NIH for Systems Immunology (Dr. Ronald Germain), Osaka University Immunology Frontier Research Center (iFReC) (Dr. Shizuo Akira) for immune imaging/dynamics, Pasteur Institute (Dr. James Di Santo) and two centers at Zurich University (Dr. Markus Manz and Dr. Christian Munz) for the 3rd generation of humanized mice with European type HLA genes and their application to establish human disease and therapy models, INSERM/Necker Hospital (Dr. Alain Fischer) for human primary immunodeficiency diseases, the Institute of Medical Science the University of Tokyo (IMSUT) (Dr. Yoshihiro Kawaoka) for pathogenic and nonpathogenic human influenza virus infection models using humanized mice, and Imperial College London (Dr. Reiko Tanaka) for mathematical modeling and simulation. Cooperation with these international institutes will complement those areas lacking in RCAI to effectively establish the system.

To realize the aims above, RCAI suggests the following 5 plans:

1. Establishment of a new generation of humanized mice and a user consortium
   There are several limitations with the present second generation of humanized mice because the immune microenvironments are entirely of mouse origin. In particular, the lack of human T cell-selecting HLA molecules and the species-specificity of several cytokines and adhesion molecules limit the efficient development of lymphocyte subsets and antigen recognition by human immune cells. In this regard, a worldwide collaboration with research centers focusing on humanized mouse models will facilitate the investigation of human immunity and immunological diseases.
2. Using humanized mice to understand the human immune system and immune responses
   Humanized mice will be integral components for understanding the human immune system and recapitulating disease onset and progression. Thus, they are quite useful for vaccine/drug development programs and to examine the safety and efficacy of candidate vaccines and drugs *in vivo*, particularly as to whether these agents effectively protect against human disorders and if there are any side effects. In order to assess functional contributions of human immune cell types to the disease of interest, protocols and reagents will be shared among the participating research groups. Although it is obviously likely that the humanized mice will only partly recapitulate human diseases, the comparative analysis of the accumulated data from patients and from the humanized mouse models should ultimately allow us to understand more precisely the differences between human and mouse immune systems as well as any advantageous similarities between humanized mice and the human system, both of which will have predictive value for future analyses. However, this outcome will occur only when a solid informational platform is established for this purpose.
3. Integrative immunobiology and disease targets
   The immune system and immune responses will be systematically elucidated by using the quantitative measurements and mathematical/statistical tools of systems biology. For this purpose, the analysis of molecular and cellular behavior will be helpful to understand spatio-temporal dynamics of immune cells in tissues and organisms. These studies will include quantitative analyses at the single molecule and single cell level and in both

time and space (systems dynamics), with the goal of assembling such data into a computable immune network. In parallel, a pipeline will be developed to mine the data with bioinformatics tools and hopefully then construct useful disease models that will allow us to obtain clues as to how to eventually set up intervention strategies. Diseases and therapeutic targets will be 1) allergic diseases, 2) primary immunodeficiencies, 3) influenza virus infection, 4) human tumor virus infection and immune control, 5) immune therapy and 6) normal immune and memory responses.

4. Establishment of an integrated disease database
   A disease-oriented database will be established by integrating immune cell data obtained directly from patient specimens or via analysis of the corresponding humanized mouse models. Humanized mice are of particular value here because they can provide us with human tissues/cells that are not routinely available from patients. In this new platform, the clinical information is integrated with various lines of information obtained by laboratory experiments, including genetic, proteomic and cellular information obtained from various tissues and immune cells of humanized mice.
5. Nurturing young investigators bridging immunology and other fields of science
   One of the objectives for starting MIWI is to nurture talented young investigators who will lead human immunology in the next generation and accelerate the establishment of interdisciplinary research areas. To facilitate integrative biology and human immunology research, the following directions in recruiting and developing young investigators will be pursued: a) researchers bridging biology and informatics, b) researchers who have clinical experience and understand both on human immunity and diseases pathogenesis for successful clinical translation in the future, c) researchers innovating new technologies that can be applied to immunology and other biomedical sciences. The global network through MIWI will encourage young investigators to strengthen communication skills and foster collaboration among different fields of science.

MIWI will also coordinate with RIKEN Yokohama Institutes for whole genome sequencing, genome-wide medical sciences (GWAS) and disease biomarkers, and with QBiC (Quantitative Biology Center) for integrative biology and the development of methods for mathematical modeling.

Dr. Shizuo Akira named the Senior Advisor and Dr. Shigeo Koyasu and Dr. Haruhiko Koseki new Deputy Directors

Dr. Shizuo Akira

Dr. Shigeo Koyasu

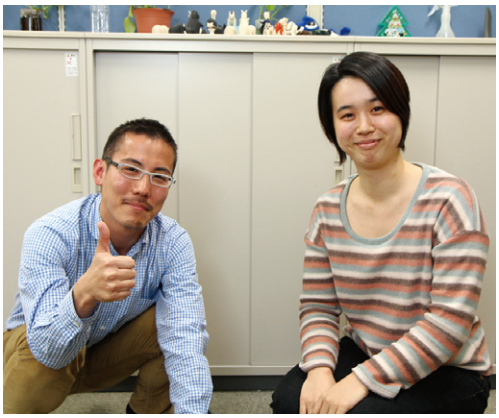
Dr. Haruhiko Koseki

Two professors, Dr. Shizuo Akira (Osaka University) and Dr. Shigeo Koyasu (Keio University) joined the advisory and management boards of Medical Immunology World Initiative (MIWI). To take on the advisory task for MIWI, Dr. Akira became the new Senior Advisor of RCAI, in addition to the current advisor, Dr. Kimishige Ishizaka. For the management of MIWI, Dr. Koyasu became a new Deputy Director of RCAI, and Dr. Haruhiko Koseki, Group Director of Laboratory for Developmental Genetics, was also promoted to a Deputy Director position. Now three Deputy Directors, Dr. Takashi Saito, Dr. Shigeo Koyasu and Dr. Haruhiko Koseki share the tasks of management of RCAI and MIWI.

Takaharu Okada

Ensuring the persistence of immune memory

A gene regulatory factor promotes long-term protection against infectious threats by effecting the maturation of a variety of immune cells



Masahiro Kitano *(left)* and Saya Moriyama *(right)*

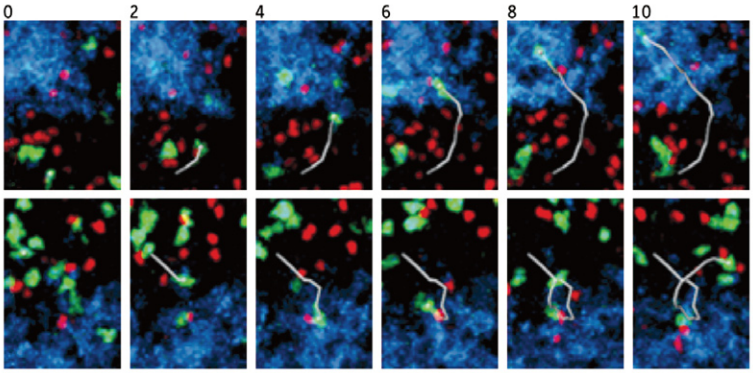


Figure: Time-lapse photography at two-minute intervals shows the migration (*white trail*) of a Bcl6-expressing B cell (*green*) into the GC (*blue*) following induction of an immune response (*top*). By comparison, B cells with inhibited Bcl6 function fail to migrate to the GC (*bottom*). © 2011 Elsevier, Inc.

Structures within the lymph nodes known as germinal centers (GCs) help the body to maintain long-term immune defense against foreign threats. The GCs essentially act as sites where antibody-producing B cells undergo a process of 'evolution' to generate higher-quality antibodies. This evolutionary mechanism is in turn supported by a class of T cells known as follicular helper T (Tfh) cells.

Takaharu Okada and colleagues at the RIKEN Research Center for Allergy and Immunology in Yokohama recently tracked the process of B cell development in mouse GCs by monitoring expression of Bcl6. This protein facilitates B cell evolution by regulating expression of key developmental genes.

The researchers were surprised to note a broader scope of Bcl6 activity than expected. "It became apparent that Bcl6 is important for Tfh cells as well," says Okada, "and I felt that we should track [the levels of] Bcl6 expression in both B and T cells at the same time."

They quantified Bcl6 levels by generating transgenic mice in which a DNA fragment encoding a fluorescent protein had been inserted into one copy of the Bcl6 gene. Since this insertion partially disrupted Bcl6 function, this reporter system enabled the researchers to examine the effects of inhibiting this protein.

Following the induction of an immune response in the animals, Okada and colleagues observed an increase of Bcl6 expression in antigen-specific B cells within peripheral regions of the lymph node; these subsequently migrated to the GC, where they continued to strongly express Bcl6 (Figure).

This migration and maturation process is dependent upon interaction with Bcl6-expressing helper T cells. All T cells expressing high levels of Bcl6 became Tfh cells, but many Tfh cells subsequently reduced Bcl6 production to varying degrees. Okada was surprised that the dynamics of Bcl6 expression differed between B and T cells. "This suggests that they employ different mechanisms for regulating expression of this important transcription factor," he says.

Okada and his team also recorded observations suggesting that Tfh cells expressing low levels of Bcl6 may give rise to 'memory cells', which enable the immune system to react quickly to recurring threats. However, this will require further investigation.

Understanding how these various cells employ this shared regulatory factor will also be a top priority moving forward. "We would like to learn the molecular mechanisms of Bcl6 expression in B and T cells by setting up collaborations with experts in the field of gene and protein expression control," says Okada.

Original Research Paper

Masahiro Kitano, Saya Moriyama, Yoshikazu Ando, Masaki Hikida, Yasuo Mori, Tomohiro Kurosaki, and Takaharu Okada. Bcl6 protein expression shapes pre-germinal center B cell dynamics and follicular helper T cell heterogeneity. ***Immunity*** Vol. 34, 961-972, 2011.

Masato Kubo

Preventing overreactions

Identification of the transcription factor that regulates a protein that dampens immune responses could aid the fight against autoimmune disease

Yasutaka Motomura

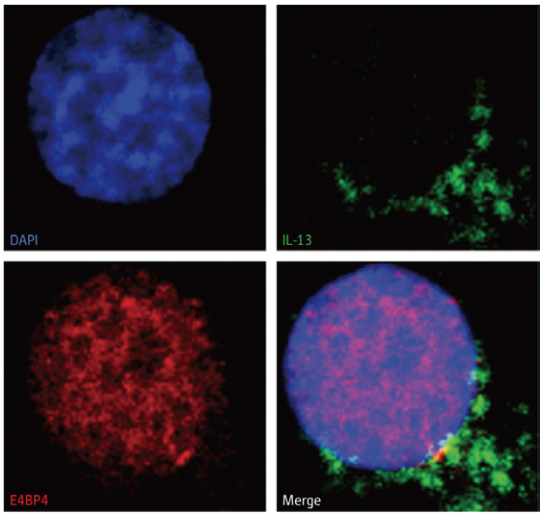


Figure: In T cells stained blue (*top left*), the transcription factor E4BP4 (*red*) regulates that production of IL-13 (*green*) and IL-10 (not shown).

Interleukin-10 (IL-10) is an anti-inflammatory cytokine protein that reduces immune responses and staves off autoimmune disease. Now, a research team led by Masato Kubo at the RIKEN Research Center for Allergy and Immunology, has identified a transcription factor called E4 promoter-binding protein (E4BP4) that is responsible for driving the expression of IL-10 in multiple types of immune cells.

The researchers investigated E4BP4 because of a unique property of a subset of immune cells called T helper type 1 (T_H1) cells, which generally enhance immune responses by secreting pro-inflammatory cytokines. However, under chronic stimulation with foreign antigens—that occur during chronic infection—T_H1 cells can also produce cytokines, such as IL-10 and IL-13, which are normally made only by other immune-cell types. While the immune system is fighting the infection, IL-13 modulates allergic responses, and IL-10 prevents the immune system from attacking the body.

Kubo and colleagues compared genes expressed in T_H1 cells with and without chronic antigen stimulation, and found that E4BP4 was expressed only in instances of chronic antigen stimulation. When they expressed E4BP4 in T_H1 cells that had not been chronically infected, it induced production of IL-10 and IL-13 in conditions in which those cytokines would not normally occur (Figure). E4BP4-deficient T_H1 cells could not increase expression of IL-10 and IL-13 after chronic antigen stimulation. The researchers found that other T cell subsets also required E4BP4 to modulate the expression of IL-10, but not IL-13.

Transcription factors can control the expression of genes by binding to a region on the genomic DNA called the promoter. Kubo and colleagues observed that E4BP4 bound to the IL-13 promoter in T_H1 cells that had been chronically stimulated with antigen. No binding occurred with T_H1 cells lacking chronic stimulation. Kubo explains, however, that: "E4BP4 seems to regulate the expression of IL-10 in a totally different way—by altering the chromosomal structure in the region of that gene."

Mice lacking IL-10 can spontaneously develop intestinal autoimmune disease. Interestingly, Kubo and his team found that E4BP4-deficient mice produced lower levels of IL-10 than control mice, and showed some symptoms of gastrointestinal inflammation along with diarrhea. The mice lacking E4BP4 also developed more severe symptoms of a neurological autoimmune disease caused by exposure to brain antigens. E4BP4 is therefore a key factor in preventing the immune system from attacking the body's own organs, and "induction of expression of E4BP4 may cure many types of autoimmune inflammatory diseases," says Kubo.

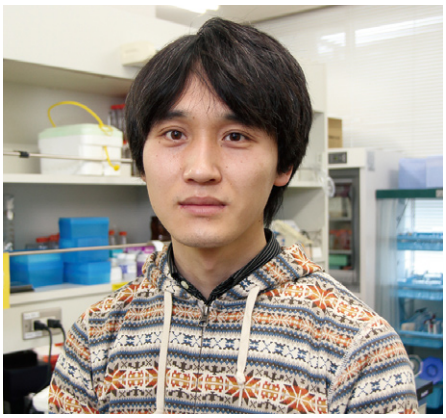
Original Research Paper

Yasutaka Motomura, Hiroshi Kitamura, Atsushi Hijikata, Yuko Matsunaga, Koichiro Matsumoto, Hiromasa Inoue, Koji Atarashi, Shohei Hori, Hiroshi Watarai, Jinfang Zhu, Masaru Taniguchi & Masato Kubo. The transcription factor E4BP4 regulations the production of IL-10 and IL-13 in $CD4^+$ T cells. ***Nature Immunology***, Vol. 12, 450-459, 2011.

Shohei Hori

Following an immune cell's career path

A protein 'fingerprint' used to identify certain immune cells is expressed more broadly than first thought, raising new questions about how these cells develop



Takahisa Miyao

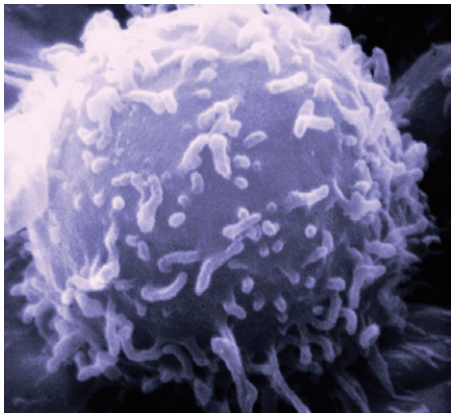


Figure: The various classes of T cells are capable of responding to pathogenic threats and restraining the resulting immune response to avoid inflicting damage on host tissues.(Source: Dr. Triche, National Cancer Institute, USA)

The immune system produces diverse varieties of T cells (Figure), such as pathogen-destroying cytotoxic T cells and immune response-boosting helper T cells. Regulatory T (T_{reg}) cells restrain these other cells and prevent the body from overreacting to threats or generating a dangerous autoimmune response.

T_{reg} cells are usually identified by expression of the transcriptional regulator protein Foxp3, but new work from a team led by Shohei Hori of the RIKEN Research Center for Allergy and Immunology has demonstrated that this is not a reliable signature.

Several groups have obtained data suggesting that T_{reg} cells can essentially 'change careers', losing their Foxp3 expression and transforming into other T cell types. However, findings from Hori and colleagues led them to propose an alternative 'heterogeneity model'. "Our observations suggested that these phenomena can be fully explained by a minor 'uncommitted' population of $Foxp3^+$ T cells without assuming reprogramming," he says. His group has now provided compelling evidence for this hypothesis by using a labeling technique that allowed them to distinguish cells currently expressing Foxp3 from those that are not, but which have expressed this protein in the past.

The researchers identified two groups of Foxp3-expressing cells that responded differently to an immune stimulus. Most expressed this protein stably and at high levels, and exhibited the functional characteristics of T_{reg} cells. A minority fraction displayed transient bursts of Foxp3 expression, but ultimately developed into other T cell types. These 'exFoxp3' cells did not appear to represent reprogrammed T_{reg} cells, but rather a separate pool of T cells that only produce this protein sporadically.

Interestingly, his team also learned that some T_{reg} cells do enter a state where they stop expressing Foxp3, although they retain 'memory' of their identity as T_{reg} cells. This is achieved via chemical modifications to the DNA within the gene encoding Foxp3, and immune stimulation promptly leads to robust re-expression of this protein. "This should force people to reconsider the popular but oversimplified view of Foxp3 as the master regulator of T_{reg} cells," says Hori.

The events that determine this expression profile are therefore likely to prove more important in establishing cellular identity than the presence or absence of Foxp3. "It has been speculated that the T_{reg} lineage is determined by a higher-order regulatory pathway upstream of Foxp3, but the nature of this system is unknown," says Hori. In future work, he plans to partner with colleagues at other RIKEN laboratories to investigate this question more closely.

Original Research Paper

Takahisa Miyao, Stefan Floess, Ruka Setoguchi, Herve Luche, Hans Joerg Fehling, Herman Waldmann, Jochen Huehn and Shohei Hori. Plasticity of $Foxp3^+$ T cells reflects promiscuous Foxp3 expression in conventional T cells but not reprogramming of regulatory T cells. ***Immunity***, 36, 161-163, 2012

Masato Kubo

CNS2 is a critical regulator of IL-4 production by follicular helper T cells

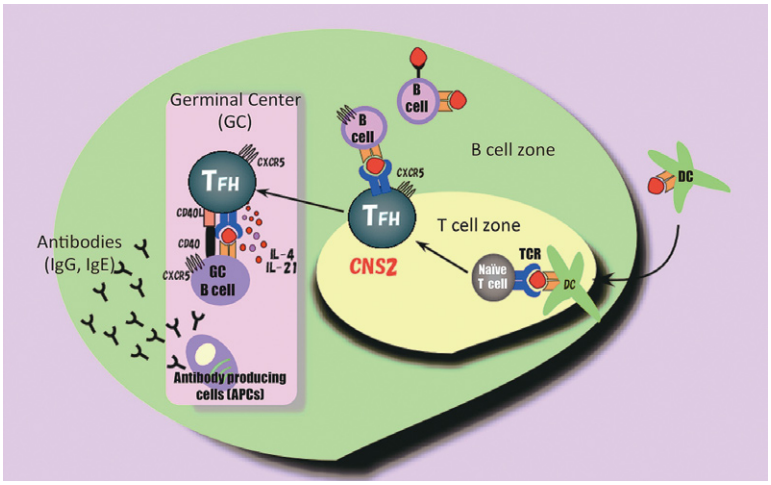


Figure: T_{FH} cells stimulate IgG1 and IgE antibody production

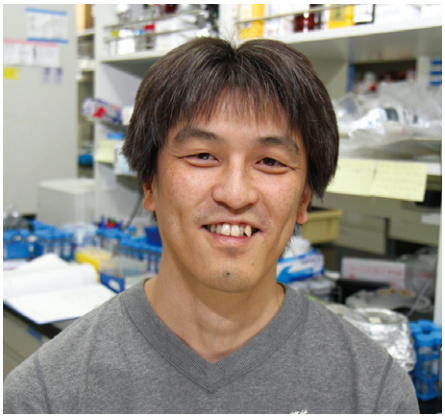
Upon antigen stimulation, naïve helper T cells differentiate into various types of effector helper T cells. Type 2 helper T (Th2) cells are effector helper T cells that secret the cytokine IL-4 and stimulate B cells to produce IgG1 and IgE antibodies, thus mediating atopic and allergic diseases.

Follicular T (Tfh) cells are a recently defined subset of helper T cells that are distinguishable from other helper T cells in two important ways: by their location in the B cell follicles and by their function - to activate B cells and trigger germinal center formation. This results in clearance of viral and bacterial infections through induction of potent humoral immunity. Like Th2 cells, the Tfh cells also secrete IL-4 and stimulate antibody class switching by B cells to produce IgG1 and IgE antibodies. Differences in the regulatory mechanisms and functions between Th2 and Tfh remained unknown.

To elucidate the mechanisms of IL-4 secretion by Th2 and Tfh cells, Masato Kubo (Open Laboratory for Signal Network) and his team generated a series of mutant mice that genetically lack each *cis*-acting gene regulatory element in the noncoding regions of the *Il-4* locus. They found that mice lacking CNS2, an enhancer located ~10 kb downstream of the *Il-4* coding exons, had a reduced production of IgG1 and IgE. To understand the function of CNS2, they next generated CNS2 reporter mice, in which CNS2 activated cells could be detected by GFP expression. GFP positive cells were localized in B cell follicles and their gene expression pattern was similar to Tfh cells. From these results, Kubo and the team discovered that CNS2 is the cell type-specific *Il-4* enhancer in Tfh cells. They also analyzed the development of CNS2-active Tfh cells, and found that these cells developed from naïve T cells 3-5 days after immunization with antigen.

To test the impact of CNS2-deficiency on allergic diseases, they induced bronchial asthma in CNS2 deficient mice. Asthma was observed in CNS2 deficient mice, indicating that development of asthmatic disease is mediated by Th2 and not by Tfh cells.

"We revealed a unique role of CNS2 during the induction phase of *Il-4* gene expression in the Tfh cell lineage. Tfh cells are important in stimulating B cell antibody production and clearing viral and bacterial infections. Our discovery will be an important step toward the understanding of antibody production and the development of effective vaccines," said Kubo.



Yohsuke Harada

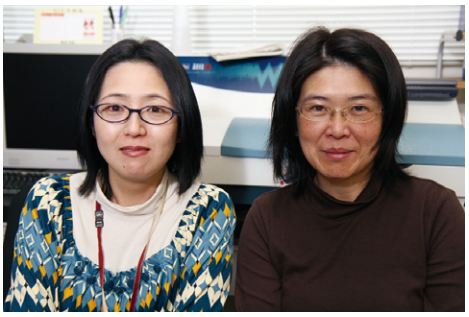
Original Research Paper

Harada, Y., Tanaka, S., Motomura, Y., Harada, Y., Ohno, S., Ohno, S., Yanagi, Y., Inoue, H., Kubo, M.The 3' enhancer CNS2 is a critical regulator of interleukin-4-mediated humoral immunity in follicular helper T cells. ***Immunity***. 36(2):188-200. (2012)

Takashi Saito

Keeping the immune system on track

Specialized motor proteins help control immune activation by physically hauling clusters of signaling receptors to a central site for eventual disposal



Akiko Hashimoto-Tane *(left)* and Machie Sakuma *(right)*

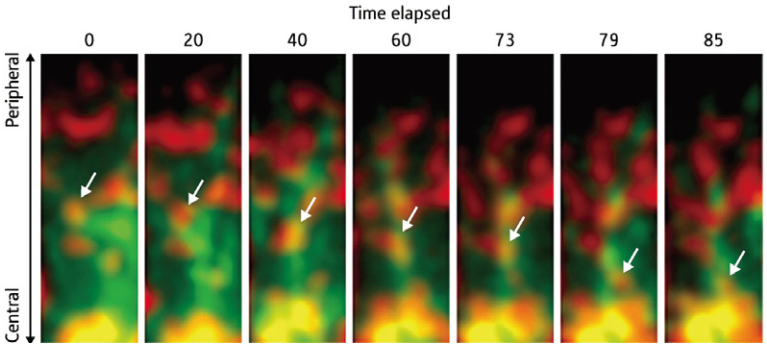


Figure: Following activation, a TCR-MC (*white arrow*) travels along microtubules of the cytoskeleton (*green*), making its way from the periphery to the cSMAC (time scale, seconds). © 2011 Elsevier Inc.

Specialized immune cells called T cells can recognize threats and induce immune responses through T cell receptors (TCRs), but these receptors do not act alone. Multiple receptors gather together at the cell surface to cooperatively switch on T cells. "The minimum unit for triggering T lymphocyte activation is known as the TCR microcluster [TCR-MC]," explains Takashi Saito of the RIKEN Research Center for Allergy and Immunology. "These are the key structure for T cells to recognize antigens and become activated."

At the interface between T cells and the antigen-presenting immune cells that switch them on, TCR-MCs accumulate at a structure called the central supramolecular activation cluster (cSMAC). Now, research from Saito and colleagues has revealed unexpected insights into how this accumulation occurs.

Saito and his team were the first to characterize TCR-MC function, but they were uncertain how these clusters make their way from the periphery to the core of the cSMAC. To understand this phenomenon, they performed a series of experiments in which T cells were placed on an artificial lipid layer that mimics the membrane of an antigen-presenting cell, allowing them to microscopically visualize activation-related events at the T cell surface.

Cellular structures are reinforced by protein fibers that form a network called the cytoskeleton, and Saito and colleagues revealed that TCR-MC movement is mediated by dynein, a 'motor protein' that shuttles cargos along these fibers. "We knew lymphocyte activation was regulated through the cytoskeleton," he says. "But it was most surprising that TCR complexes are physically associated with dynein and that their movement is mediated by assembling with this complex."

Upon TCR activation, the dynein-facilitated movement drags TCR-MCs laterally along the surface of the membrane towards the cSMAC (Figure), a function previously unseen for this motor protein. Pharmacological inhibition of dynein strongly impaired migration of TCR-MCs and undermined their assembly within the cSMAC, as did the selective reduction of a key subunit of the dynein complex. Intriguingly, the same treatments that impaired TCR-MC migration also enhanced T cell activation. Saito and colleagues therefore concluded that once these clusters reach the center of the cSMAC, they become internalized within the cell and thereby taken out of action.

Saito hopes to exploit this effect by learning how the TCR-MC-dynein complex is assembled. "It would be ideal if we had a specific inhibitor of this assembly," he says, "which could lead to stronger immune status with enhanced activation of T cells."

Original Research Paper

Akiko Hashimoto-Tane, Tadashi Yokosuka, Kumiko Sakata-Sogawa, Machie Sakuma, Chitose Ishihara, Makio Tokunaga, and Takashi Saito. Dynein-driven transport of T cell receptor microclusters regulates immune synapse formation and T cell activation. ***Immunity***, Vol. 34, 919-931, 2011.

Related paper

Tadashi Yokosuka, Kumiko Sakata-Sogawa, Wakana Kobayashi, Michio Hiroshima, Akiko Hashimoto-Tane, Makio Tokunaga, Michael L Dustin and Takashi Saito. Newly generated T cell receptor microclusters initiate and sustain T cell activation by recruitment of Zap70 and SLP-76. ***Nature Immunology*** Vol. 6, 1253-1262, 2005.

Katsuaki Sato

Reacting to changing circumstances

An analysis of a subset of immune cells reveals how these cells rally defenses against infection while keeping potentially harmful inflammatory reactions in check

Hideaki Takagi

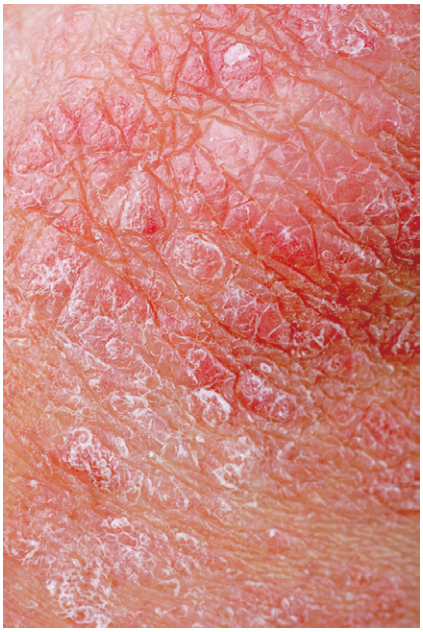


Figure: In autoimmune conditions such as the skin condition psoriasis, the immune system attacks tissues in the body, with painful consequences for patients. © 2012 iStockphoto/QUAYSIDE

T cells represent a significant component of the 'muscle' in the immune system, promoting aggressive action against perceived threats or restraining fellow immune cells from launching an unhealthy autoimmune response (Figure). Dendritic cells (DCs) help to manage these cells, presenting bits of antigen to T cells in a context that allows them to react appropriately.

As with everything in the immune system, however, the biological details are considerably more complex. "DCs consist of heterogeneous subsets, including conventional DCs (cDCs) and plasmacytoid DCs (pDCs)," explains Katsuaki Sato of the RIKEN Research Center for Allergy and Immunology, "and the precise functional role of each DC subset in immune responses remains unclear." Sato is especially interested in pDCs, as the experimental data obtained to date have done little to clarify their behavior within the body.

Sato and his colleagues from Japan and France recently published a detailed analysis of pDC function, achieved by selectively obliterating this cell population in mice. To do this, the team inserted a toxin gene into the gene encoding Siglec-H, a protein uniquely expressed by pDCs; by chemically activating this fatal factor, the researchers could rapidly eliminate Siglec-H-producing cells. As an added benefit, this insertion effectively knocked out Siglec-H expression, revealing the functional contributions of this protein in otherwise normal pDCs.

pDCs express a protein called toll-like receptor 9 (TLR9), which responds particularly to the presence of pathogens such as viruses and bacteria. The researchers determined that pDCs generate various inflammatory signals in response to TLR9 activation, but that the levels of these signals are normally modulated by the inhibitory action of Siglec-H, which appears to be a key regulatory molecule in these cells.

Their experiments confirmed a central role for pDCs in responding to infection, driving both the inflammatory response pathway as well as the production of pathogen-destroying cytotoxic T lymphocytes. However, pDCs also appear to make an important contribution to the process of 'peripheral tolerance', which holds the immune system in check and prevents it from overreacting to non-threatening antigens. Specifically, pDC signals inhibited the production of antigen-specific helper T cells, which activate other immune cells, and favored the formation of regulatory T cells, which help to restrain the immune response.

These latter findings were somewhat surprising, and Sato hopes to delve further into their implications for human health in future studies. "We have a plan to analyze the role of pDCs and their regulation in the control of autoimmune disease," he says.

Original Research Paper

Hideaki Takagi, Tomohiro Fukaya, Kawori Eizumi, Yumiko Sato, Kaori Sato, Azusa Shibazaki, Haruna Otsuka, Atsushi Hijikata, Takashi Watanabe, Osamu Ohara, Tsuneyasu Kaisho, Bernard Malissen, and Katsuaki Sato. Plasmacytoid dendritic cells are crucial for the initiation of inflammation and T cell immunity *in vivo*. ***Immunity***, Vol. 35, 958-971, 2011

Takashi Tanaka

PDLIM2 inhibits T helper 17 cell development and inflammation

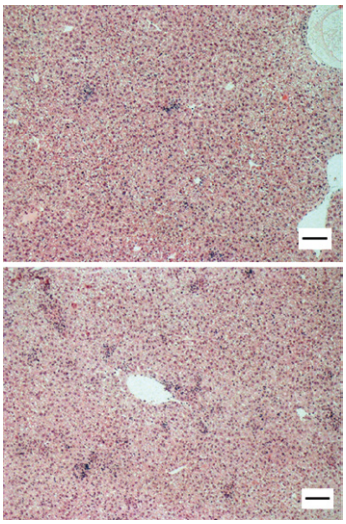


Figure: Relative to wild-type mice (*top*), the livers of PDLIM2-deficient animals (*bottom*) show considerably higher levels of granuloma formation. Granulomas are indicated by dark spots formed following staining with hematoxylin and eosin (scale bars, 100 μm).

The T helper 17 (T_H17) cell belongs to a newly identified helper T cell subset that is distinct from T_H1 and T_H2 cells. Whereas T_H1 and T_H2 cells characteristically produce the cytokines interferon-γ and interleukin (IL)-4, respectively, T_H17 cells secrete IL-17, IL-21, and IL-22 as effector cytokines. T_H17 cells can induce a massive inflammatory response and have essential roles in eliminating microbial pathogens. However, T_H17 cells are also potentially highly pathogenic, because excessive and prolonged activation of T_H17 cells may cause human autoimmune and inflammatory diseases, including rheumatoid arthritis and inflammatory bowel diseases. T_H17 cells are also critically involved in the development of granulomatous inflammation, which suggests that controlling the development/function of T_H17 cells will be an important strategy to prevent and treat granulomatous diseases.

Although much is known about the differentiation of T_H17 cells, little is known about how these cells are inhibited. Takashi Tanaka (Research Unit for Inflammatory Regulation, RCAI) in collaboration with Tadashi Matsuda (Hokkaido University) discovered that the ubiquitin ligase PDLIM2 inhibits T_H17 cell differentiation.

To analyze T_H17 induced inflammation, they used an established bacterial-induced inflammation model, *Propionibacterium acnes*-induced liver granuloma. The granulomatous inflammation was significantly enhanced in PDLIM2-deficient mice compared to wild-type mice (Figure). T cells isolated from PDLIM2 deficient mice produced two- to fivefold more IL-17, IL-21 and IL-22 than the control mice due to the enhanced differentiation of T_H17 cells.

To clarify how PDLIM2 was involved in the regulation of T_H17 cell differentiation, the researchers analyzed signaling molecules that interact with PDLIM2. Using coimmunoprecipitation and reporter assays, they found that PDLIM2 interacts with STAT3 and terminates STAT3-mediated signaling. Furthermore, they found that PDLIM2 mediates ubiquitination of STAT3, and that the ubiquitinated STAT3 molecules are degraded through the proteasome pathway. Indeed, STAT3 was more stable in PDLIM2-deficient cells, and STAT3-dependent gene expression was substantially increased when PDLIM2 was knocked-down by small interfering RNA (siRNA).

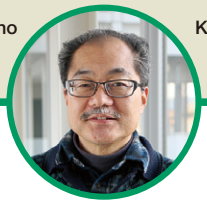
STAT3 is a master regulator of the development of highly proinflammatory T_H17 cells, and T_H17 cells are critically involved in several inflammatory and autoimmune diseases. Therefore, as a suppressor of STAT3, PDLIM2 may be a therapeutic target to prevent T_H17 cell-mediated diseases. "Recent studies suggest that T_H1 and T_H17 cell subsets are not mutually exclusive, but cooperatively induce inflammatory responses," says Tanaka. "In this study and in our previous work, we have demonstrated that PDLIM2 can negatively regulate the development of both cell types, and thus represents a useful new target for the treatment of human autoimmune and inflammatory diseases."

Original Research Paper

Tanaka, T., Yamamoto, Y., Muromoto, R., Ikeda, O., Sekine, Y., Grusby, M.J., Kaisho, T. & Matsuda, T. PDLIM2 inhibits T helper 17 cell development and granulomatous inflammation through degradation of STAT3. ***Science Signaling*** 4, ra85 (2011).

Related paper

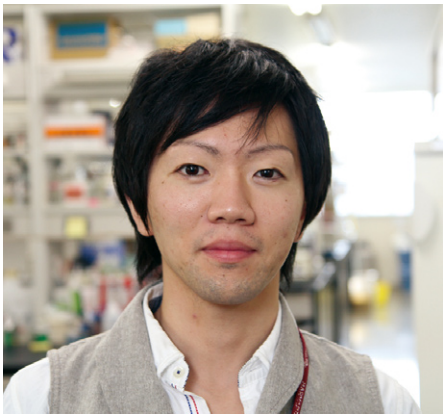
Tanaka, T., Soriano, M.A. & Grusby, M.J. SLIM is a nuclear ubiquitin E3 ligase that negatively regulates STAT signaling. ***Immunity*** 22, 729-736 (2005).


Hiroshi Ohno


Koji Hase

Sorting out gut health

Gastrointestinal inflammation is prevented by a protein sorting factor found in cells lining the gut


Daisuke Takahashi

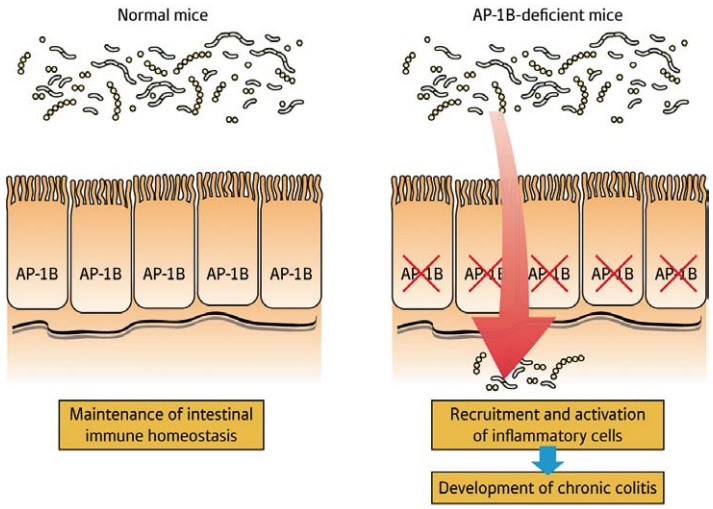


Figure : AP-1B expression in gut epithelial cells prevents bacteria from entering the gut and causing inflammatory bowel diseases.

The gastrointestinal tract is lined with intestinal epithelial cells (IECs) that maintain gut health by keeping bacteria and pro-inflammatory immune cells from infiltrating gut tissues. Now, a team of researchers in Japan has shown that a protein in these cells, which is responsible for sorting many proteins to particular portions of the IEC surface, plays a key role in IEC modulation of gut inflammation.

IECs are polarized cells, with a bottom surface that attaches to deeper gut tissues, and a top surface that faces the inside of the gut, where it is exposed to ingested food and gut-resident bacteria. Proteins that are created in the cell are sorted preferentially to either the top or the bottom portion of the IEC. For example, cytokine receptors are shuttled mainly to the bottom of IECs so they can respond to cytokines released by immune cells within deeper gut tissues. Led by Koji Hase at the RIKEN Research Center for Allergy and Immunology, the researchers thought that disruption of proper protein sorting could affect the ability of IECs to properly respond to their environment.

To test their theory, the researchers generated mice lacking the μ1B subunit for a sorting protein called adaptor protein-1B (AP-1B). These mice developed an inflammatory gut disease called colitis, in which large number of immune cells infiltrated the gut. Mice lacking AP-1B expressed fewer antibacterial proteins, allowing bacteria to attack gut tissue (Figure). Hase and colleagues showed that this bacterial entry enhanced immune cell recruitment into the gut, because antibiotics could reduce the inflammation in these mice.

Cytokines such as interleukin-17 (IL-17) are responsible for inducing antibacterial protein expression in IECs. However, the researchers found that cells lacking AP-IB were unable to properly sort cytokine receptors, including the IL-17 receptor, to the appropriate portion of the IEC membrane. This suggested that IECs may have failed to properly respond to IL-17 because its receptors were in the wrong part of the cell.

When Hase and colleagues examined IECs in humans with an inflammatory bowel condition called Crohn's disease, they found that expression of the μ1B subunit was reduced, and that one cytokine receptor seemed to sort to the wrong portion of the IEC surface. "AP-1B-dependent protein sorting therefore seems to control epithelial immune functions that keep the human gut healthy," explains Hase. Enhancing the expression of μ1B could be a potential therapy for Crohn's disease, the team concludes.

Original Research Paper

Daisuke Takahashi, Koji Hase, Shunsuke Kimura, Fubito Nakatsu, Masumi Ohmae, Yasushi Mandai, Toru Sato, Yasuhiro Date, Masashi Ebisawa, Tamotsu Kato, Yuuki Obata, Shinji Fukuda, Yuki I. Kawamura, Taeko Dohi, Tatsuro Katsuno, Osamu Yokosuka, Satoshi Waguri, Hiroshi Ohno. The epithelia-specific membrane trafficking factor AP-1B controls gut immune homeostasis in mice. ***Gastroenterology*** Vol. 141, 621-632, 2011.

Tomohiro Kurosaki

CIN85 drives B cell responses by linking the BCR to the NF-κB signaling pathway

Kohei Kometani *(left)* and Tadashi Yokosuka *(right)*

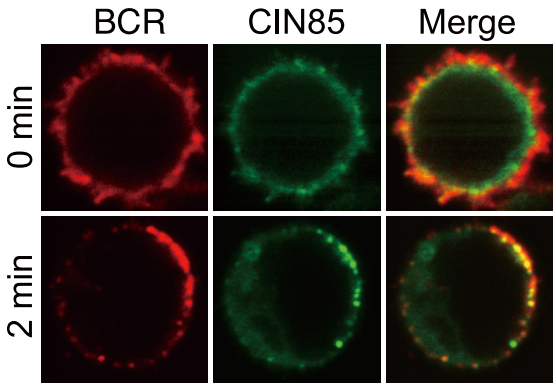


Figure : Colocalization of CIN85 clusters and BCR

When pathogens invade the body, B cells quickly respond to them by producing antibodies. B cells can recognize the antigens on pathogens because they bind to receptors on their cell surface (B cell receptor (BCR)). Binding of antigen stimulates downstream signaling pathways that promote activation, proliferation and differentiation of B cells to produce antibodies. Defects in these signaling pathways would lead to insufficient antibody production and immunodeficiency, whereas excess activation could cause autoimmune diseases or B cell malignancies. Thus, elucidation of BCR-mediated signaling pathways is essential for understanding the cause of such diseases and for the development of new therapies.

Dr. Tomohiro Kurosaki and his group (Laboratory for Lymphocyte Differentiation) previously discovered that the adaptor protein BLNK is a key molecule in BCR signaling and subsequent B cell responses. To further understand the role of BLNK in BCR signaling, they decided to identify proteins that bind to BLNK.

Using a yeast two-hybrid screen, they found that CIN85, another adaptor molecule, interacts with BLNK. Because CIN85 is expressed in many different immune cell types, they generated a B cell-specific CIN85 knockout mice to analyze its function in B cells. To determine its role in antibody production, they immunized the mice with a T cell independent type II (TI-II) antigen. TI-II antigens are known to stimulate antibody production in the absence of T cell help. The TI-II response was barely detectable in the CIN85 knockout mice, indicating the importance of CIN85 in T cell independent antibody production.

To further dissect the proliferative signaling pathway involving CIN85, they gave various stimuli to CIN85 knockout B cells *in vitro*. The mutant B cells exhibited defective survival after anti-IgM stimulation, demonstrating CIN85 involvement in BCR-mediated proliferation and survival. Because BCR stimulation induces multiple signaling pathways, they next decided to address which pathways are perturbed by the CIN85 knockout. They identified a defect in the NF-κB signaling pathway in the mutant B cells and could correct this defect *in vivo* by introduction of a constitutively active form of IKK-β, an NF-κB activating enzyme, into the knockout mice. Thus, they confirmed that CIN85 participates in the BCR-mediated NF-κB signaling pathway.

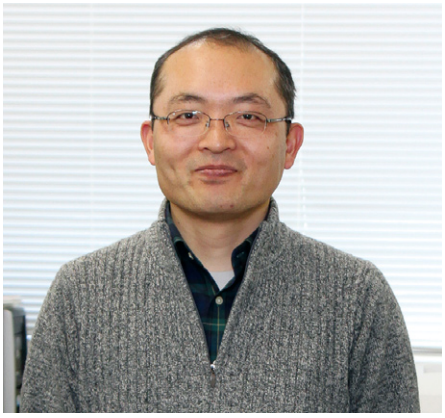
In this study, the RCAI investigators discovered the importance of CIN85 in BCR-mediated survival, proliferation and antibody production due to its association with BLNK and participation in NF-κB signaling. This finding suggests that a functional defect in CIN85 would induce immunodeficiency, or that hyperactivation of CIN85 could induce autoimmune diseases or cancer. Therefore, CIN85 may be a promising therapeutic target for regulation of B cell antibody production and control of immune diseases.

Original Research Paper

Kometani, K., Yamada, T., Sasaki, Y., Yokosuka, T., Saito, T., Rajewsky, K., Ishiai, M., Hikida, M. and Kurosaki, T. CIN85 drives B cell responses by linking BCR signals to the canonical NF-κB pathway. ***J Exp Med***. 208 (7): 1447-57. 2011

Masaru Taniguch

Functional and developmental heterogeneity of invariant natural killer T cells



Hiroshi Watarai

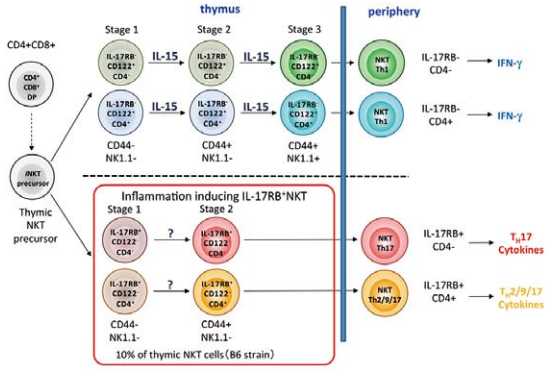


Figure: Newly proposed model of NKT cell development and function

Allergy has become one of the most prevalent diseases in Japan, affecting 30% of the population. Atopic asthma is one of the more serious allergic diseases and it afflicts 300 million people worldwide, causing 250 thousand deaths every year. The numbers are increasing; childhood asthma is found in 6% of children these days, six times more than in the 1980's.

A research team lead by Masaru Taniguchi and Hiroshi Watarai (Group Director and Senior Researcher, respectively, at the Laboratory for Immune Regulation) had previously identified a subtype of Natural Killer T (NKT) cells expressing IL-17 receptor B (IL-17RB$^+$) that triggers the pathogenesis in airway hyper reactivity, an animal model for asthma. IL-17RB$^+$ NKT cells produce IL-4, IL-5, IL-13, IL-17A and IL-22, cytokines that cause airway inflammation and damage bronchial mucosa. In further investigating this NKT subtype, they recently discovered that there are distinct developmental lineages and functions among NKT cells (Figure).

It is known that NKT cells change their cell surface marker expression patterns during development; NKT cells develop through Stage 1 (CD44$^-$ NK1.1$^-$), Stage 2 (CD44$^+$ NK1.1$^-$) and Stage 3 (CD44$^+$ NK1.1$^+$) in the thymus and then move to the periphery. When the team analyzed the expression of IL-17RB in mouse thymus, they found that only 10% of thymic NKT cells were IL-17RB$^+$, and most of them were in Stage 1 and 2. Surprisingly, these IL-17RB$^+$ NKT cells did not require IL-15, which, until now, was thought to be essential for NKT cell maturation. IL-17RB$^+$ NKT cells maturated from Stage 1 to Stage 2 and then, without changing to IL-17RB$^-$, they moved to the periphery. This observation suggested that IL-17RB$^+$ cells constitute a distinct NKT subtype, one which follows a different developmental pathway from the IL-17RB$^-$ NKT cells. Based on cytokine production, two distinct subpopulations of IL-17RB$^+$ NKT cells could be identified, IL-17RB$^+$CD4$^+$ and IL-17RB$^+$CD4$^-$ NKT cells. IL-17RB$^+$CD4$^+$ NKT cells responded to IL-25 and produced T_H2 cytokines (IL-13, IL-4), T_H9 cytokines (IL-9, IL-10) and T_H17 cytokines (IL-17A, IL-22), while IL-17RB$^+$CD4$^-$ NKT cells responded to IL-23 and produced T_H17 cytokines (IL-17A, IL-22). These cytokines are known to induce allergic reactions directly but also to stimulate other immune cells and cause inflammation.

Given this developmental heterogeneity, the team next analyzed *in vivo* functions of IL-17RB$^+$ NKT cells. They found that the allergy prone BALB/c mouse strain had 3-4 fold more IL-17RB$^+$ NKT cells than the less allergic C57BL/6 strain. Indeed, *Il17rb* knockout mice had a defective production of T_H2, T_H9, and T_H17 cytokines, and when they were infected with respiratory syncytial virus (RSV), which causes bronchitis and pneumonia, *Il17rb* knockout mice did not develop any airway inflammation.

"IL-17RB$^+$ NKT cells were responsible for exacerbating the inflammation caused by RSV infection. RSV infection is one of the major causes of pneumonia in asthmatic babies and children. Regulation of IL-17RB$^+$ NKT cells would be a key for prevention of inflammation in childhood asthma," said Hiroshi Watarai.

This research was supported by Japan Science and Technology Agency's Precursory Research for Embryonic Science and Technology (PRESTO).

Original Research Paper

Hiroshi Watarai, Etsuko Sekine-Kondo, Tomokuni Shigeura, Yasutaka Motomura, Takuwa Yasuda, Rumi Satoh, Hisahiro Yoshida, Masato Kubo, Hiroshi Kawamoto, Haruhiko Koseki and Masaru Taniguchi. Development and function of invariant natural killer T cells producing T_H2- and T_H17-cytokines. ***PLoS Biol***. Vol. 10, e1001255, 2012.

Related paper

Asuka Terashima, Hiroshi Watarai, Sayo Inoue, Etsuko Sekine, Ryusuke Nakagawa, Koji Hase, Chiaki Iwamura, Hiroshi Nakajima, Toshinori Nakayama, and Masaru Taniguchi. A novel subset of mouse NKT cells bearing the IL-17 receptor B responds to IL-25 and contributes to airway hyperreactivity. ***J. Exp. Med.***, Vol. 25, 2727-2733, 2008.

Fumihiko Ishikawa

Membrane-bound human SCF/KL promotes *in vivo* human hematopoietic engraftment and myeloid differentiation in mice

Shinsuke Takagi

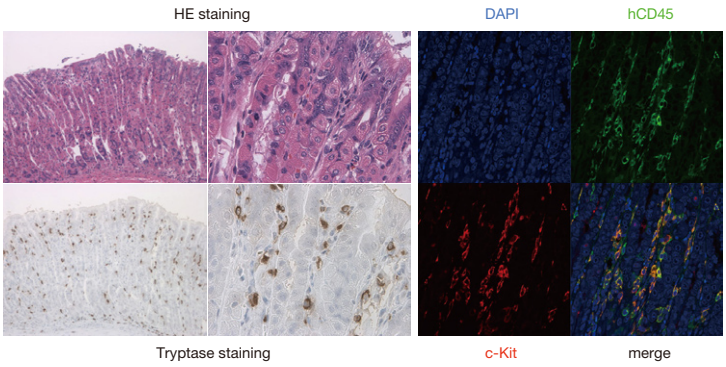


Figure: Distribution of human mast cells in the stomach of a humanized mouse. The left panel shows H&E (*top*) and mast cell tryptase (*bottom*) staining of humanized hSCF Tg NSG mice. The right panel shows staining for human CD45 to identify human hemopoietic cells, human c-kit to identify mast cells, and a merged image. DAPI staining identifies nucleated cells in this section of mouse stomach.

The humanized mouse model system has served as a valuable tool to investigate human hematopoiesis, immunity, and diseases *in vivo*, because biomedical research involving living humans is severely limited by ethical and technical constraints. However, one of the major limitations of the humanized mouse model is that the tissue microenvironments are primarily of mouse origin. The lack of an appropriate microenvironment contributes to the impaired development of human T-lymphoid and myeloid cells in this system, thus limiting investigation of human hematopoiesis and immunity in the humanized mouse. To help overcome this problem, in collaboration with Dr. Leonard D. Shultz at the Jackson Laboratory, Dr. Fumihiko Ishikawa and his team (Laboratory for Human Disease Model) had previously reported the development of functional human T cells in NOD/SCID/IL2rγKO (NSG) mice expressing human HLA class I, a molecule important for human T cell selection/development and function in the mouse. Recently, they further modified this NSG mouse and successfully created an improved mouse (hSCF Tg NSG mouse) that expresses a membrane bound form of human stem cell factor (SCF), which is critical for the maintenance of stem and progenitor cell activities. When this hSCF Tg NSG mouse was transplanted with human hematopoietic stem cell to generate a humanized mouse, chimerism of human hematopoietic cells was dramatically increased. The frequency of human lymphoid and myeloid cells that developed in the bone marrow of hSCF Tg NSG mice was significantly higher (97.1% and 49.7% respectively) compared with non-Tg NSG mice (63.1% and 26.2% respectively).

Among the leukocytes, the researchers identified human mast cells, granulocytes and antigen-presenting cells. Notably, the greatest subfraction among engrafted human myeloid cells consisted of granulocytes including neutrophils and mast cells. Both mature and immature human mast cells were present in bone marrow, and human mast cells were identified not only in spleen and hematopoietic organs but also in lung, gastric tissue, and intestinal tissues of hSCF Tg NSG recipients.

In human hematopoiesis, SCF-cKit signaling is critical for the maintenance of stem and progenitor cell activities. When human mast cells from gastric tissues of hSCF Tg recipients were examined, human cKit was present in these cells. The observed significant improvement in human hematopoietic cell chimerism could be attributed to preferential binding of human SCF to human c-Kit in hematopoietic stem cells, resulting in efficient SCF-c-Kit signaling.

To further improve human hematopoietic cell development in the humanized mouse, it is clearly necessary to humanize the microenvironment. This is also required for *in vivo* investigation of the interactions between hematopoietic stem cells and their microenvironment. "The hSCF Tg NSG humanized mice will facilitate investigation of *in vivo* differentiation, migration, function, and pathology of human mast cells," Dr. Ishikawa noted.

Original Research Paper

Takagi S, Saito Y, Hijikata A, Tanaka S, Watanabe T, Hasegawa T, Mochizuki S, Kunisawa J, Kiyono H, Koseki H, Ohara O, Saito T, Taniguchi S, Shultz LD, Ishikawa F. Membrane-bound human SCF/KL promotes *in vivo* human hematopoietic engraftment and myeloid differentiation. ***Blood***. 19(12):2768-77. 2012

Outstanding Contribution of the Year 2011

RIKEN RCAI Outstanding Contribution of the Year 2011 was given to three researchers, Toshio Hirano (photo 1), Group Director of Laboratory for Cytokine Signaling, Takaharu Okada (photo 2 left) Leader of the Research Unit for Immunodynamics and Tomokatsu Ikawa (photo 3 left), Senior Researcher of the Laboratory for Lymphocyte Development. This award was established in 2006 to recognize RCAI investigators who make outstanding contributions to the Center.

Dr. Toshio Hirano received the award for his contribution to promotion of Japanese and RIKEN's Immunology research by the discovery of IL-6, analysis of IL-6 receptor signaling, and further development of the new area of zinc signaling. It is increasingly expected that immunologists will elucidate disease mechanisms and therapeutics. Dr. Hirano's discovery of IL-6 contributed greatly to our understanding of the pathogenic mechanisms of rheumatoid arthritis and other autoimmune diseases (Hirano, Proc. Jpn. Acd., Ser. B, 2010). Dr. Hirano had already been awarded the Crafoord Prize from the King of Sweden and he also received the Japan Prize last year for "Discovery of interleukin-6 and its application in treating diseases." These awards made impacts throughout the world and encouraged RIKEN researchers, especially the young scientists. Dr. Hirano's pioneering research on zinc and zinc transporters also contributed to the outstanding reputation of RIKEN's science.

Dr. Hirano and his team discovered that zinc has roles in mediating and controlling intracellular signaling events. In 2004, they found that the Zn transporter Slc39a6/Zip6/Liv1 is a STAT3 target gene and showed that it has a role in cell migration during early zebrafish development (Yamashita et al., *Nature*, 2004). They then found in dendritic cells that LPS-induced maturation is partly mediated through lowering the intracellular concentration of free zinc (Kitamura et al., *Nat Immunol*, 2006). To physiologically analyze the function of zinc, they generated knockout mice of various zinc transporters. Using *ZIP13* deficient mice, they found that ZIP13 is required for bone, teeth and connective tissues development, and is involved in BMP/TGF-β signaling, and that the same genetic defect causes Ehlers-Danlos syndrome in humans (Fukada et al., *PLoS ONE*, 2008). Deletion of the *ZIP14* gene resulted in growth retardation and dysregulation of G-protein coupled receptor signaling (Hojyo et al., *PLoS One*, 2011). They also reported that the zinc transporter ZnT5 is required for mast cell-mediated delayed type allergic responses. (Nishida et al., *J Exp Med*, 2009). In addition to the role of zinc transporters, Dr. Hirano's group found that zinc functions as a second messenger capable of converting an external signal into internal events (Yamasaki et al., *J Cell Biol*, 2007). They named this the "Zinc wave". Based on these results, Dr. Hirano proposed two types of zinc signalings, "late zinc signaling" dependent on changes in the transcription of Zn transporter genes and "early zinc signaling" which occurs without new transcription (Fig. 1) (Hirano et al., Adv Immunol, 2008 , Fukada et al., *J. Biol. Inorg*. Chem, 2011).

Photo 1 : Dr. Toshio Hirano

Photo 2 : Dr. Takaharu Okada (*left*) and Dr. Masaru Taniguchi (*right*)

Photo 3 : Dr. Tomokatsu Ikawa (*left*) and Dr. Masaru Taniguchi (*right*)

Dr. Hirano also contributed to the management of RCAI as Deputy Director. He organized the annual international symposium on immunology in collaboration with JSI, and contributed to the establishment of a collaborative relationship between RCAI and Osaka University. Dr. Hirano was the Dean of Graduate School of Medicine in Osaka University from 2008 till March 2011, and in August of 2011 he became the President of Osaka University. In addition, he has recently become an Executive Member of the Council for Science and Technology Policy (CSTP) for the Japanese Cabinet. To avoid any appearance of conflict of interest, he decided to leave RCAI at the end of March 2012.

In his speech, Dr. Hirano recalled the time he spent at RCAI. "I still remember the day when Dr. Taniguchi was formally appointed as the

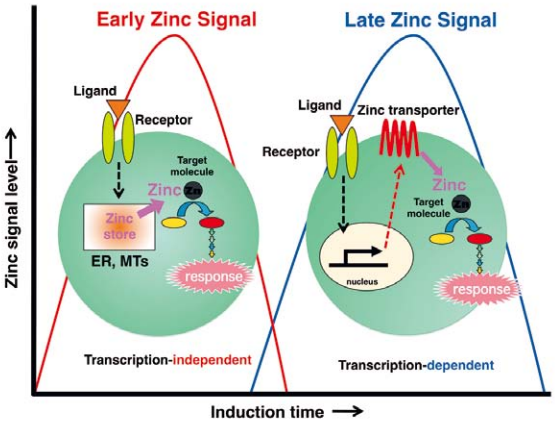


Figure 1: Early Zn signaling and late Zn signaling. Intracellular Zn signaling can be classified into at least two categories: early Zn signaling, involving the Zn wave, which is directly induced by an extracellular stimulus, and late Zn signaling, which is dependent on a transcriptional change in Zn transporter expression

Director of RCAI in June of 2001. Since then, under his excellent leadership, everything began. This building was completed in October 2003. All people gathered here and started activities in April 2004. Then the time passed and we are now celebrating the 10th anniversary. During this 10-year period, RCAI made major contributions to immunology, not only in Japan but also in the world. RIKEN's Second Midterm Plan will end next year, and for the Third Midterm Plan, a new initiative will start under a new director, focusing on integrative biology. It is very sad for me that I have to leave here formally in order to avoid any conflict of interests after appointment to the CSTP. However, I think the passion of this Center, which has developed in the last 10 years, will remain in each of us forever." Director Taniguchi congratulated Dr. Hirano on his decision to take on the most important burden for science in Japan, although it saddened him that Dr. Hirano officially left RCAI.

Dr. Takaharu Okada was awarded for his contribution for establishing "*in vivo* imaging of the dynamics of immune cells by two-photon laser scanning microscopy". This is becoming a powerful new method in immunological studies, and his lab has developed this technique to a remarkably high level that has been reached by only a handful of groups in the world. Recently, his lab further demonstrated the effectiveness of this technique by making important findings regarding the dynamics of germinal center formation, the key event in humoral immune responses. This technique is expected to play an important role in RCAI's future strategy to promote integrative biology for understanding the mechanisms of inflammatory and immunological disease development.

Dr. Okada tracked the process of B cell development in the mouse germinal center (GC) by monitoring expression of Bcl6, a transcription factor that facilitates B cell evolution by regulating expression of key developmental genes. In GCs, antibody-producing B cells undergo differentiation to generate higher-quality antibodies, a process that is supported by a class of T cells known as follicular helper T (Tfh) cells. To track the events *in vivo*, the team generated transgenic mice in which *Yellow Fluorescent Protein* had been inserted into one copy of the *Bcl6* gene. Since this insertion partially disrupted Bcl6 function, this reporter system also enabled them to examine the effects of partial Bcl6 deficiency. Following the induction of an immune response in the BCL6 reporter mouse, Okada and colleagues observed an increase of Bcl6 expression in antigen-specific B cells within peripheral regions of the lymph node; these cells subsequently migrated to the GC, where they continued to express high levels of Bcl6. This migration and maturation process was dependent upon interaction with Bcl6-expressing helper T cells. All T cells expressing high levels of Bcl6 became Tfh cells, but many Tfh cells subsequently reduced Bcl6 production to

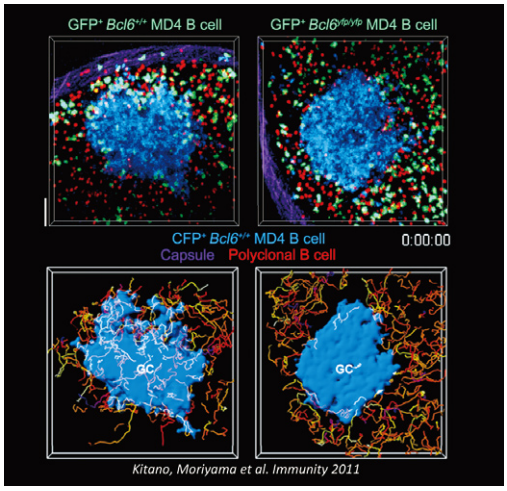


Figure 2: Two-photon imaging of the B cell entry into the pre-formed GC. The top panels are representative images from 60 min recordings. The bottom panels show the surface of GCs reconstructed from the CFP images and the trajectories of the cells.

varying degrees (Fig. 2). Based on these findings, the authors suggest that Tfh cells expressing low levels of Bcl6 may give rise to 'memory cells', which enable the immune system to react quickly to recurring threats. (Kitano et al., *Immunity*, 2011)

Dr. Okada said in his speech that he took the award as encouragement to move towards integrative biology. "We

Photo 4 : Dr. Takaharu Okada (*left*) and Dr. Gib Bogle (*right*) at Dr. Bogle's office

actually would like to do some modeling, but it will be really hard. It is necessary to incorporate many kinds of factors like G-protein coupled receptors, various other receptors and also adhesion molecules, and it will also be hard to model that immune response in tissues. This is a great opportunity RCAI has given me. In 2010, a group of researchers from New Zealand visited us and we had a joint workshop with New Zealand and Chiba University. Two of the researchers, Rod Dunbar (Maurice Wilkinson Center) and Gib Bogle (Bioengineering Institute in University of Auckland) (photo 4 right), invited me for two weeks to launch the collaboration for modeling of immune responses." Drs. Dunbar and Bogle had developed an agent-based model for T cell activation in a lymph node. Agent based models are computational models for simulating action and interaction of autonomous agents, and such models incorporate processes at a range of scales: cell receptor-ligand dynamics, cell motility, TCR stimulation leading to T cell activation and proliferation, and cell trafficking. They are focusing on understanding of the immune response by integrating knowledge, to build an *in silico* laboratory for biological experiments, and to provide a new tool for immunology research. "I think the purpose of our modeling will be to try to extract the information that is hard to get through wet lab experiments, such as lipid mediator micro-distribution; for example S1P or LPA, those really important lipids for B cell and T cell dynamics. It is really hard to get the real data, but how is it if we put X in a model and then are able to get some information like that through the agent-based model of the B cell follicle? The ultimate hope may be to make a virtual lymph node that helps us to improve the design of the synthetic lymph node, by providing this type of information," said Dr. Okada.

Dr. Tomokatsu Ikawa was awarded for his contribution on the "elucidation of molecular mechanisms of T cell lineage commitment". Dr. Ikawa and colleagues established a unique culture system that can induce development of myeloid-T (MT) bipotent progenitors from multipotent hematopoietic progenitors. Using this culture system, Dr. Ikawa discovered Bcl11b as a master regulator for T lineage commitment and also provided molecular evidence in support of the "Myeloid-based model" in T cell development, which was discovered by Dr. Kawamoto at RCAI. This study has had a huge impact on the field of developmental immunology, and Dr. Ikawa has been recently selected to receive the Minister of Education, Culture, Sports, Science and Technology (MEXT) Prize for Young Investigators 2012.

Dr. Ikawa started his research career in 1997 when he was a graduate student in Dr. Katsura's laboratory in Kyoto University. His first project was to understand how T or NK lineage commitment occurs. He established a novel clonal culture system to study T or NK commitment status, and he also showed that Id2 is essential for NK lineage commitment from bipotent progenitors (Ikawa et al., *Proc Natl Acad Sci U S A*, 2001). After obtaining a Ph.D. degree, he moved to Cornelis Murre's lab at UCSD as a postdoc. Dr. Murre had identified E2A, a famous transcription factor essential for B cell lineage differentiation. In his lab, using E2A knockout progenitor cells, Dr. Ikawa found that these E2A knockout hematopoietic progenitor cells have self-renewal activity with multipotentcy (Ikawa et al., *Immunity*, 2004).

He joined Dr. Kawamoto's lab in 2006 and started research on T cell lineage commitment. Their main interest was how hematopoietic stem cells or early multipotent progenitors commit to the T cell lineage in the prethymic or early thymic stages. Dr. Masuda found that the commitment occurs in the thymic DN2 stage, so that in the earlier stages, MT progenitors still maintain other lineage potentials, such as macrophage or NK cells (Masuda et al., *J Immunol*, 2007; Wada et al., *Nature*, 2008) (Fig. 3). So the next question was how this commitment occurs. To address this question, Dr.

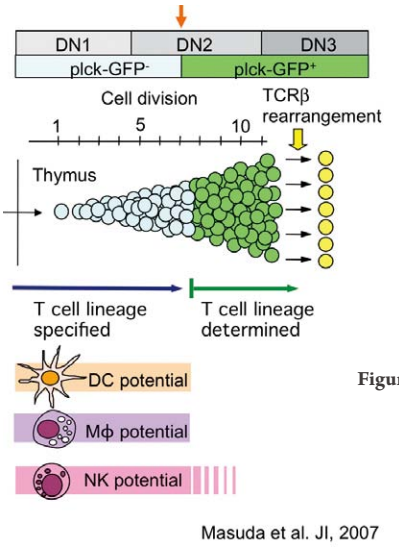


Figure 3: Intrathymic progenitors become fully committed to the T cell lineage in the midst the DN2 stage by terminating myeloid potential

Ikawa established a stromal cell-free culture system because the only culture system to induce T cells was using stromal cells with Notch ligand. He tried various different culture conditions and finally found out that reduction of the concentration of IL-7 in the middle of the culture period induces T cell development without the need for any stromal cells. This was the first example to show that T cell can be induced in stroma-free conditions. He also found that Bcl11b is an essential transcriptional factor in T cell lineage commitment. In Bcl11b knockout mice, T cell development was blocked at

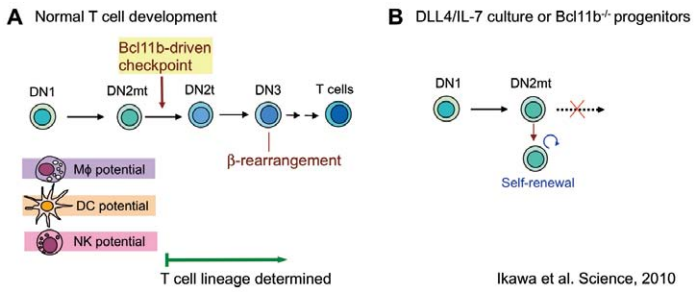


Figure 4: DN2-determiantion step is a critical checkpoint of T cell development, and Bcl11b is a key factor that drives T cell lineage determination. The blockage of Bcl11b driven checkpoint induces self-renewal of arrested progenitors

the DN2 stage, and these cells can be maintained *in vitro* with self-renewal capacity. From these data, he showed that Bcl11b is a key factor for T cell lineage determination (Ikawa et al., Science, 2010) (Fig. 4).

In his speech, he mentioned several ongoing projects. One was on transcriptional networks in T cell lineage commitment, in collaboration with the Omics Science Center (OSC). Using the culture system he established together with the CAGE (Cap Analysis of Gene Expression) system in OSC, they are now working to draw transcriptional networks involved in T cell commitment. Another one was on the maintenance of T cell progenitor fate by epigenetic factors, in collaboration with Dr. Koseki of RCAI. Dr. Ikawa has been able to reprogram T cells to B cells by inactivating epigenetic factors. "I want to expand our knowledge of lineage commitment during lymphocyte development. I'd like to thank Dr. Kawamoto for giving me the opportunity to work here in RIKEN, probably the best institute in the world, and I hope to continue to contribute to the Center," said Dr. Ikawa.

During the awards ceremony, Mr. Kenji Okuma, Director of RIKEN Yokohama Institute (Photo 5), explained about the new direction of the Center. RIKEN's 5-year Mid-term Plan will be finished in March next year, and RIKEN decided to establish a new center focusing on integrative medical sciences. During the first five-year mid-term plan, RCAI initiated its research efforts in immunology. The management system of RCAI was extraordinary and its advisory council system was unique. In the second mid-term plan, RCAI strengthened and expanded its efforts, and received excellent evaluations. However, after these 10 years, RIKEN is required to make qualitatively different research achievements and has to consider some new direction for the future. "I respect RCAI because it has been making tremendous efforts to rethink the direction of future immunology. Still maintaining its central focus in immunology, RCAI tries to think about more integrative medical life sciences in the next third mid-term plan. This could not have been done without RCAI's achievements during the last 10 years, and I think today's awardees of the Outstanding Contribution of the Year 2011 are also contributors to this new direction. I believe RCAI will play a central role for RIKEN to make a new step forward towards the new direction," said Mr. Okuma. Mr. Okuma will resign his position at the end of March, 2012. A large flower bouquet was given to him by Ms. Sonoko Watanabe, Director of RIKEN Yokohama Institute Research Promotion Division .

Photo 5 : Mr. Kenji Okuma, Director of RIKEN Yokohama Institute

Dr. Taniguchi, RCAI's Director said to the members of RCAI, "I believe we've made extraordinary achievements and contributions to the immunology field during these 10 years. This is the result of everyone's tremendous continuous efforts. I would like to thank all of you and we will never forget the efforts by all the RCAI members to cooperate for the Center. "

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Prize Winners 2011

Photo 1 : Makio Tokunaga

Makio Tokunaga (Photo 1), Leader of the Research Unit for Molecular Systems Immunology received The Commendation for Science and Technology from the Minister of Education, Culture, Sports, Science and Technology, 2011. He won the Research Category Prize for his studies on elucidation of molecular dynamics and interactions by the development of single molecular imaging microscopy. This prize is awarded to investigators whose research is highly original and contributes to the science and technology of Japan.


賞状

Photo 2 : Tomokatsu Ikawa

Tomokatsu Ikawa (Photo 2), Researcher of the Laboratory for Lymphocyte Development, received the Research Encouragement Award of the Japanese Society for Immunology for his research on transcriptional regulation of T cell/ B cell lineage commitment. He developed a unique cell culture system to analyze cell lineage commitments from hematopoietic progenitors. He found that Id2 is essential for NK lineage commitment from T/NK bipotent progenitors, and that Notch signals are essential for early T cell development. Recently, he showed that BCL11B is essential for T cell lineage commitment. This award is given to young investigators, age 40 or younger, who have performed distinguished immunological research.

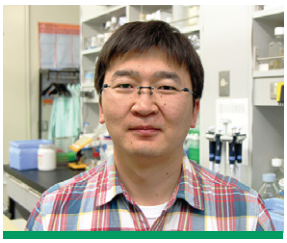


Photo 3 : Nyambayar Dashtsoodol

Nyambayar Dashtsoodol (Photo 3), Researcher of the Laboratory for Immune Regulation, received the President of Mongolia's Prize for the Distinguished Young Scientist of the Year 2011. He was awarded for his study of the thymic development of NKT cells: identification of CD4, CD8 double-negative NKT cell precursors. This award is given to a young Mongolian scientist (under the age of 35 years) whose research has greatly contributed to the advancement of science aimed for human benefit and representation of Mongolia at the international level.

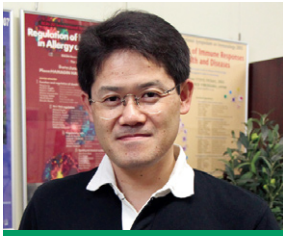


Photo 4 : Toshiyuki Fukada

Toshiyuki Fukada (Photo 4), Researcher of the Laboratory for Cytokine Regulation, received the Kanagawa Nambyo Study Foundation Award 2011 for elucidation of the mechanisms of zinc-related diseases and received 400 thousand yen. This award is given to scientists working in Kanagawa Prefecture who contributed research advancements aimed at conquering intractable diseases.

RIKEN Research and Technology Incentive Awards 2011 were presented to two RCAI researchers, **Masahiro Kitano** (Photo 5) and **Atsushi Hijikata** (Photo 6). Kitano, Special Postdoctoral Researcher of the Research Unit for Immunodynamics, received the award for his study on *in vivo* imaging of immune cell dynamics by two-photon laser scanning microscopy. Hijikata, a Research Associate of the Laboratory for Immuno-

Photo 5 : Masahiro Kitano

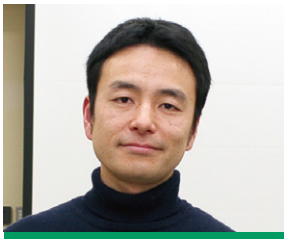


Photo 6 : Atsushi Hijikata

Photo 7 : Shinji Fukuda

genomics, received the award for the establishment of a high precision gene testing method for mosaic autoinflammatory diseases. The RIKEN Research and Technology Incentive Award was established in FY2009 to recognize young researchers and technicians under age 40 who have contributed to furthering RIKEN's ideals by achieving exemplary results in their research or research support activities. Kitano also received the Medical Science Prize at RIKEN FY2011 SPDR & FPR Presentation of Research Results for his poster presentation, "Bcl6 Protein Expression Shapes Pre-Germinal Center B Cell Dynamics and Follicular Helper T Cell Heterogeneity."

Shinji Fukuda (Photo 7) and **Hiroshi Ohno**, Laboratory for Epithelial Immunobiology, received the Bioengineering Research Paper Award, in collaboration with Yasuhiro Date, Yumiko Nakanishi, Tamotsu Kato and Jun Kikuchi of RIKEN Plant Science Center in September 2011. They were awarded for the paper "New monitoring approach for metabolic dynamics in microbial ecosystems using stable-isotope-labeling technologies", Vol. 110, pages 87-93, *Journal of Bioscience and Bioengineering*, 2010. In the paper, they reported a newly developed approach for monitoring the metabolic dynamics in microbial ecosystems using a combination of DNA fingerprinting and metabolome analysis based on stable-isotope-labeling technologies. This award is given to selected papers published in the Journal of Bioscience and Bioengineering for their contribution to the advancement of bioengineering.

Shintaro Hojyo (Photo 8), Researcher of the Laboratory for Cytokine Regulation, received The Best Poster Award at The 6th Annual Meeting of the Japan Transporter Research Association. He was awarded for his poster presentation "Zinc Transporter, SLC39A14/ZIP14, regulates the endocrine signals mediated by G protein-coupled receptors (GPCR)"

Saya Moriyama (Photo 9), Graduate Student of the Research Unit for Immunodynamics, received the Incitement Award at The 3rd Meeting of Signal Network Society for her presentation, "BCL6 Protein Expression Shapes Pre-Germinal Center B Cell Dynamics and Follicular Helper T Cell Heterogeneity." She also received a Keystone Symposia Scholarship to attend the meeting, "B Cells: New Insights into Normal versus Dysregulated Function (D6)" held on Apr 12-17, 2011, in Whistler, Canada.

Kohei Kometani (Photo 10) of the Laboratory for Lymphocyte Differentiation, **Takayuki Imanishi** (Photo 11) of the Laboratory for Cell Signaling, **Rika Ouchida** (Photo 12) of the Laboratory for Immune Diversity, **Yuuki Obata** (Photo 13), **Daisuke Takahashi** (Photo 14) and **Shinji Fukuda** (Photo 7) of the Laboratory for Epithelial Immunobiology received Tadamitsu Kishimoto International Travel Award from the Japanese Society for Immunology to attend international immunology meetings held overseas.

Photo 8 : Shintaro Hojyo

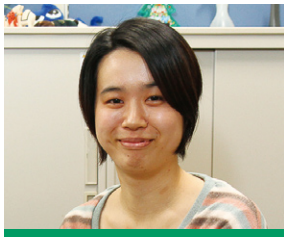


Photo 9 : Saya Moriyama

Photo 10 :Kohei Kometani

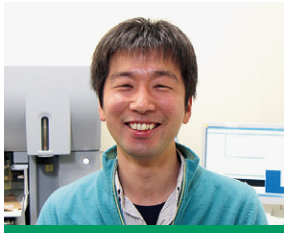


Photo 11 : Takayuki Imanishi

Photo 12 : Rika Ouchida

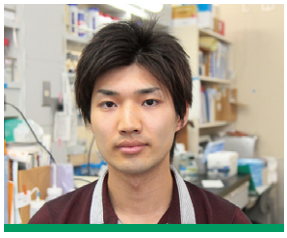


Photo 13 : Yuuki Obata

Photo 14 : Daisuke Takahashi

Excellent Paper of the Year 2011

The RCAI Award for Excellent Paper was originally established in 2004 with donations from Dr. Masaru Taniguchi and Dr. Toshio Hirano. The annual award aims to recognize excellent publications by RCAI scientists. Although the funds were depleted by 2008, RCAI's strategic committee decided that there was great value in awarding excellent achievements by young researchers and encouraging their efforts, so they provided the funding to continue this prize.

In 2011, 11 excellent papers were selected from 13 candidates for this award.

Masahiro Kitano, Saya Moriyama and Takaharu Okada
Research Unit for Immunodynamics and Laboratory for Lymphocyte Differentiation
"Bcl6 protein expression shapes pre-germinal center B cell dynamics and follicular helper T cell heterogeneity"
Immunity, Vol. 34, pp. 961-972, 2011

Yasutaka Motomura and Masato Kubo
Open Laboratory for Signal Network
"The transcription factor E4BP4 regulates the production of IL-10 and IL-13 in $CD4^+$ T cells"
Nature Immunology, Vol. 12, pp. 450-459, 2011

Takahisa Miyao and Shohei Hori
Research Unit for Immune Homeostasis
"Plasticity of $Foxp3^+$ T cells reflects promiscuous Foxp3 expression in conventional T cells but not reprogramming of regulatory T cells"
Immunity, Vol. 36, pp. 262-275, 2012

Hideaki Takagi and Katsuaki Sato
Laboratory for Dendritic Cell Immunobiology
"Plasmacytoid dendritic cells are crucial for the initiation of inflammation and T cell immunity in vivo"
Immunity, Vol. 35, 958-971, 2011

Akiko Hashimoto-Tane and Takashi Saito
Laboratory for Cell Signaling
"Dynein-driven transport of T cell receptor microclusters regulates immune synapse formation and T cell activation"
Immunity, Vol. 34, 919-931, 2011

Yohsuke Harada and Masato Kubo
Open Laboratory for Signal Network
"The 3′ enhancer CNS2 is a critical regulator of interleukin-4-mediated humoral immunity in follicular helper T cells"
Immunity, Vol. 36, 188-200, 2012

Takashi Tanaka
Research Unit for Inflammatory Regulation
"PDLIM2 inhibits T helper 17 cell development and granulomatous inflammation through degradation of STAT3"
Science Signaling, Vol 4, ra85, 2011

Daisuke Takahashi, Koji Hase and Hiroshi Ohno
Laboratory for Bioenvironmental Epigenetics and Laboratory for Epithelial Immunobiology
"The epithelia-specific membrane trafficking factor AP-1B controls gut immune homeostasis in mice"
Gastroenterology, Vol. 141, pp. 621-632, 2011

Kohei Kometani and Tomohiro Kurosaki
Laboratory for Lymphocyte Differentiation
"CIN85 drives B cell responses by linking BCR signals to the canonical NF-κB pathway"
The Journal of Experimental Medicine, Vol. 208, pp.1447-1457, 2011

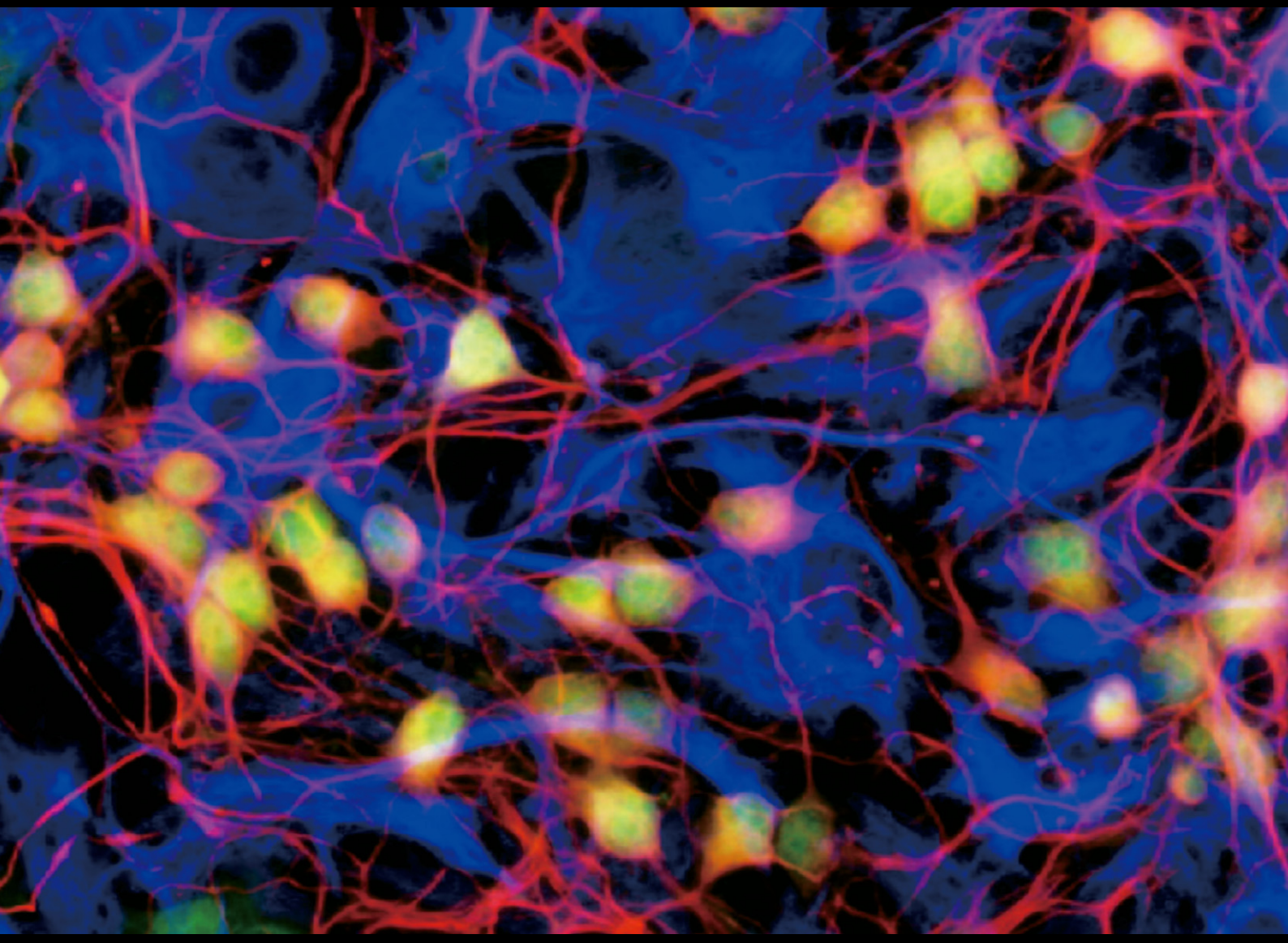
Hiroshi Watarai and Masaru Taniguchi
Laboratory for Immune Regulation
"Development and function of invariant natural killer T cells producing T_H2- and T_H17-cytokines"
PLoS Biology, Vol. 10, e1001255, 2012

Shinsuke Takagi and Fumihiko Ishikawa
Laboratory for Human Disease Model
"Membrane-bound human SCF/KL promotes in vivo human hematopoietic engraftment and myeloid differentiation"
Blood, Vol. 119, pp. 2768-2777, 2012

2011

Part 2

Technology and Innovation



RCAI and Torii collaborate for development of an allergy vaccine

Japanese cedar (*Cryptomeria japonica*) pollinosis is a common allergy in Japan, with a prevalence estimated to be 26% based on a nationwide survey conducted in 2008. Impaired performance due to pollinosis and medications used for treating pollinosis is thought to be an important cause of loss of concentration and productivity of patients in the workplace. Antigen-specific immunotherapy (SIT) is considered to be the only curative therapy for allergy. Vaccines using allergoids and modified Cry j 1, a major allergen of Japanese cedar pollen, have been developed and used for pre-clinical trials; however, none of them has been commercially available for medical use either due to poor clinical outcomes of late stage clinical trials or the failure to find a cooperative pharmaceutical company to introduce the vaccines into the market. To fill the critical gap between basic research and later stage of drug development, RCAI offered a program named 'exchange zone' where RCAI, universities, hospitals, and pharmaceutical companies work together for drug development, including allergy vaccine development especially for Japanese cedar pollinosis. An exchange zone is based on the concept that sprinters in a relay race run together in the exchange zone, where the baton is passed firmly and smoothly. Based on this program, RIKEN and Torii Pharmaceutical Co., Ltd. set up joint research laboratory in RCAI in May 2010 and started research and development of a recombinant SIT vaccine, taking into consideration GLP toxicity tests and GMP. Our aims are to develop a recombinant SIT vaccine with high safety, tolerability and efficacy, and to introduce the vaccine into the Japanese market.

Development of the recombinant SIT vaccine

The vaccine was originally developed in RCAI as a safer SIT vaccine, one that is less dangerous in inducing adverse events, such as anaphylaxis, after administration. For the vaccine, recombinant technology is used to conjugate two major allergens from Japanese cedar pollen, namely Cry j 1 and Cry j 2. The fusion protein is then further modified with polyethylene glycol (PEG) to inhibit binding with immunoglobulin E (IgE), the allergy-inducing antibody that is likely to pre-exist in the target patient population. PEG is conjugated to the recombinant Cry j 1/Cry j 2 fusion protein via a cysteine residue on the fusion protein. All cysteine residues except one in the fusion protein were substituted with serine residues to control the number and location for conjugation with PEG. The vaccine is expected to lose potential to bind human IgE, which may induce adverse events after vaccine administration *in vivo*.

The stability and yield of the recombinant fusion protein were compared among several host/vector combinations including eukaryote and prokaryote. It is also necessary to develop a simple method for the purification of the recombinant SIT vaccine expressed in prokaryotes or eukaryotes, taking into consideration cost-performance and purity. To date, they have set up a procedure to purify the recombinant protein followed by conjugation with PEG. It is now necessary to optimize the procedure for large scale industrial production. They are also preparing monoclonal antibodies specific to the vaccine and will develop vaccine-specific quantification systems such as a sandwich ELISA.

Therapeutic potential of the vaccine in a mouse model of Japanese cedar pollinosis

Systemic injections of the vaccine prevent the increase of serum Cry j 1-specific IgE following subsequent secondary or tertiary sensitization with native Cry j 1 in mice. In humans, the major clinical symptoms of the pollinosis are local inflammation like conjunctivitis, rhinorrhea, and sneezing. They established a mouse model of the pollinosis by Cry j 1 sensitization resulting in infiltration of inflammatory cells such as eosinophills and mast cells into ocular and nasal mucosa. They are assessing improvement in the ocular and nasal inflammation after treatment by local or systematic vaccination. They will then evaluate the therapeutic potential of the vaccine by comparing the reduction of local inflammation or IgE production with various doses and frequencies of vaccination using the sensitized mouse and mouse model of pollinosis. To improve the therapeutic effects of the vaccine, elucidation of the therapeutic mechanisms and identification of predictive biomarkers are important issues. Therefore, they are searching for biomarkers to monitor therapeutic responses during or after the vaccination. They plan to evaluate the therapeutic effects of the vaccine in humans using the biomarkers identified in this translational research, after which they will produce the vaccine as GMP grade.

Members of RIKEN-TORII Joint Research Team

Team Leader :	**Masaru Taniguchi**
Senior Scientist :	**Takashi Fujimura**
Visiting Scientists :	**Hiroyuki Miyazaki, Yasushi Okumura, Koji Fujinami, Masao Matsuda, Ryosuke Ishikawa**
Technical Staff :	**Kyounga Seo**

Recent Publications :

1. Fujimura T, Okamoto Y, Taniguchi M. Therapeutic effects and biomarkers in sublingual immunotherapy: a review. ***J. Allergy (Cairo)*** 2012: 381737. (2012)
2. Fujimura T, Yonekura S, Horiguti S, Taniguchi Y, Saito A, Yasueda H, Inamine A, Nakayama T, Takemori T, Taniguchi M, Sakaguchi M, Okamoto Y. Increase of regulatory T cells and the ratio of specific IgE to total IgE are candidates for response monitoring or prognostic biomarkers in two-year sublingual Immunotherapy (SLIT) for Japanese cedar pollinosis. ***Clin. Immunol***.139:65-74. (2011)
3. Fujimura T, Yonekura S, Taniguchi Y, Horiguti S, Saito A, Yasueda H, Nakayama T, Takemori T, Taniguchi M, Sakaguchi M, Okamoto Y. The induced regulatory T cell level, defined as the proportion of $IL10^{+}Foxp3^{+}$ cells among $CD25^{+}CD4^{+}$ leukocytes, is a potential therapeutic biomarker for sublingual immunotherapy: a preliminary report. ***Int. Arch. Allergy. Immunol***. 153: 378-387. (2010)
4. Fujimura T and Okamoto Y. Antigen-specific immunotherapy against allergic rhinitis: the state of the art. ***Allergol. Int***. 59: 21-31. (2010)

iPS technology development for immunological research and therapeutics

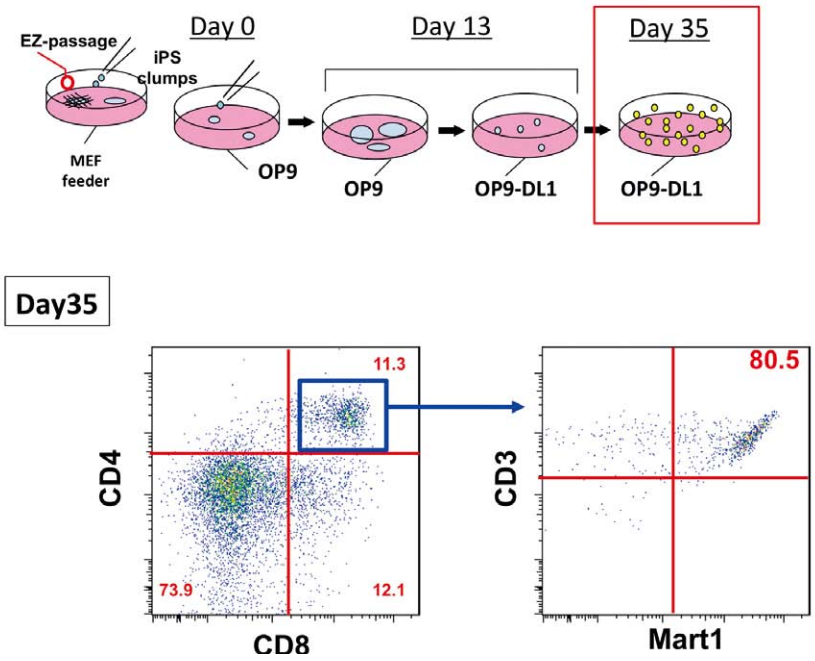


Figure : **Induction of antigen specific CTL cells from CTL derived iPS cells.** MART1, melanoma antigen recognized by T cells, is a protein antigen expressed on the surface of melanocytes and melanoma cells. Human iPS colonies derived from Mart1 antigen-specific CTL were cut into small clumps and plated on OP9 stromal cells. On day 13, cells were harvested by tripsinization and transferred to co-cultures with OP9-DLL1 stromal cells. At day 35, DP cells were generated *in vitro* and most of the DP cells were Mart1 tetramer positive T cells.

Induced pluripotent stem (iPS) cells possess tremendous therapeutic potential not only in the field of regenerative medicine but also for immune therapy. RCAI has started an activity to apply iPS technology for mouse and human immunology research and therapeutic development. The core facility for iPS research is engaged in developing efficient protocols to reprogram various lymphocytes and induce differentiation of iPS cells into lymphoid lineage cells. These studies are being done on a collaborative basis with individual research activities in RCAI, and this activity is partly supported by CREST from JST.

Generation of iPSCs from mature lymphocytes and identification of genes that resist reprogramming

Mature lymphocytes are believed to be more resistant than embryonic fibroblasts to the genetic reprogramming needed to generate iPS cells. Since gene expression profiles and global epigenetic status are quite different between lymphocytes and fibroblasts, the iPS group hypothesized the presence of genes in mature lymphocytes that are difficult to fully reprogram. To test this hypothesis, Daisuke Yamada and colleagues established 20, 2 and 5 independent iPSC clones from mature B, T and NKT cells (B-, T-, NKT-iPSC) in mouse. All of them were qualified as authentic lymphocyte-derived iPSCs by analyzing the genomic configurations of immunoglobulin or T cell receptor loci, gene expression profiles, and the ability to form teratomas and chimeric mice.

However, they found that only 7 of them were competent to differentiate into the germ cell lineage. By comparing gene expression profiles between B-iPSCs and ESCs, they found that the Polycomb target genes in ESCs tend to be more repressed in B-iPSCs. This over-repression of Polycomb targets turned out to associate with over-loading with Polycomb repressive complex-1 (PRC1). Interestingly, they did not observe such PRC1 over-loading in the few B-iPSCs that are competent for germline transmission. Consistent with these observations, B-iPSCs can more efficiently differentiate upon conditional depletion of the PRC1 components Ring1A/B than ESCs. These results suggest that Polycomb target genes are epigenetically unstable during reprogramming of B-lymphocytes and easily mis-reprogrammed. To test this possibility, the investigators further analyzed local levels of Histone H3K9 trimethylation (H3K9me3), which are known to compete for the targets with Polycomb, and again found its over-loading at Polycomb target genes in B-iPSCs that are incompetent for germline transmission. These results suggest that reprogramming of Polycomb target genes is a rather unstable process during induction of iPSCs. This observation implies that local levels of PRC1 and H3K9me3 could be used as molecular markers to predict pluripotency of iPSCs.

Generation of $CD8^+$ singlepositive T cells *in vitro* from iPS cells derived from human mature T cells

Induced pluripotent stem (iPS) cells are expected to be used as a source for production of various types of cells to be used in regenerative medicine. In this study Kawamoto and colleagues established iPS cells from human mature T cells (T-iPS cells), namely from total $CD3^+$ cells or $CD4^-CD8^+$ (CD8SP) cells of cord blood as well as of adult peripheral blood. These T-iPS cells were confirmed to bear productively rearranged TCRβ chain genes. When co-cultured with OP9/DLL1 cells, T-iPS cells efficiently generated $TCR\beta^+CD4^+CD8^+$ cells on day 40 of cultivation, in contrast to iPS cells derived from human cord blood $CD34^+$ cells. They further showed that T-iPS cells derived from CD8SP cells formed substantial numbers of mature CD8SP cells on day 60 of the culture. The present study thus provides a novel method for cloning and expansion of CD8SP cells with defined antigen specificity, which can then be applied for cell therapy against cancer.

Members of iPS Group

Leaders :	**Haruhiko Koseki** (Group Director)
	Hiroshi Kawamoto (Team Leader)
	Fumihiko Ishikawa (Group Director)
	Osamu Ohara (Group Director)
Senior Scientist :	**Hiroshi Watarai**
Research Scientists :	**Daisuke Yamada**
	Kyoko Masuda
Technical Staff :	**Momoko Okoshi-Sato, Genta Kitahara, Masako Fujita, Chieko Tezuka, Sakura Sakata, Yuko Nagata, Mariko Tomizawa, Raul Eduardo Vizcardo Sakoda**

Recent Publications :

1. Generation of functional NKT cells in vitro from embryonic stem cells bearing rearranged invariant Vα14-Jα18 TCRα gene. Watarai H, Rybouchkin A, Hongo N, Nagata Y, Sakata S, Sekine E, Dashtsoodol N, Tashiro T, Fujii S, Shimizu K, Mori K, Masuda K, Kawamoto H, Koseki H, and Taniguchi M. ***Blood*** 115:230-237 (2010)

2. Watarai H, Fujii S, Yamada D, Rybouchkin A, Sakata S, Nagata Y, Iida-Kobayashi M, Sekine-Kondo E, Shimizu K, Shozaki Y, Sharif J, Matsuda M, Mochiduki S, Hasegawa T, Kitahara G, Endo TA, Toyoda T, Ohara O, Harigaya K, Koseki H, Taniguchi M. Murine induced pluripotent stem cells can be derived from and differentiate into natural killer T cells. ***J Clin Invest***. 120:2610-2618 (2010)

The PIDJ Network develops a new genetic diagnosis method

The clinical archive for the Primary Immunodeficiency in Japan (PIDJ) is steadily growing. The number of PIDJ entries was only 260 in 2008, but now exceeds 1500. Importantly, the data entries are geographically widely distributed, deposited by more than 300 different hospitals, in addition to RCAI's direct collaborators, the Japan PID study group supported by the Ministry of Health, Labour, and Welfare. This outcome clearly indicates that the PIDJ network is establishing firm roots in the clinician community in Japan.

As another important activity of PIDJ, RCAI, in collaboration with the Kazusa DNA Research Institute, analyzed approximately 250 primary immunodeficiency (PID) samples during the 2011 fiscal year. In other words, the number of analyzed known and/or candidate PID genes exceeds 100 and the total number of the PID genes actually sequenced is more than 600 (Figure 1). Among PIDs, there are three major disease entities that clinicians frequently submit for genetic testing because they are difficult to accurately diagnose only from the symptoms; autoinflammatory diseases, mendelian susceptibility to mycobacterial disease, and common variable immunodeficiency. Because each of these diseases has multiple known causative genes, their genetic analysis is highly labor-intensive and time-consuming. This fact has strongly motivated Dr. Ohara (RCAI's Laboratory for Immunogenomics and Kazusa DNA Research Institute) to develop a more efficient method of genetic testing than the conventional one based on amplification of genetic regions of interest by polymerase chain reaction (PCR) followed by capillary DNA sequencing.

Massively parallel DNA sequencing (MPS) technology is an obvious method for this purpose. However, a well-known caveat of MPS is the high frequency of sequencing errors, which is fine for exploratory high throughput purposes, but which is a particularly serious problem when the sequence is used for conclusive genetic tests. To solve this problem, Ohara and his team tried to establish an MPS-assisted statistically robust method for diagnosis of chronic infantile neurological cutaneous and articular syndrome (CINCA), also known as neonatal-onset multisystem inflammatory disease (NOMID). This disease is a dominantly inherited autoinflammatory disease that is characterized by neonatal onset and a triad of symptoms, including urticarial-like skin rash, neurological manifestations, and arthritis/arthropathy. CINCA is known to be caused by mutations in the *NLRP3* gene, which is a member of the Nod-like receptor family and a component of the inflamasome of the innate immune system. Dr. Ohara's collaborators in Kyoto University previously showed that there can be somatic mosaicism of *NLRP3* gene mutations as a cause of CINCA/NOMID and that this explains the pathogenesis in about half of CINCA/NOMID patients who are undiagnosed by a conventional direct PCR sequencing of the *NLRP3* gene (Saito et al., *Blood*, 2008, 111, 2132–41; Tanaka et al. *Arthritis Rheum*., 2011, 63, 3625–32). Because the occurrence of somatically mutated cells is frequently less than 50% in the presence of normal cells, the diagnosis of this form of the disease requires higher accuracy than that for diagnosis of conventional Mendelian diseases. The conventional approach for diagnosis of NLRP3 somatic mosaicism relied on cloning of PCR products of exons of the *NLRP3* gene followed by capillary DNA sequencing. Because somatic mosaicism higher than 5% is usually considered diagnostic of the disease, more than 100 clones have to be analyzed for each exon, which inevitably imposes a heavy workload when diagnosing somatic mosaicism. Thus, a

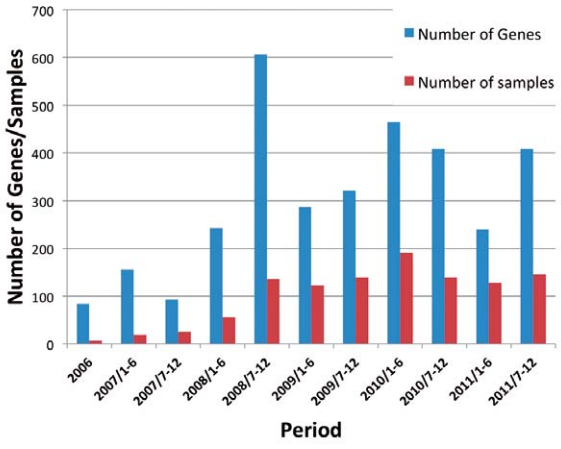


Figure 1: Statistics of PIDJ Genetic Tests

problem is how to avoid tedious cloning steps for diagnosis of NLRP3 somatic mosaicism while still maintaining high accuracy in the genetic test. The investigators succeeded in addressing this difficult issue by making error-occurrence reference maps for a gene to be analyzed by MSP (Fig. 2: Izawa et al., *DNA Res*., 2012, 19, 143-52). By comparing the error-occurrence map with actual data, they could eventually discriminate sequencing errors from real genetic variations with statistical confidence. In fact, this method was successfully applied for diagnosis of CINCA/NOMID. This approach is, as obvious in its principle, applicable to other conventional genetic tests, and has the potential to greatly reduce the cost and labor of conventional genetic tests as well. Based on the pipeline already developed for diagnosis of somatic mosaicism of the *NLRP3* gene, development of an MPS-assisted genetic testing pipeline for autoinflammatory diseases, Mendelian susceptibility to mycobacterial disease and common variable immunodeficiency is now underway.

The success rate of conventional genetic tests of known PID genes in PIDJ is 20-30% on average. Thus, more than 500 clinical cases are currently accumulated in the PIDJ resource as genetically unidentified. This resource is a great outcome of the PIDJ collaboration to date in searching for new PID-causing mutations. Because technical advances in DNA sequencing make it possible to sequence all the exons in the human genome at a reasonable cost, the team has actually started to perform a genome-wide search of disease causative genetic variations for some of the unidentified PIDs. However, to understand the genotype-phenotype relationship of PIDs, knowledge of basic immunology is indispensable. In particular, because the number of PID patients is very limited in Japan and the PIDs are frequently sporadic, the functional analysis of the detected genetic variation(s) eventually plays a key role in such an exploratory project for the identification of PID genes. In addition, the role of bioinformatics becomes more and more critical than before because the amount of the information drastically increases. In this situation, it is strong advantage that the PID collaboration network is composed of multidisciplinary researchers, including clinical PID specialists, genome scientists, and basic immunologists. By fully exploiting the PIDJ network, they will continue to tackle and try to clarify the mechanism through which dysregulation of the homeostasis of the immune system takes place.

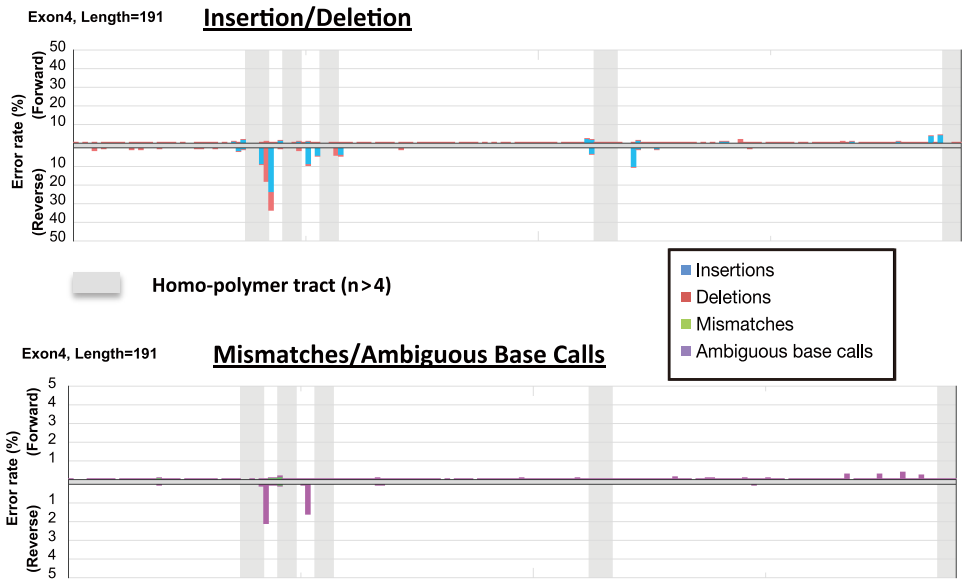


Figure 2: Example of an Error-Reference Map
The occurrence rates of sequencing errors (Insertion/Deletion in the upper panel, Mismatches/Ambiguous calls in the lower panel) for exon 4 of the *NLRP3* gene are shown in a strand-specific manner (indicated as "Reverse" or "Forward").

NKT cell-targeted adjuvant cell therapy for cancer patients

独立行政法人
国立病院機構
National Hospital Organization

RCAI has been developing a Phase I/IIa clinical study of the application of NKT cell-targeted therapy for advanced lung cancer (stage IIIB, IV or recurrence), in collaboration with Chiba University Hospital (Prof. Toshinori Nakayama, Prof. Takehiko Fujisawa and Associate Prof. Shin-ichiro Motohashi). α-GalCer-pulsed APCs ($1x10^9/m^2$ PBMC-derived DCs) were intravenously administered four times. In this study, the MST (median survival time) of the responder group (defined by an increased number of IFN-γ producing NKT cells compared to the poor-responder-group) was significantly longer than the poor-responder group (29.3 months versus 9.7 months). Thus, the increased IFN-γ production by NKT cells upon α-GalCer stimulation showed a significant association with clinical outcome. These phase I/IIa trial results are encouraging and warrant further evaluation of the survival benefit of this immunotherapy.

In September, 2011, this NKT cell-targeted therapy for lung cancer was approved by The Advanced Medical Care Assessment System by the Japanese Ministry of Health, Labour and Welfare (MHLW). The Advanced Medical Care Assessment System was introduced in Japan in 1985 to ease the financial burden on patients who need state-of-the-art medical treatment that is safe and effective, but still not covered by health insurance because it is not yet approved under the Pharmaceutical Affairs Law (PAL). In response to recent rapid progress in medical technology and the patients' need to have safe and lower cost treatment with advanced technologies, this system was introduced to allow partial health insurance coverage, such as for clinical evaluations, medications and hospital stays. It is also the case that the collection of appropriate clinical research data is facilitated by this system, and thus can then lead to approval of the advanced medical technologies under the PAL.

After the approval, RIKEN and the National Hospital Organization (NHO) agreed to an integrative collaboration for NKT cell-targeted therapy and medical innovations in March, 2012. NHO manages a Japanese network of 144 hospitals, including 53 thousands beds and a staff of 54 thousands. It supports high-quality clinical trials in the hospitals and facilitates collection of appropriate clinical research data. They plan to start clinical trials of NKT cell-targeted therapy at the Nagoya Center of the National Health Organization.

Development of artificial adjuvant vector cells (aAVC) as a novel therapy

In collaboration with Dr. Kakimi (Tokyo Univ.), Dr. Maeda (Iwate Medical Univ.) and Dr. Mizuno (Yamaguchi Univ.), RCAI's Drs. Fujii (Research Unit for Cellular Immunotherapy), Shimizu (Research Unit for Immunotherapeutics) and Ishii (Laboratory for Vaccine Design) have been developing unique adjuvant vector cells that stimulate NKT cell activation.

These investigators previously demonstrated that allogeneic fibroblast cells loaded with α-GalCer and transfected with antigen-encoding mRNA have a combination of effects; adjuvant effects due to *i*NKT cell activation and the delivery of antigen to DCs *in vivo* (*Blood* 2009). These cells produced antigen protein and activated NK and *i*NKT cells. When injected into mice, they

elicited antigen-specific T cell responses and provided tumor protection. Thus, glycolipid-loaded, mRNA-transfected allogeneic fibroblasts act as adjuvant vector cells (aAVCs) to promote *i*NKT cell activation, leading to DC maturation and antigen-specific T cell immunity. In preclinical studies, they have set up canine studies to observe the safety profile and immune response. As was the case in mice, administration of these aAVCs to dogs activates *i*NKT cells as well as elicits antigen-specific T cell responses with no adverse events. This unique tool could prove clinically beneficial in the development of immunotherapies for malignant and infectious diseases.

Development of humanized mouse models

RCAI's Laboratory for Human Disease Model (Group Director: Fumihiko Ishikawa), Laboratory for Developmental Genetics (Group Director: Haruhiko Koseki) and Laboratory for Immunogenomics (Group Director: Osamu Ohara) aim to develop the next generations of humanized mice that carry essential human genes, including MHC class I and II, cytokines and adhesion molecules. Their goal is to develop humanized mice in which the immune responses to various pathogens and environmental stresses will precisely recapitulate those seen in humans. These next generation mice will enable us to create useful models to better reconstitute human immune system and to recapitulate specific human diseases.

In 2010 in collaboration with The Jackson Laboratory, the team developed humanized mice carrying a human leukocyte antigen (HLA). They showed that the expression of human genes is essential to fully recapitulate human lymphopoiesis in immunodeficient NOD/SCID/IL2rγKO (NSG) mice. In the humanized mice that they created (HLA class1 A02 transgenic NOD/SCID/IL2rγKO mice), human cytotoxic T lymphocytes ($CD8^+$T cells) developed successfully and these human T cells were functional in terms of cytokine production and cytotoxicity. They recognized Epstein-Barr virus (EBV)-infected cells and virus-associated peptides, and showed cytotoxicity in an HLA-restricted manner. Moreover, this antiviral cytotoxic T lymphocyte response was inhibited by the addition of an anti-HLA class I antibody (Shultz et al., *Proc Natl Acad Sci USA*, 2010).

In 2012, to humanize bone marrow microenvironment, they further modified this humanized mouse model and created an NSG mouse that expressed human membrane-bound Kit ligand/stem cell factor (hSCF Tg NSG mouse), a cytokine that plays an important role in hematopoiesis. Transplantation of cord blood-derived human HSCs into the hSCF Tg NSG mouse resulted in significantly higher long-term engraftment of human leukocytes in the bone marrow, spleen, and peripheral blood compared with non-hSCF transgenic NSG recipients. Humanization of the mouse bone marrow microenvironment with hSCF has led to nearly 100% human hematopoietic repopulation and efficient myeloid cell development. The frequency of human $CD33^+$ myeloid cells within the total human $CD45^+$ population was significantly higher in the hSCF Tg NSG recipients compared to the non-Tg NSG recipients and constituted the majority of human hematopoietic cells. The reconstituted mice also have abundant human mast cells in spleen and gastrointestinal mucosa. These findings demonstrate the essential role of membrane-bound SCF in human myeloid cell development and, moreover, the new hSCF Tg NSG humanized mouse model may be useful in studies of human allergy and innate immunity *in vivo*. (Takagi et al., *Blood*, 2012)

These newly created humanized mouse models are expected to be essential tools for understanding the human immune system and recapitulating disease states. RCAI recently launched the Medical Immunology World Initiative to facilitate a worldwide collaboration with research centers focusing on the investigation of human immunity and immunological diseases using humanized mice. The next generations of humanized mice will be reciprocally exchanged with other members of the research centers, thereby optimizing studies of human diseases and pioneering human immunology research (See *Research Highlights* section).

Calpis-RIKEN Integrated Collaborative Research conducted in RCAI

In 2004, RIKEN started the Integrated Collaborative Research with Industry Program to promote joint research focused on meeting industry needs that emphasizes industry initiatives to make use of RIKEN's considerable and ever-growing research assets. The Gut Microbiology Research Team was established under this program in RCAI in 2010 to conduct collaborative research between Calpis Co. Ltd., a Japanese manufacturer of an uncarbonated milk and lactic acid-based soft drink, and RCAI's Laboratory for Epithelial Immunobiology. Dr. Naoyuki Yamamoto conducted immunology research on probiotics at RCAI in collaboration with RCAI's Hiroshi Ohno.

RCAI Innovation Projects in Drug Discovery and Medicine

In order to conduct research and development of novel drugs and medical technologies, RIKEN established the Program for Drug Discovery and Medical Technology Platforms (DMP) in April 2010. Since then, based on its guidelines, DMP has selected theme leaders, who promote the projects for drug discovery and medical technology, focusing on areas of diseases with low therapeutic satisfaction, orphan drugs and new concepts. The theme leaders are researchers at RIKEN, but DMP supports the project by assistance of the portfolio manager with experience in drug discovery and by technical platforms established in DMP, linked to RIKEN Research Centers.

Several RCAI researchers collaborate with DMP. Toshitada Takemori became a unit leader of Drug Discovery Antibody Platform Unit of DMP, in order to develop monoclonal antibodies that can be used as therapeutic drugs for treatment of cancer and other diseases. The activity of the Antibody Platform Unit is supported by RCAI Leaders Takashi Saito, Hiroshi Kawamoto and Fumihiko Ishikawa, who are experts in monoclonal antibody production, artificial lymph nodes, and humanized mice, respectively.

Besides the Antibody Platform Unit, four other R&D projects were conducted under the collaboration between RCAI and DMP. "NKT cell cancer therapy" (Project Leader: Masaru Taniguchi), "Novel allergy therapy" (Project Leader: Masaru Taniguchi), "Novel therapy for leukemia targeting cancer stem cells" (Theme Leader: Fumihiko Ishikawa) and "CD8T therapeutic antibody drugs" (Theme Leader: Tsuneyasu Kaisho).

Considering this direction, RCAI launched a new program "Innovation Projects in Drug Discovery and Medicine" in 2010. The aim of this program is to develop seeds that can be candidate projects for DMP. During 2011, two projects were conducted at RCAI.

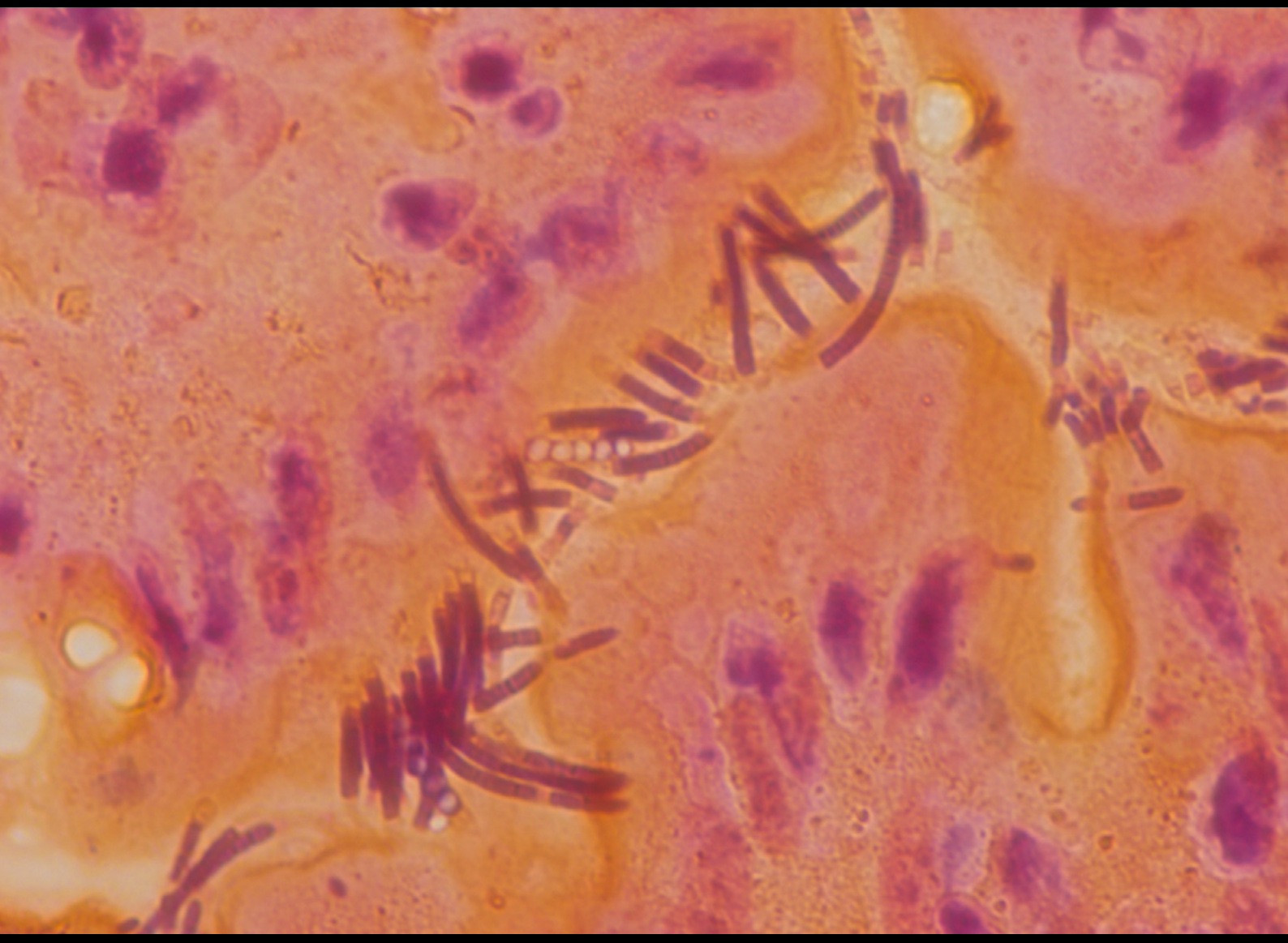
Table : RCAI Innovation Projects in Drug Discovery and Medicine 2011

1.	Shin-ichiro Fujii **"Development of artificial adjuvant vector cells"**
2.	Takashi Tanaka **"Development of a novel treatment for wounds using siRNA against PDLIM2"**

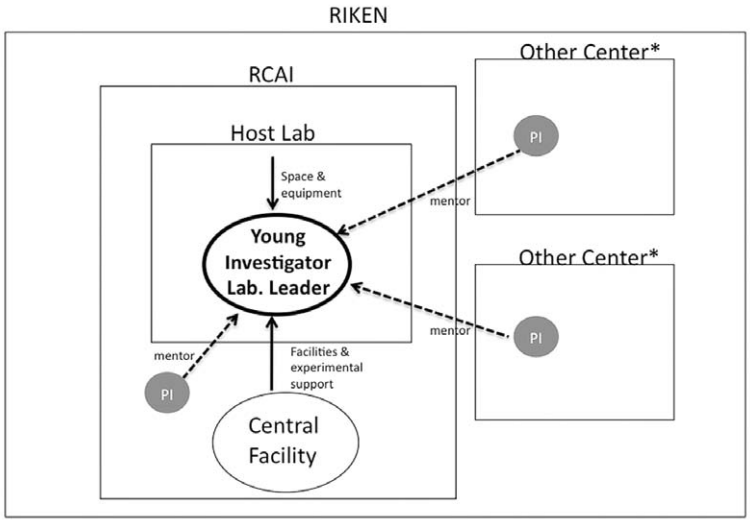
2011

Part 3

Nurturing Young Scientists



Young Chief Investigator Program



* Other Centers : all the research centers in RIKEN including PSC, CGM, SSBC, OSC, BASE, CRNID, ASI, BSI, CDB, CMIS, BRC, SPring-8, XFEL, Nishina Center, Senadi Facility, Nagoya Facility, Quantitative Biology Center, etc.

Figure : Scheme of the Young Chief Investigator Program
The Young Chief Investigator (YCI) will run a laboratory independently in terms of funding and research. The laboratory will, however, share space, equipment and facilities with a host lab (RCAI Research Group or Team). YCIs are supported by a mentor system. Three or four specialists (PIs) from the related fields inside or outside RCAI will be the mentors and will provide guidance for experimental design, preparation of papers and presentations, promotion of international visibility, and obtaining research funding.

RCAI launched a new program, Young Chief Investigator Program, in 2010 to provide a career path for young investigators who conduct multidisciplinary research that will bridge immunology with other research fields. In this program, the selected Young Chief Investigator (age below 40) will head an independent research laboratory but will have an access to mentoring by multiple senior specialists in related research fields, i.e. the leaders in various RIKEN Institutes. Mentors provide guidance for experimental design, preparation of papers and presentations, promotion of international visibility, and obtaining research funding. The YCI laboratory will also share space, equipment and facilities with a host laboratory in RCAI (Groups, Teams or Units) (Fig.) To consider necessary changes in the level of RCAI support for YCI, the YCI Program Committee meets twice a year. The committee will also discuss the relevance and value of the research project as part of the core research projects at RCAI.

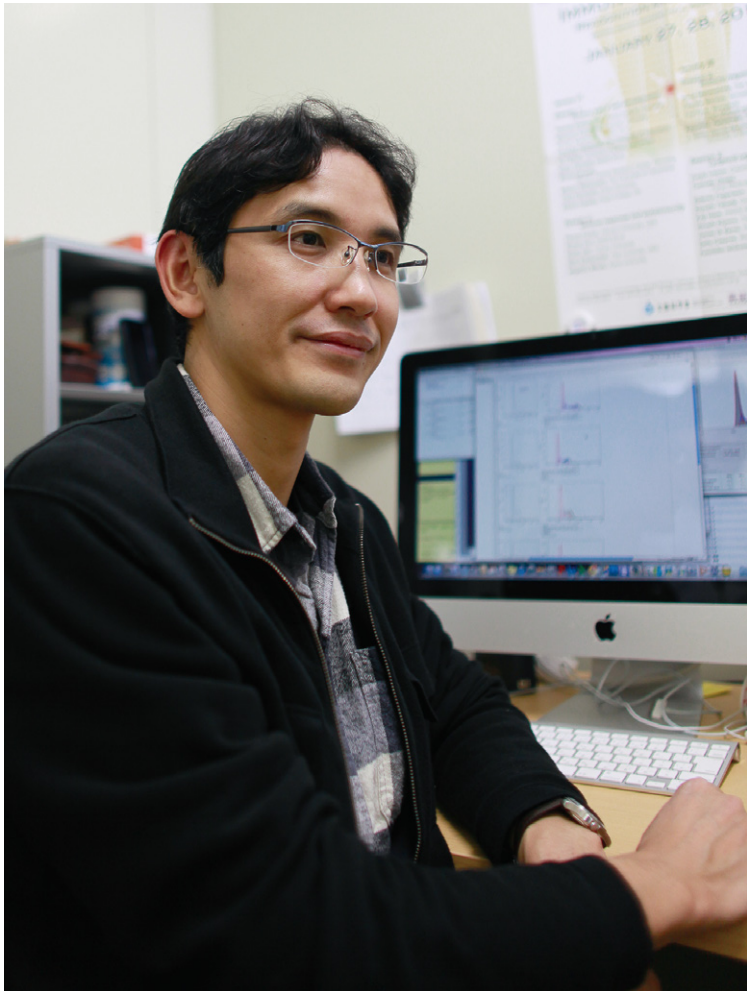
There will be an initial 5-year appointment, with the possibility of extending for an additional 2 years after evaluation by the Director and an internal committee. At that point, a Young Chief Investigator can leave RCAI to take a position at another institution or be promoted to another type of position within RCAI.

Three researchers were selected for the Young Chief Investigator Program, Dr. Hayato Naka-Kaneda (stem cells and aging reversal), Dr. Shinji Nakaoka (development of interface between integrative biology and mathematics), and Dr. Koji Hase (mucosal flora and epigenetic analysis). Their mentors and host laboratories are listed in Table.

Table: Young Chief Investigators and their mentors 2011

YCI lab	Mentors	Affiliation
Shinji Nakaoka "Laboratory for Mathematical Modeling of Immune System" Host Lab: **Osamu Ohara**, RCAI	**Masahiro Ueda**	Group Director, Laboratory for Cell Signaling Dynamics, RIKEN QBiC
	Kenji Kabashima	Associate Professor, Department of Dermatology, Graduate School of Medicine, Kyoto University
	Osamu Ohara	Group Director, Laboratory for Immunogenomics, RIKEN RCAI
	Masato Kubo	Professor, Research Institute for Biological Science, Tokyo University of Science Senior Visiting Scientist, Open Lab. for Signal Network, RIKEN RCAI
	Ronald Germain	National Institutes of Health, USA
Hayato Kaneda "Laboratory for Stem Cell Competency" Host Lab: **Ichiro Taniuchi**, RCAI	**Hitoshi Niwa**	Team Leader, Laboratory for Pluripotent Stem Cell Studies, RIKEN CDB
	Piero Carninci	Team Leader, Functional Genomics Technology Team, RIKEN OSC
	Hiroshi Kawamoto	Team Leader, Laboratory for Lymphocyte Development, RIKEN RCAI
	Haruhiko Koseki	Group Director, Laboratory for Developmental Genetics, RIKEN RCAI
Koji Hase "Laboratory for Bioenvironmental Epigenetics" Host Lab: **Haruhiko Koseki**, RCAI	**Minoru Yoshida**	Chief Scientist, Chemical Genetics Laboratory, RIKEN ASI
	Piero Carninci	Team Leader, Functional Genomics Technology Team, RIKEN OSC
	Yoichi Shinkai	Chief Scientist, Cellular Memory Laboratory, RIKEN ASI
	Haruhiko Koseki	Group Director, Laboratory for Developmental Genetics, RIKEN RCAI

YCI Laboratory for Bioenvironmental Epigenetics



Young Chief Investigator :

Koji Hase

Student Trainee : **Yukihiro Furusawa**

Epigenetics is a mechanism that imposes a specific and heritable pattern of gene expression on the progeny of differentiating cells, without affecting the base sequence of the DNA. The major epigenetic mechanisms include DNA methylation, chemical modifications of histone tails and higher order chromatin organization. Emerging evidence indicates the importance of epigenetic events in the development and proper functions of the immune system. Furthermore, it is assumed that transcriptional regulation via epigenetic mechanisms may be influenced by environmental factors, e.g., microbial products and cytokines. However, identification of such environmental factors and defining their impact on immunological homeostasis remain elusive goals.

Humans harbor over 100 trillion bacteria in the distal intestine. These commensal (meaning 'to share a dining table' in Latin) bacteria have long been appreciated for the benefits they provide to the host, the most obvious being their capacity to metabolize indigestible food components to small metabolites that are utilized as nutrients by host cells. Moreover, it is now clear that the presence of commensal bacteria contributes to shape the gut immune system through promoting the development of gut-associated lymphoid tissues, the largest collection of secondary lymphoid organs, which are necessary for induction of mucosal IgA responses. Certain enteric bacteria also facilitate differentiation of type17 helper T (Th17) and regulatory T (Treg) cells, both of which are major T cell populations in the intestinal mucosa. In support of this concept, the development of Th17 and Treg cells as well as IgA production are defective in mice housed in a germ-free isolator (denoted as "germ-free mice"). These previous observations led to the notion that host-microbe interactions establish immunological homeostasis in the gut, which further raises the important question: how do commensal bacteria affect the host immune system?

Epigenetic modifications of host cells by intestinal commensal bacteria

We have so far conducted studies to understand the maintenance of intestinal immune homeostasis and have demonstrated the biological significance of intestinal M cells localized at gut-associated lymphoid tissues. During the course of these studies, we noticed that the expression level of DNA methyltransferases is upregulated in the colonic

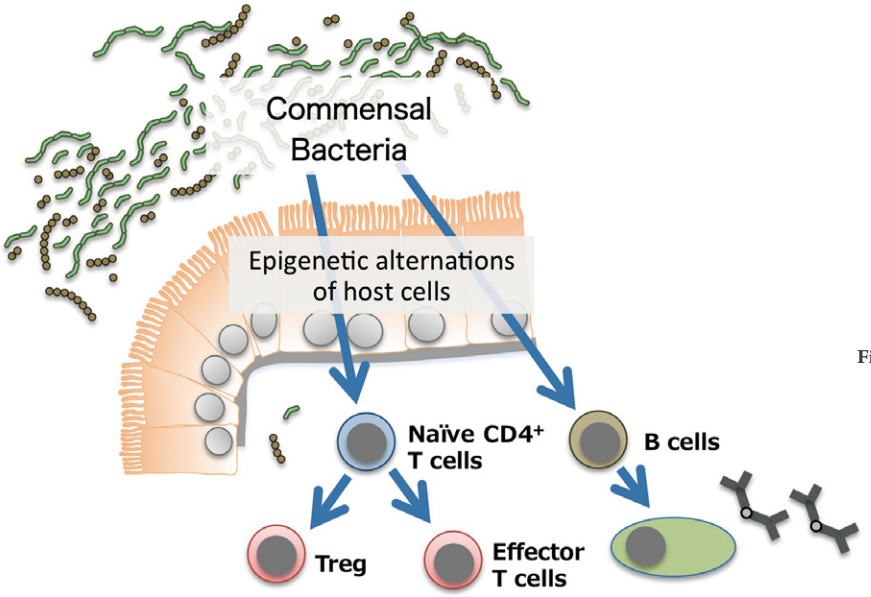


Figure : **Schematic of epigenetic regulation of intestinal immunity by commensal bacteria.** Intestinal commensal bacteria actively produce small molecule metabolites. These metabolites may directly or indirectly influence cell differentiation and function of immunocompetent cells such as T and B lymphocytes via epigenetic mechanisms.

mucosa of mice maintained under conventional conditions compared to that in germ-free mice. This observation suggests that bacterial colonization may influence the DNA methylation status of host cells. Indeed, our methyl DNA immunoprecipitation-on-chip (MeDIP-chip) assay revealed that the global DNA methylation level is substantially higher in colonic epithelium prepared from conventional mice compared to that from germ-free mice.

The enteric microflora constitute a potent bioreactor that controls several metabolic functions. The principal metabolic functions include the fermentation of indigestible food substances such as dietary fibers and resistant starch into simple sugars, absorbable nutrients, and short-chain fatty acids (SCFAs). Notably, certain SCFAs have been reported to exert histone deacetylase (HDAC) inhibitory activity, at least *in vitro*. Based on these observations, we hypothesize that intestinal microbiota may secure mucosal immune homeostasis via epigenetic mechanisms by the action of metabolites that they produce (see Fig.)

Goal of the study: Identification of the missing link between commensal bacteria and the development of gut immune system

This newly launched laboratory aims to explore the epigenetic regulation of the immune system by intestinal microbiota, and also to elucidate the biological significance of this epigenetic regulation in gut immunity. To simplify the host-bacteria interactions, I will employ germ-free mice and various gnotobiotic mice that will be colonized with one or only a few bacterial strains. Mucosal immunocompetent cells from these mice will be subjected to a genome-wide analysis of epigenetic modification (i.e. DNA methylation and histone tail trimethylation such as H3K4me3, H3K9me3 and H3K27me3). Furthermore, transcriptome analysis of the host cells as well as metabolic profiling of fecal samples will be performed. Bioinformatic analysis will be applied to define positive correlations between epigenetic changes in host cells and specific metabolites. Thus, identification of a key metabolite(s) that facilitates epigenetic changes in host cells is currently underway.

Recent publications

1. Fukuda S, Toh H, Hase K, Oshima K, Nakanishi Y, Yoshimura K, Tobe T, Clarke JM, Topping DM, Suzuki T, Taylor TD, Itoh K, Kikuchi J, Morita H, Hattori M, Ohno H. Bifidobacteria protect host from enteropathogenic infection through production of acetate. ***Nature*** 469, 543-7, (2011)

2. Takahashi D, Hase K, Kimura S, Nakatsu F, Ohmae M, Mandai Y, Sato T, Date Y, Ebisawa M, Kato T, Obata Y, Fukuda S, Kawamura Y. I, Dohi T, Katsuno T, Yokosuka O, Waguri S and Ohno H. The Epithelia-specific membrane trafficking factor AP-1B controls gut immune homeostasis in mice. ***Gastroenterology*** 141, 621-32 (2011)

3. Hase K, Kawano K, Nochi T, Pontes G.S., Fukuda S, Ebisawa M, Kadokura K, Tobe T, Fujimura Y, Kawano S, Yabashi A, Waguri S, Nakato G, Kimura S, Murakami T, Iimura M, Hamura K, Fukuoka S-I, Lowe AW, Itoh K, Kiyono H and Ohno H. Uptake via Glycoprotein 2 of FimH$^+$ bacteria by M cells initiates mucosal immune response. ***Nature*** 462, 226-230 (2009)

4. Hase K, Kimura S, Takatsu H, Ohmae M, Kawano S, Kitamura H, Ito M, Watarai H, Hazelett CC, Yeaman C and Ohno H. M-Sec promotes membrane nanotube formation by interacting with Ral and the exocyst complex. ***Nat. Cell Biol.*** 11, 1427-32 (2009)

YCI Laboratory for

Mathematical Modeling of Immune System

Young Chief Investigator :

Shinji Nakaoka

The skin tissue is a first line of host defense that acts as barrier and sensor for invading pathogens. Atopic dermatitis is a common skin disease that could occur as a consequence of loss of skin barrier function, followed by extensive allergic/inflammatory immune responses. Despite extensive basic and clinical research, definitive components and mechanisms that would mediate chronic allergic immune responses in the skin tissue remain unknown. Dysregulated immune responses in the skin tissue of atopic dermatitis patients are typically observed at heterogeneous spatio-temporal scales. Spatio-temporal multiscaleness and participation of multiple types of immune cells are key issues to be elucidated for obtaining a comprehensive view of the progression of atopic dermatitis.

The main goal of the research in my laboratory is to construct multi-scale mathematical models for atopic dermatitis, psoriasis vulgaris and other eczemas. The entire research plan consists of the following four essential building blocks: (i) Construction of a resource/information-base for atopic dermatitis, (ii) Exploration of intracellular gene expression dynamics of skin and immune cells, (iii) Quantitative study of immune cell dynamics in the skin tissue, and (iv) Development of supportive theory for multi-scale mathematical modeling -- *in silico* representation of immunological events and rules.

Construction of resource/information-base for atopic dermatitis

Current existing subfields in atopic dermatitis study include various disciplines such as skin biology, immunology, neuroendocrinology and psychology. Encouragement of multidisciplinary collaborations over different subfields of atopic dermatitis study holds the promise of bringing comprehensive understanding to this disease. Construction of a resource/information-base for atopic dermatitis is an essential step to facilitate multidisciplinary study by offering various types of structured knowledge. Although existing versatile database and computational tools developed in bioinformatics enable us to obtain relevant information about atopic dermatitis, integration of existing vast yet dispersed knowledge in subfields is required to mediate interdisciplinary collaborations. The main purpose of this project is to

Multiscale mathematical modeling of immune responses

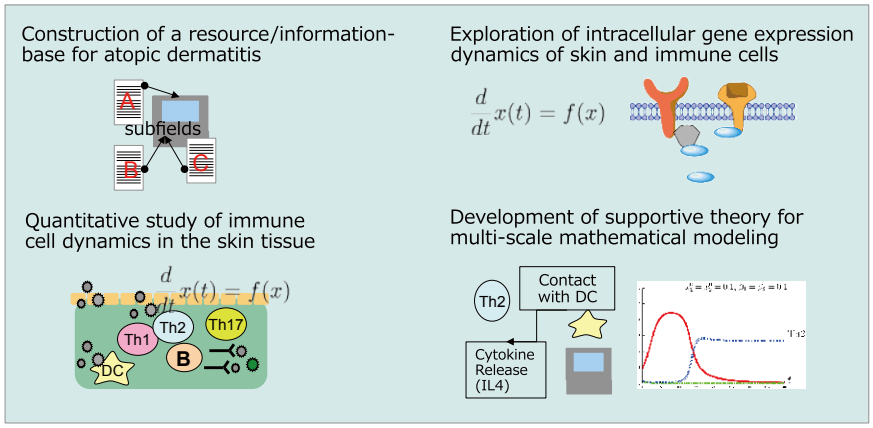


Figure : **A schematic representation of the four building blocks for multi-scale mathematical modeling of immune responses.**

construct an integrated knowledge-base for atopic dermatitis. As a first step, a reference-database system for atopic dermatitis is now under construction.

Exploration of intracellular gene expression dynamics of skin and immune cells

The first step to clarify the onset of atopic dermatitis is to define basic characteristics of the initial allergic and inflammatory reactions. Exploration of differences in gene expression profiles and progression of allergic inflammation processes between normal and atopic dermatitis mice should provide informative guides to identify definitive components and mechanisms that mediate chronic and allergic immune responses. In this project, several atopic dermatitis mouse models are used to investigate gene expression dynamics of skin and immune cells. The main purpose of this project is to investigate the role of the JAK/STAT signaling pathway: how differences in signal transduction would result in diverse outcomes. The project is currently in progress.

Quantitative study on immune cell dynamics in the skin tissue

The first step to clarify the progression (post-initial phase) of atopic dermatitis is to understand the dynamic interplay among skin and immune cells. Newly developed measurement devices offer quantitative, multivariate, high-throughput and time-series data. Mathematical modeling and statistical/information-theoretic analyses are essential to extract qualitative insights from this kind of data. The main purpose of this project is to investigate immune cell dynamics in the skin tissue under tight collaboration with experiments on the basis of quantitative research design. The theoretical goal of this project is to extract essential control mechanisms that are dysregulated during the progression of atopic dermatitis. The project is now being developed.

Development of supportive theory for multi-scale mathematical modeling

In general, the whole process of immune responses is achieved under complex dependencies in terms of a wide range of spatio-temporal interactions and participation of multiple types of immune cells. Any mathematical models that describe process and dynamics of immune responses should appropriately reflect such complex dependencies. However, a lack of theoretical guidance to describe complex immune responses *in silico* hampers construction of multiscale mathematical models. The main purpose of this project is to develop a supportive theory for representing events and rules of immune responses *in silico*. Our primary aim is to extract *in silico*-translatable rules underlying allergic (Th2) immune reactions from the existing literature. The project is currently in progress.

Recent publications

1. Katsuyama C., Nakaoka S., Takeuchi Y., Tago K., Hayatsu M., Kato K., Complementary cooperation between two syntrophic bacteria in pesticide degradation, ***J. Theor. Biol.*** 256 644-654 (2009).
2. Nakaoka S., Wang W., Takeuchi Y., Effect of parental care and aggregation on population dynamics, ***J. Theor. Biol.*** 260 161-171 (2009).
3. Iwami S., Nakaoka S., Takeuchi Y., Miura Y., Miura T., Immune impairment thresholds in HIV infection, ***Immunol. Letters*** 123 149-154 (2009).
4. Diekmann O., Gyllenberg M., Metz J.A.J., Nakaoka S. and de Roos A., Daphnia revisited: local stability and bifurcation theory for physiologically structured population models explained by way of an example, ***J. Math. Biol.*** 61 277-318 (2010).
5. Nakaoka S., Aihara K., Mathematical study on kinetics of hematopoietic stem cells -- theoretical conditions for successful transplantation --, ***J. Biol. Dyn.*** IFirst, 1–19 (2011).

YCI Laboratory for Stem Cell Competency

Young Chief Investigator :

Hayato Kaneda

Technical staff : **Shiho Nakamura**

Age-related disruption of tissue homeostasis induces reduction of regenerative ability, resulting in age-associated pathologies and a lower quality of life (QOL). Japan has already become an ageing society and the demographic trends predict a doubling of the world population over 65 years of age within the next 30 years. However, we know little about the mechanisms of ageing and even much less about how to counteract it. A tight balance between cellular proliferation and cell death maintains tissue homeostasis. In the former case, somatic stem cells (SSCs) play important roles by supplying tissue-specific cells over the lifespan of the animal. SSCs are generally defined by multipotency and the ability to self-renew. However, recent studies have demonstrated that SSCs themselves undergo ageing, changing their functions with age. Their dysfunction and decreased regenerative capacity can cause physiological deficiencies, e.g., inefficient muscle repair, reduced bone mass, neurodegenerative diseases, and dysregulation of hematopoiesis. Therefore, we predict that the restoration of SSCs functions towards those in young healthy individuals would contribute to recovery of tissue homeostasis and improvements in our health.

Competence regulation of neural stem cells

We have been investigating the molecular mechanisms governing the differentiation of neural stem cells (NSCs) using an *in vitro* neurosphere culture system that we had originally developed. In this system, NSCs are induced from embryonic stem cells (ESCs) through embryoid body (EB) formation and selectively amplified as neurospheres (Fig. 1). NSCs have multipotency to differentiate into neurons and glial cells. Neurogenesis largely precedes gliogenesis during central nervous system (CNS) development in vertebrates and this neurogenesis-to-gliogenesis switching requires the temporal identity transition of NSCs. This transition can also be observed in our culture system as it recapitulates *in vivo* mouse CNS development. We previously identified *Coup-tfI* and *II (Coup-tfs)* as critical molecular switches for the neurogenic-to-gliogenic transition of NSCs (Naka H. et al. *Nature Neuroscience* 2008). Their knockdown caused sustained generation of the early-born neurons and loss of gliogenesis (Fig. 2). Remarkably, *Coup-tfs* neither repress neurogenesis nor promote gliogenesis. Instead, we found that they are only involved in the temporal “competence change” of NSCs to permit the transition of their developmental status by changing their responsiveness to extrinsic gliogenic signals (Fig.3).

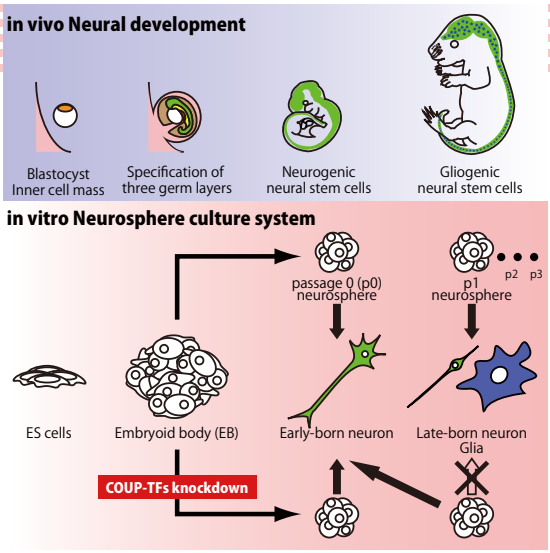


Figure 1: ***In vitro*** **neurosphere culture system**
NSCs are induced from ESCs as neurospheres. *Coup-tfs* knockdown caused sustained generation of early-born neurons and loss of gliogenesis.

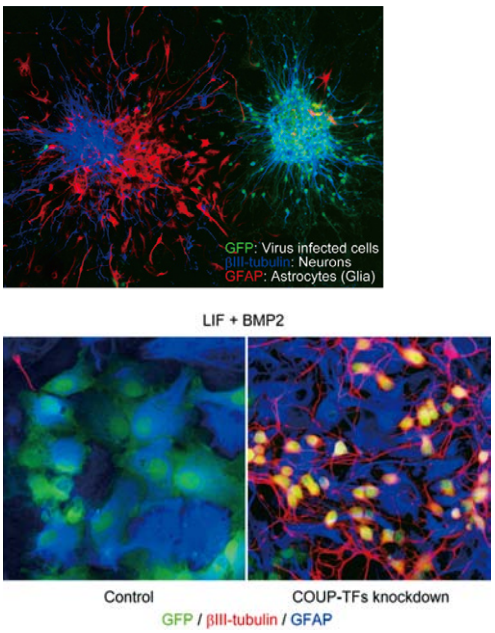


Figure 2: **Alteration of responsiveness to gliogenic cytokines**
Coup-tfs knockdown neurospheres continue to produce neurons even in the p2 stage and resist the induction of gliogenesis by gliogenic cytokines, LIF and BMP2.

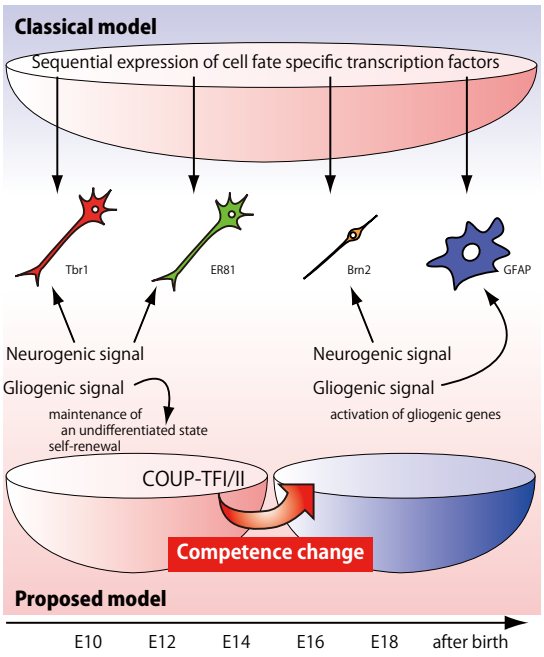


Figure 3: **Model for the temporal identity transition of NSCs that incorporates the concept of competence regulation**
Cytogenesis is integration of outputs from multiple dynamic regulatory programs in stem cells. Expression of COUP-TFI/II triggers NSC competence change. Responsiveness to the gliogenic signals is altered and the NSCs acquire gliogenic competence. At that point, gliogenic signals induce gliogenesis only from the gliogenic NSCs.

Stem cell ageing

Recently, we could further reveal the molecular mechanisms underlying the NSC competence change. The results led us to hypothesize that competence change, which is reflected by changes in responsiveness to extrinsic signals, is a common and fundamental molecular mechanism among various SSCs in the regulation of their properties. Thus, we are trying to expand our discovery made in NSC development to the regulation of a variety of SSC regulations and are now focusing on stem cell ageing.

Among various SSCs, stem cell ageing is best studied in hematopoietic stem cells (HSCs). Ageing induces expansion of the HSC population, but its capacity for long-term blood cell reconstitution becomes reduced and also its differentiation potency becomes biased towards myelopoiesis at the expense of lymphocyte production. Numerous cell-intrinsic and extrinsic mechanisms connecting stem cell ageing and age-associated pathologies have been identified as HSC ageing modulators. They include DNA damage accumulation, oxidative stress, chromatin dysregulation, induction of senescence-associated proteins and ageing of niche cells, for example. However, no critical molecular regulators of stem cell ageing have been identified. Thus, we are trying to understand the contributions of competence regulation to stem cell ageing.

Recent publications

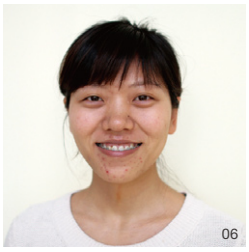
1. Sato T., Shimazaki T., Naka H., Fukami S., Satoh Y., Okano H., Lax I., Schlessinger J., Gotoh N. FRS2alpha regulates Erk levels to control a self-renewal target Hes1 and proliferation of FGF-responsive neural stem/progenitor cells. ***Stem Cells*** 28, 1661-1673 (2010).

2. Naka-Kaneda H., Shimazaki T., Okano H. Neurogenesis to gliogenesis switching. ***Experimental Medicine (Jikken Igaku)*** 28, 815-822 (2010).

3. Tao O., Shimazaki T., Okada Y., Naka H., Kohda K., Yuzaki M., Mizusawa H., Okano H. Efficient generation of mature cerebellar Purkinje cells from mouse embryonic stem cells. ***J. Neurosci. Res.*** 88, 234-247 (2010).

RIKEN Joint Graduate School Program International Program Associate

RCAI accepted eight international students as RIKEN International Program Associates (IPA). Under this IPA program, RCAI lab heads host international students from collaborating graduate schools and supervise their Ph.D. program as Joint Supervisors. The students receive a daily living allowance and housing costs for up to a maximum of three years.



The IPA students who studied at RCAI in 2011 were

Li Yingqian
(Nanjing University, China) studied in the Laboratory for Immune Diversity (Photo 1)

Sebastian Nieke
(Tübingen Univ., Germany) studied in the Laboratory for Transcriptional Regulation (Photo 2)

Li Shuyin
(China Agriculture University, China) studied in the Laboratory for Immune Diversity (Photo 3)

Yue Ren
(Jilin University, China) studied in the Laboratory for Immune Regulation (Photo 4)

Mohamed El Sherif Gadelhaq Gadelhaq Badr
(Tokyo Medical and Dental University) from Edypt studied in the Laboratory for Cell Signaling (Photo 5)

Zhang Yanfei
(Peking University, China) studied in the Laboratory for Immune Diversity (Photo 6)

Joo Ann Ewe
(Universiti Sains Malaysia, Malaysia) studied in the Laboratory for Epithelial Immunobiology (Photo 7)

Huey Shi Lye
(Universiti Sains Malaysia, Malaysia) studied in the Laboratory for Epithelial Immunobiology (Photo 8)

RIKEN Foreign Postdoctoral Researcher

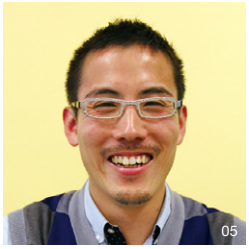
The RIKEN Foreign Postdoctoral Researcher (FPR) program offers aspiring young foreign researchers with creative ideas and who show promise of becoming internationally active in the future the opportunity to pursue innovative research at RIKEN under the direction of a RIKEN laboratory head. The FPR program is one of RIKEN's initiatives to open up its facilities and resources to the world and create a stimulating research environment that will place RIKEN at the forefront of global science and technology.

In 2011, Jafar Sharif (Photo) studied in the Laboratory for Developmental Genetics as a RIKEN FPR.

RIKEN Special Postdoctoral Researcher (SPDR) Program

RIKEN's program for Special Postdoctoral Researchers was instituted to provide young and creative scientists the opportunity to be involved in autonomous and independent research in line with RIKEN objectives and research fields. The positions are competitive, but if selected, researchers receive salaries and research budgets (1 million yen) from RIKEN and they are able to conduct their research at one of its laboratories.

This year, nine postdocs conducted their research at RCAI through the SPDR program.



Shinsuke Ito (Laboratory for Developmental Genetics) (Photo 1)
Mizuho Kajikawa (Laboratory for Infectious Immunity) (Photo 2)
Takashi Kanaya (Laboratory for Epithelial Immunobiology) (Photo 3)
Shimpei Kawamoto (Laboratory for Mucosal Immunity) (Photo 4)
Masahiro Kitano (Research Unit for Immunodynamics) (Photo 5)
Yoshitaka Shirasaki (Laboratory for Immunogenomics) (Photo 6)
Yuki Horisawa-Takada (Laboratory for Developmental Genetics) (Photo 7)
Hirokazu Tanaka (Laboratory for Transcriptional Regulation) (Photo 8)
Nayuta Yakushiji (Laboratory for Developmental Genetics) (Photo 9)

RIKEN's Junior Research Associate (JRA) Program

The Junior Research Associate program was launched in 1996 to encourage young scientists with fresh ideas and youthful enthusiasm to collaborate with, and learn from, senior scientists with years of experience. This program provides part-time positions at RIKEN for young researchers enrolled in university Ph.D. programs. The JRA program serves the dual purpose of fostering the development of these young scientists while also energizing RIKEN with their innovative thinking.

This year, eleven JRA students studied in RCAI.

Yuki Aoki (Laboratory for Human Disease Model) (Photo 1)
Misao Hanazato (Laboratory for Epithelial Immunobiology) (Photo 2)
Rikiya Ishikawa (Laboratory for Infectious Immunity) (Photo 3)
Toshi Jinnohara-Yuyama (Laboratory for Epithelial Immunobiology) (Photo 4)
Chie Kano (Laboratory for Immune Diversity) (Photo 5)
Tamotsu Kato (Laboratory for Epithelial Immunobiology) (Photo 6)
Nanako Shimura (Laboratory for Immunogenomics) (Photo 7)
Masanaka Sugiyama (Research Unit for Inflammatory Regulation) (Photo 8)
Shinsuke Takagi (Laboratory for Human Disease Model) (Photo 9)
Hideaki Takagi (Laboratory for Dendritic Cell Immunobiology) (Photo 10)
Yuuhou Najima (Laboratory for Human Disease Model) (Photo 11)

Adjunct Professorship Programs

RCAI accepts graduate students through the mechanism of adjunct professorships at various Japanese universities. RCAI collaborates with and accepts graduate students from 10 domestic university graduate schools. There are now a total of 25 adjunct professors/associate professors in RCAI. Seventy-eight students studied at RCAI in 2011.

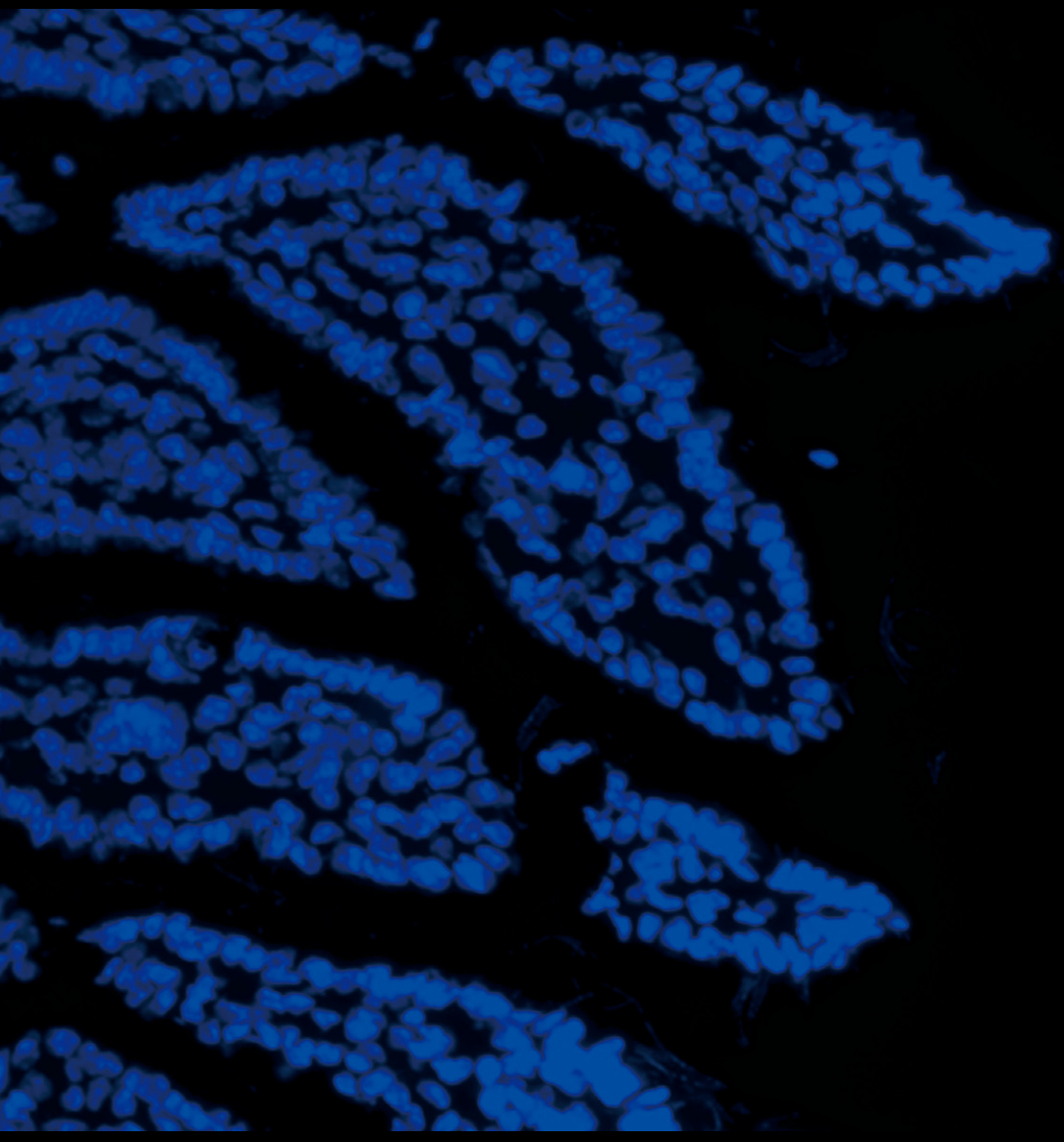
Table: Adjunct professorship programs

Graduate Program	Affiliated RCAI Investigator
Graduate School of Frontier Bioscience, Osaka University	**Tomohiro Kurosaki** (Professor)
	Ichiro Taniuchi (Visiting Professor)
	Keigo Nishida (Visiting Associate Professor)
Graduate School of Medicine, Osaka University	**Takashi Saito** (Visiting Professor)
	Toshiyuki Fukada (Visiting Associate Professor)
Department of Immunology, Graduate School of Medicine, Chiba University	**Takashi Saito** (Visiting Professor)
	Haruhiko Koseki (Visiting Professor)
	Hiroshi Ohno (Visiting Professor)
	Shin-ichiro Fujii (Visiting Associate Professor)
	Yasuyuki Ishii (Visiting Associate Professor)
	Fumihiko Ishikawa (Visiting Associate Professor)
Graduate School of Pharmaceutical Sciences, Chiba University	**Osamu Ohara** (Visiting Professor)
School of Biomedical Science, Tokyo Medical and Dental University	**Takashi Saito** (Visiting Professor)
	Tomohiro Kurosaki (Visiting Professor)
	Masato Kubo (Visiting Professor)
	Mariko Okada (Visiting Professor)
	Sidonia Fagarasan (Visiting Associate Professor)
International Graduate School of Arts and Sciences, Yokohama City University	**Hiroshi Ohno** (Visiting Professor)
	Tsuneyasu Kaisho (Visiting Professor)
	Satoshi Ishido (Visiting Associate Professor)
Research Institute of Biological Sciences, Tokyo University of Science	**Masato Kubo** (Professor)
	Osamu Ohara (Visiting Professor)
	Shohei Hori (Visiting Associate Professor)
	Tadashi Yokosuka (Visiting Associate Professor)
Department of Computational Biology, Graduate School of Frontier Sciences, The University of Tokyo	**Mariko Okada** (Visiting Associate Professor)
Graduate School of Medicine, Kyoto University	**Fumihiko Ishikawa** (Visiting Associate Professor)
Graduate School of Medicine, Kobe University	**Masaru Taniguchi** (Adjunct Lecturer)

2011

Part 4

Collaborative Networks



Open Laboratory at RCAI

RCAI's Open Laboratory for Allergy Research was established in 2009 to provide a framework for researchers from external institutes, universities or hospitals that would allow them access to the Center's resources and the opportunity for collaborative allergy research projects.

In 2011, two researchers, Drs. Masato Kubo (Open Laboratory for Signal Network) and Dr. Noriko M Tsuji (Open Laboratory for Allergy and Mucosal Immune Tolerance), were promoted to Senior Visiting Scientists. They have their own laboratory space and staff in RCAI and have joined various internal research meetings and discussions to promote extensive research communication with RCAI investigators.

Two other projects were also conducted at RCAI under the Allergy Open Laboratory system. Dr. Kenji Matsumoto (National Research Institute for Child Health and Development) and Dr. Naoki Shimojo's (Chiba University) project "Cohort study of allergy in infants and the generation of a humanized mouse model" was hosted by Dr. Toshitada Takemori (Laboratory for Immunological Memory), and Dr. Taeko Dohi's (National Center for Global Health and Medicine) project "Intestinal flora and epigenomic imprinting" was hosted by Dr. Hiroshi Ohno (Laboratory for Epithelial Immunobiology).

In addition to allergy research, RCAI also established an Open Laboratory for Systems Biology, where multidisciplinary researchers can interact at RCAI to model the complex immune system using mathematical and computational approaches.

Open Laboratory for Allergy Research
Masato Kubo (Research Institute for Biological Science, Tokyo University of Science) "Laboratory for Signal Network"
Noriko M Tsuji (Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST)) "Laboratory for Allergy and Mucosal Immune Tolerance"
Kenji Matsumoto (National Research Institute for Child Health and Development) and **Naoki Shimojo** (Chiba University) "Cohort study of allergy in infants and the generation of a humanized mouse model"
Taeko Dohi (National Center for Global Health and Medicine) "Intestinal flora and epigenomic imprinting"
Open Laboratory for Systems Biology
Kazuyuki Aihara (Institute of Industrial Science, The University of Tokyo)
Tatsuji Hara (Graduate School of Information Science and Technology, The University of Tokyo)
Yoshiharu Yamamoto (Graduate School of Education, The University of Tokyo)
Kenko Uchida (Graduate School of Advanced Science and Engineering, Waseda University)
Jun-ichi Imura (Graduate School of Information Science and Engineering, Tokyo Institute of Technology)
Koji Tsumura (Graduate School of Information, Science and Technology, The University of Tokyo)
Hidenori Kimura (RIKEN BSI-TOYOTA Collaboration Center)
Shinji Nakaoka (Graduate School of Mathematical Sciences, The University of Tokyo)

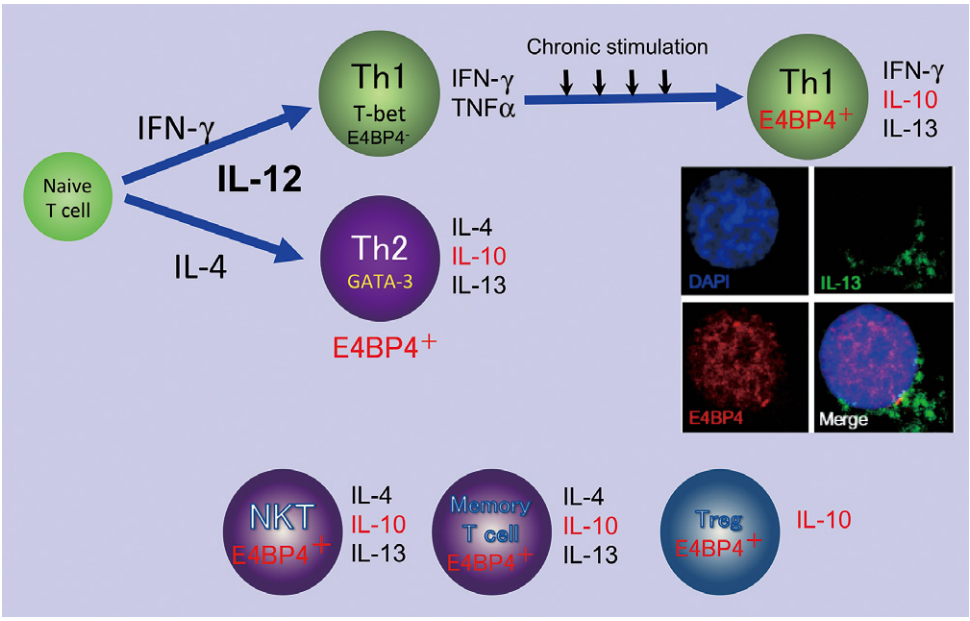


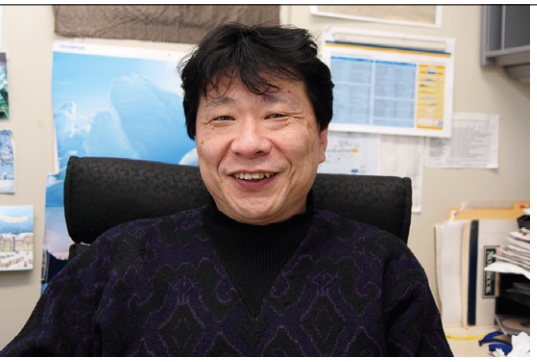
Figure : Expression of E4BP4 regulates IL-10 expression in several T cell subsets. E4BP4 controls the plasticity of IL-13 production by Th1 cells following chronic antigen stimulation and functions as a master regulator for IL-10 expression by Th1, Th2, Treg and NKT cells.

T cells play a central role in the effector and regulatory functions in immunological surveillance, and aberrations in these functions can lead to various immunological disorders. T helper 1 (T_H1) cells secrete interleukin-2 (IL-2), IFN-γ and TNF-α during the cellular immune response to intracellular pathogens and viruses. T_H2 cells, which are mainly protective against extracellular pathogens but also account for allergic immune responses, produce IL-4, IL-5, IL-6, IL-10 and IL-13. These cytokines secreted by the different effector helper T cells play a critical role in controlling the outcome of immunological surveillance. The helper T cell subsets differentiate from common precursor cells, the naïve T cells, and this differentiation is accompanied by acquiring the capacity to produce certain types of cytokines. Recent studies suggest that TH2 cytokines that are tightly associated with allergic immune responses are also expressed by innate type immune cells, such as mast cells and basophils, as well as by type II innate lymphoid cells, including natural helper cells and neocytes. These innate cell-derived as well as TH2-derived cytokines play a critical role in eliciting allergic disease. The overriding goal of our laboratory is to understand the molecular mechanisms underlying cytokine gene expression in helper T cell subsets, innate type immune cells and innate lymphoid cells.

E4bp4, a mammalian basic leucine zipper (bZIP) transcriptional factor, is a critical regulator of IL-10 and IL-13 production in CD4 T cells

Interleukin-10 (IL-10) is an anti-inflammatory cytokine that downregulates immune responses and staves off autoimmune disease. This cytokine is secreted mainly by TH2 cells but also by TH1 cells during chronic infections. We observed plasticity of IL-10 and IL-13 expression following chronic TH1 stimulation and found that IL-10 and IL-13 are regulated by the basic leucine zipper type of transcriptional factor, E4BP4/NFIL-3. TH1 cells lacking *E4bp4* had attenuated IL-10 and IL-13 expression following chronic antigen stimulation and, consistently, forced expression of *E4bp4* initiated IL-10 and IL-13 expression in conventional TH1 cells. On the other hand, TH2 cells lacking *E4bp4* had a marked impairment in IL-10 production with no effect on IL-13. Moreover, Treg and NKT cells derived from mice lacking *E4bp4* had a reduction in IL-10 expression.

Transcription factors can control the expression of genes by binding to a specific region of the enhancer



and the promoter. We observed that E4BP4 bound to the *Il13* promoter in T_H1 cells that had been chronically stimulated with antigen, but not in T_H1 cells that had not been chronically stimulated. However, E4BP4 regulates the expression of IL-10 in an epigenetic manner by altering the chromosomal structure in the region of the *Il10* gene. These results indicate that E4BP4 has multiple functions in controlling the plasticity of IL-13 in Th1 cells and in functioning as a master regulator for IL-10 expression in Th1, Th2, Treg and NKT cells. Interestingly, E4BP4-deficient mice, which produced lower levels of IL-10 than control mice, also showed some symptoms of gastrointestinal inflammation along with diarrhea. Therefore, E4BP4 is also a key transcriptional factor in preventing autoimmune inflammation.

Role of basophils and mast cells in allergic responses and helminth infections.

We established a diphtheria toxin (DT)-based conditional deletion system using *Il4* enhancers specific for either mast cells (MCs) or basophils to drive the expression of the DT receptor (DTR) (Mas-DTR and Bas-DTR mice). While diphtheria toxin treatment of Bas-DTR mice resulted in specific deletion of basophils, treatment of Mas-DTR mice resulted in deletion of both MCs and basophils. Using these mice, we found that MCs and basophils played distinctive roles in the early and chronic phases of the IgE-mediated allergic response. Basophils were dispensable for asthmatic responses, and for IgE production induced by systemic antigen immunization. However, basophil deletion partly impaired IL-4 production by activated T cells from *Trichinella* infected mice. We also identified heterogeneity between TSLP-elicited versus IL-3-elicited basophils. In humans, TSLP expression is associated with asthma, atopic dermatitis and food allergies, and studies in murine systems demonstrated that TSLP promotes TH2 cytokine-mediated immunity and inflammation. We demonstrated that TSLP induces TH2 cytokines by eliciting basophil responses.

Furthermore, in Mas-TRECK mice, contact hypersensitivity (CHS) was attenuated when MCs were depleted during the sensitization phase. We examined the role of mast cells in CHS and in FITC-induced cutaneous DC migration and found that both maturation and migration of skin DCs were abrogated by MC depletion. CHS was attenuated when MCs were depleted during the sensitization phase. Consistent with these findings, co-culture with bone marrow-derived MCs enhanced the maturation and chemotaxis of BMDCs *in vitro*. These results suggest that MCs interact with DCs in the skin and enhance DC functions. This interaction might be essential for establishing the sensitization phase of CHS.

Leader :

Masato Kubo

(Research Institute for Biological Science, Tokyo University of Science)

Recent Publications :

1. Tanaka, S., Motomura, Y., Suzuki, Y., Yagi, R., Inoue, H., Miyatake, S., Kubo, M. The enhancer HS2 critically regulates GATA-3-mediated *Il4* transcription in T_H2 cells. ***Nat Immunol.*** 12, 77-85, 2011
2. Sofi, M. H., Qiao Y., Ansel, K. M., Kubo, M., Chang, C-H. Induction and maintenance of IL-4 expression are regulated differently by the 3'enhancer in CD4 T cells. ***J Immunol***. 186, 2792-9. 2011
3. Motomura, Y., Kitamura, H., Hijikata, A., Matsunaga,Y., Matsumoto, K., Inoue, H., Atarashi, K., Hori. S., Watarai, H., Zhu, J., Taniguchi, M., and Kubo, M. The transcription factor E4BP4 regulates the production of IL -10 and IL-13 in $CD4^+$ T cells. ***Nat Immunol.*** 12, 450-459, 2011
4. Siracusa, M. C., Saenz, S. A., Hill, D. A., Kim, B. S. Headley, M. B., Doering, T. A., Wherry EJ., Jessup, H. K., Siegel, L. A., Kambayashi, T., Dudek, E. C., Kubo, M., Cianferoni, A., Spergel, J. M., Ziegler, S. F., Comeau, M. R., and Artis, D.; TSLP promotes interleukin-3-independent basophil hematopoiesis and type 2 inflammation. ***Nature*** 477, 229-233, 2011.
5. Otsuka, A., Kubo, M., Honda, T., Egawa, G., Nakajima, S., Tanizaki, H., Kim, B., Matsuoka, S., Watanabe, T., Nakae, S., Miyachi, Y., Kabashima, K. Requirement of interaction between mast cells and skin dendritic cells to establish contact hypersensitivity. ***Plos One*** 6, e25538. 2011

Open Laboratory for Allergy and Mucosal Immune Tolerance (AMIT)

To accommodate a vast antigenic exposure from food components as well as from commensal bacteria, the gut has evolved a naturally anti-inflammatory environment. Under normal physiological conditions, the GALT develops mechanisms to tolerate, or exist in a symbiotic relationship with, a large, normally innocuous community of gut microbes. In order to maintain this tolerant state, the immune system of the gut must exhibit "dominant tolerance" and possess the ability to generate a regulatory immune response that can even control systemic immunity. Oral tolerance is therefore a major physiological mechanism to maintain intestinal and systemic immune homeostasis. It is also a promising method for controlling both regional and systemic allergic inflammation.

Defects in the induction of oral tolerance against egg proteins, for instance, lead to a food allergy. Therefore co-opting the immune system towards avoiding unwanted inflammation and stabilizing its homeostasis is an ideal approach for the fundamental treatment of inflammatory disorders such as allergy. To this end, induction of Ag-specific oral tolerance is an attractive solution. Promising strategies for induction of Ag-specific regulatory T cells might include usage of soluble peptides, tolerogenic adjuvants and/or cytokines, as well as the direct application of Ag via the mucosal route.

The AMIT open lab will elucidate:

(1) the mechanism for generation and functional maturation of intestinal Ag-specific Treg and their suppression mechanism in allergic inflammatory responses.

(2) how suppression mechanisms that originate in the intestine dominantly regulate systemic immunity,

(3) the mechanism of rush specific oral tolerance induction (SOTI), a feasible clinical treatment method

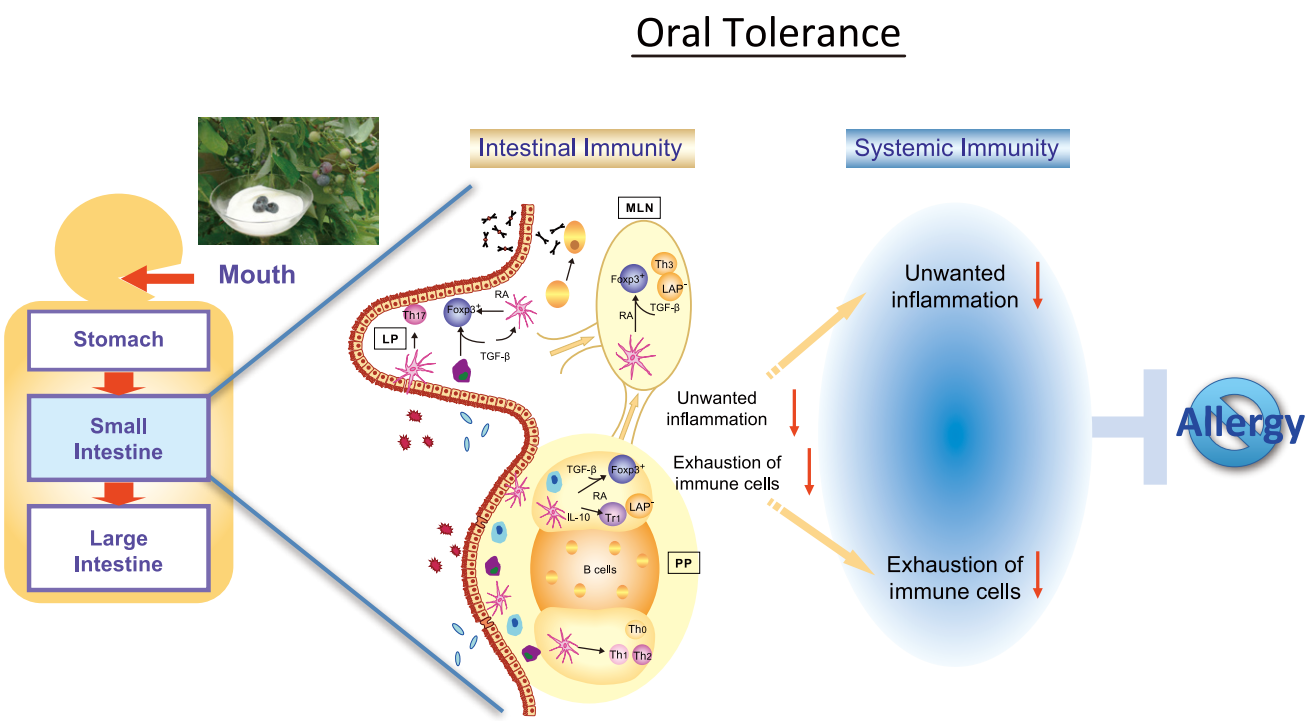
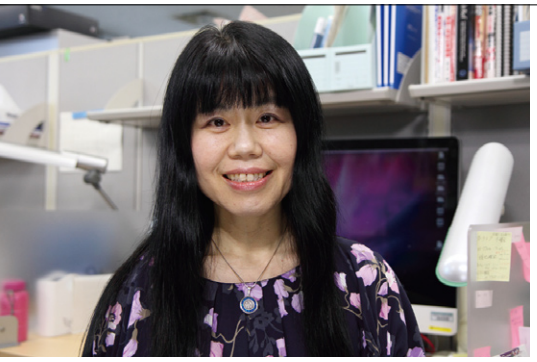


Figure 1: Oral tolerance: its suppression mechanism originates in the intestine and dominantly regulates systemic immunity, thus it is essential for maintenance of immune homeostasis. It is also a promising method of regulating both regional and systemic allergic inflammation.



for food allergy, by developing an animal model.

The AMIT open lab has established a model system for type I allergy (anaphylaxis), regular and rush SOTI in sensitized mice, and confirmed prolonged effects of the SOTI protocol in allergy model mice. These experimental models will be utilized to elucidate how gut-induced Tregs suppress allergic inflammation and related effector cells such as mast cells. Since oral tolerance is the most physiological prototype of the Ag-specific tolerogenic immune response, further understanding of antigen presenting cells and regulatory T cells in the gut should lead to effective therapeutic strategies targeting immune dysfunction.

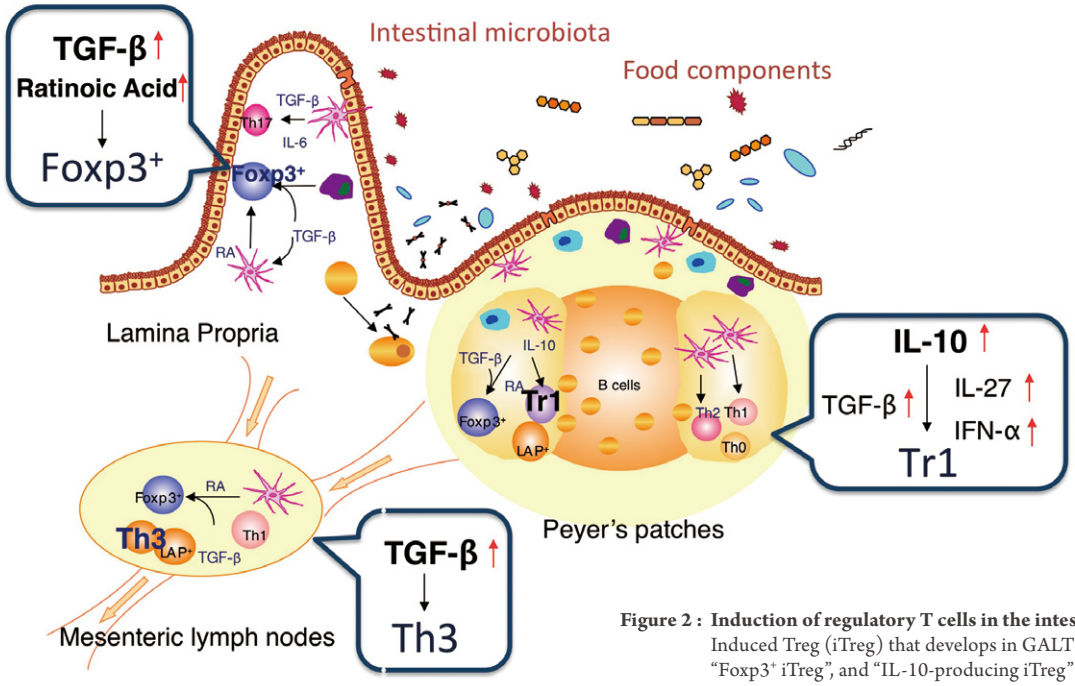


Figure 2 : Induction of regulatory T cells in the intestine
Induced Treg (iTreg) that develops in GALT is "TGF-β-producing iTreg", "Foxp3⁺ iTreg", and "IL-10-producing iTreg" whose differentiation factors are TGF-β, TGF-β plus retinoic acid, and IL-10, respectively. Although each group of Treg has the ability to inhibit Th1 immune responses and inflammation, their exact suppressive mechanisms are largely unknown. (Tsuji NM & Kosaka A, *Trends Immunol* (2008) 29: 532-540)

Leader :

Noriko M Tsuji

(Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST))

Recent Publications :

1. Kawashima T, Hayashi K, Kosaka A, Kawashima M, Igarashi T, Tsutsui H, Tsuji NM, Nishimura I, Hayashi T, Obata A. Lactobacillus plantarum strain YU from fermented foods activates Th1 and protective immune responses. ***Int Immunopharmacol.*** 11(12):2017-24. 2011
2. Yan H, Kakuta S, Nishihara M, Sugi M, Adachi Y, Ohno N, Iwakura Y, Tsuji NM. Kjellmaniella crassifolia Miyabe (Gagome) extract modulates intestinal and systemic immune responses. ***Biosci Biotechnol Biochem.*** 75(11):2178-83. 2011
3. Jeon SG, Kayama H, Ueda Y, Takahashi T, Asahara T, Tsuji H, Tsuji NM, Kiyono H, Ma JS, Kusu T, Okumura R, Hara H, Yoshida H, Yamamoto M, Nomoto K, Takeda K. Probiotic Bifidobacterium breve induces IL-10-producing Tr1 cells in the colon. ***PLoS Pathog.*** 2012 8(5):e1002714. 2012
4. Faria AM, Mucida D, McCafferty DM, Tsuji NM, Verhasselt V. Tolerance and inflammation at the gut mucosa. ***Clin Dev Immunol.*** 2012:738475. 2012

University of Michigan-RCAI Joint Workshop

December 1-2, 2011

University of Michigan
Medical School

Five immunologists from University of Michigan visited RCAI for the first University of Michigan-RCAI Joint Immunology Workshop held on December 1-2, 2011. This workshop was originally proposed by the University of Michigan in order to launch collaborative relationships with RCAI researchers. In the first session "T cell Signaling", Takashi Saito (RCAI) introduced his recent discovery of TCR signaling regulation by PD-1 microclusters. Hei-ichiro Udono (RCAI, photo 1) discussed the role of HSP90 in antigen cross-presentation, and Malini Raghavan (U of M, photo 2) discussed the functions of calreticulin in MHC class I folding. The session on "Mucosal Immunity" led to a hot discussion on commensal bacteria. Gabriel Nunez (U of M, photo 3), Naohiro Inohara (U of M, photo 4), Sidonia Fagarasan (RCAI), Hiroshi Ohno (RCAI) and Hilde Cheroutre (LIAI and RCAI, photo 5) found some differences in commensal bacteria in the same mouse strains, but it was difficult to explain the reasons. The last session of the first day was "Clinical Immunology". Sophie Paczesny (U of M, photo 6) described her studies to identify biomarkers for GVHD, Shin-ichiro Fujii (RCAI) explained his new allogeneic immunotherapy using cells loaded with NKT cell ligand, and Fumihiko Ishikawa (RCAI) introduced his anti-leukemic stem cell (LSC) therapeutic development using the humanized mouse model. The second day started with a "Lymphocyte Development" session. Wes Dunnick (U of M, photo 7) talked about class switch recombination regulated by chromosomal translocations, Hiroshi Kawamoto (RCAI) described a lineage check point during the development of T cells, and Ichiro Taniuchi (RCAI) and Hilde Cheroutre (LIAI and RCAI) discussed Thpok regulation of T cell fate. In the afternoon of the second day, each researcher from the University of Michigan held individual discussions with RCAI researchers with common interests. By the end of the workshop, all participants agreed to the continuation of communications and discussions for collaborations, and the organizers, Wes Dunnick and Haruhiko Koseki (Photo, 8) promised to make efforts for the next joint workshop, hopefully in Michigan.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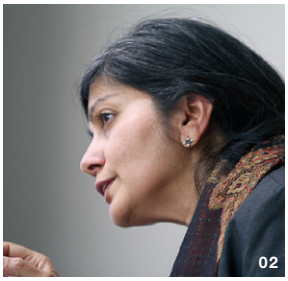
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Table: Program

Thursday, December 1

Opening remarks	Masaru Taniguchi, RCAI and Wesley Dunnick, University of Michigan
Session 1 : T cell Signaling	**Chair : Hiroshi Ohno**
Takashi Saito (RCAI)	Imaging of T cell activation regulation: -Negative regulation of TCR signaling by PD-1 microcluster.
Hei-ichiro Udono (RCAI)	Dendritic cells require HSP90 as a cytosolic translocator of extracellular antigen for cross-presentation
Malini Raghavan (University of Michigan)	Functions of calreticulin in MHC class I folding and antigen presentation
Session 2 : Inflammation	**Chair : Naohiro Inohara**
Takashi Tanaka (RCAI)	HSP70 is essential for PDLIM2-mediated termination of NF-κB signaling
Masato Tanaka (RCAI)	Immune regulation by dead cell clearance
Session 3 : Mucosal Immunity	**Chairs : Masato Tanaka & Hilde Cheroutre**
Gabriel Nunez (University of Michigan)	Role of Nod-like receptors and IL-1 signaling in intestinal immunity and host defense against enteric pathogens
Naohiro Inohara (University of Michigan)	The interaction between host immunity and commensals: A view from the bacterial side
Sidonia Fagarasan (RCAI)	Vitamin A-dependent transcriptional activation of NFATc1 is critical for the development and survival of B1 cells
Hiroshi Ohno (RCAI)	The epithelia-specific membrane trafficking factor AP-1B secures gut immune homeostasis in mice
Session 4 : Clinical Immunology	**Chairs : Malini Raghavan & Takashi Tanaka**
Sophie Paczesny (University of Michigan)	Discovery and validation of graft-versus-host disease biomarkers
Fumihiko Ishikawa (RCAI)	Understanding human immunity & diseases through a creation of humanized mouse model.
Shin-ichiro Fujii (RCAI)	Dendritic cell (DC) targeting immunotherapy by antigen mRNA transfected, allogeneic human cells loaded with NKT cell ligand as artificial adjuvant vector cells (aAVCs)

Friday, December 2

Session 5 : Lymphocyte Development	**Chairs : Takashi Saito & Wesley Dunnick**
Wesley Dunnick (University of Michigan)	Regulating class switch recombination and chromosomal translocations
Hiroshi Kawamoto (RCAI)	Lineage restriction process from hematopoietic stem cells to T cell progenitors: An essential developmental checkpoint for production of the T cell lineage.
Ichiro Taniuchi (RCAI)	Mechanisms of helper versus cytotoxic lineage choice
Hilde Cheroutre (LIAI / RCAI)	Silencing of Thpok drives plasticity of CD4 effector T cells in vivo

The 4th Joint RCAI-LIAI Workshop Program

The 4th Joint RCAI-LIAI workshop was jointly organized by the La Jolla Institute for Allergy & Immunology (LIAI) and RIKEN at LIAI on February 7-8. Nearly 100 participants, including Masaru Taniguchi, Director of RIKEN RCAI, and Mitchell Kronenberg, LIAI President and Chief Scientific Officer, attended the workshop.

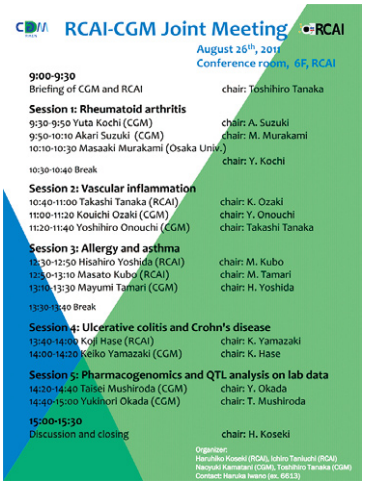
This workshop addressed current progress in six major areas, Th & Treg cells, Signaling in the immune system, Disease regulation, Antigen recognition by T cells & antibodies, and T cell development & memory. Eighteen leading scientists in these fields from the two institutes presented their recent data. Over the course of the workshop, the participants actively discussed these topics, and this opportunity facilitated further collaboration between RCAI and LIAI researchers.

Both RCAI and LIAI were established with the goals of making breakthroughs in our understanding of the immune system and improving human health through the development of treatments and cures for immune system disorders. Past collaborations resulted in the creation of a new research unit (by Hilde Cheroutre) in RCAI in 2011. The 2011 workshop also resulted in a few new collaborations. Future collaborations between the two research institutes will help in achieving their common goals.

RCAI-CGM collaboration is launched; toward integrative medical sciences

RIKEN RCAI and RIKEN Center for Genomic Medicine (CGM) launched a collaboration to link their two research fields; RCAI's mechanistic research using mice and other experimental models and CGM's research on human disease susceptibility genes. In this collaborative scheme for the analysis of common human immunological disorders, RCAI will work closely with CGM and plans to collect and integrate the data from genetically modified mice with clinical data accumulated at CGM. To discuss the practicalities of how to integrate these similar but different research fields, a kick-off meeting was held on Aug 26, 2011 (photo 1-8). Researchers from both centers introduced their themes, approaches and unpublished results to promote discussions.

At the end of the meeting, the researchers agreed to focus on five different themes and each working group will start concrete collabora-



tions; 1) Rheumatoid arthritis and autoimmune diseases, 2) Vasculitis and vascular diseases, 3) Allergy, 4) Colitis and 5) Pharmacogenetics, QTL and other diseases.

During the fall and winter of 2011, each working group continued discussions and started collaborative analysis. Based on their mouse experiments, RCAI researchers suggested candidate genes for diseases in humans, and CGM researchers suggested their candidate genes for functional analysis using genetically modified mice. In addition to these discussions and analysis, a series of joint seminars was started based on requests from researchers. For the first one, held on Jan 24, Dr. Katsushi Tokunaga (The University of Tokyo, photo 9) and Dr. Yuta Kochi (CGM, photo 10) were invited to talk about variations of HLA and diseases. For the second seminar on Feb. 21, Dr. Shigeharu Wakana (RIKEN BioResource Center) was invited to talk about the International Mouse Phenotyping Consortium, their standardized analysis and pipelines. The RCAI-CGM collaboration will continue to expand in order to reach toward the goal of establishing integrative medical sciences.

01 Naoyuki Kamatani (CGM)

02 Michiaki Kubo (CGM)

03 Masaru Taniguci (RCAI)

04 Toshihiro Tanaka (CGM)

05 Masaaki Murakami (Osaka Univ.)

06 Haruhiko Koseki (RCAI)

07 Toshitada Takemori (RCAI)

08 Kazuhiko Yamamoto (CGM)

09 Katsushi Tokunaga (Univ. Tokyo)

10 Yuta Kochi (CGM)

RCAI 10th Anniversary Seminar Series

In 2011, RCAI held a series of guest seminars to enhance communication with outside researchers, because the Center's major external events had been canceled after the Great East Japan Earthquake. A total of 35 active researchers (23 from Japan and 12 from abroad), especially young ones, were selected and invited from various fields.

Table : RCAI 10th Anniversary Seminar Series

Date	Speaker	Affiliation	Country	Title
2011.7.29	**Masahiro Yamamoto**	Department of Microbiology and Immunology, Graduate School of Medicine, Osaka University	Japan	**Exploration of Host-Parasite Interface Possibly Reveals a New Aspect of Immunology**
2011.8.2	**Yumi Matsuzaki**	Keio University, School of Medicine, Center of Integrated Medical Research	Japan	**Donor mesenchymal stem cells trigger chronic graft-versus-host disease following minor antigen mismatched bone marrow transplantation**
2011.8.4	**Richard Jones**	The University of Chicago	USA	**Proteinomic analysis of cell signaling networks with micro-western arrays**
2011.8.9	**Jun Seita**	Institute for Stem Cell Biology & Regenerative Medicine, and Department of Pathology, Stanford University School of Medicine	USA	**Systems Approach to Hematopoietic Stem/ Progenitor Cell Biology**
2011.8.30	**Taro Kawai**	Immunology Frontier Research Center, Osaka University	Japan	**Signaling pathways activated by innate receptors and their roles in host defense**
2011.9.6	**Kiichiro Yano**	Cardiovascular-Metabolic Laboratories, Daiichi-Sankyo Co., Ltd	Japan	**Role of Crosstalk between Adipocytes and Endothelium in Sepsis**
2011.9.13	**Kazuyo Moro**	Keio University School of Medicine, Department of Microbiology and Immunology	Japan	**Natural helper cells play a critical role in IL-33-dependent eosinophilia in the lung.**
2011.9.29	**Tomoya Katakai**	Department of Molecular Genetics, Institute of Biomedical Science, Kansai Medical University	Japan	**High-speed interstitial T cell migration in lymph node involves LFA-1-dependent and -independent mechanisms controlled by stromal cell network**
2011.10.7	**Taro Fukao**	Max-Planck Institute of Immunobiology and Epigenetics	Germany	**MicroRNA Regulation of Energy Metabolism in Mammalian Immune System**
2011.10.11	**J. Rodrigo Mora**	Massachusetts General Hospital & Harvard Medical School	USA	**Retinoic acid and gut-homing T cells in intestinal immune homeostasis**
2011.10.13	**Takahiro Maeda**	Department of Medicine, Harvard Medical School, Brigham and Women's Hospital	USA	**LRF Transcription Factor Maintains HSC Homeostasis by Preventing Lymphoid-Primed LT-HSCs from Excessive Differentiation**
2011.10.19	**Shinobu Saijo**	Medical Mycology Research Center, Chiba University	Japan	**C-type lectins: their roles in the host defense against fungal infection**
2011.10.25	**Atsushi Mizoguchi**	Harvard Medical School, Massachusetts General Hospital	USA	**Regulatory B cells, lessons from the model of IBD**
2011.11.2	**Taishin Akiyama**	The Institute of Medical Science, The University of Tokyo	Japan	**Molecular mechanisms required for thymic self-tolerance**
2011.11.16	**Makoto Arita**	Graduate School of Pharmaceutical Sciences, The University of Tokyo	Japan	**Mediator Lipidomics in Inflammation Research**
2011.11.21	**Takeshi Egawa**	Department of Pathology and Immunology Washington University School of Medicine	USA	**Transcription factors in cytotoxic T cell development**

Taro Fukao

J. Rodrigo Mora

Shinobu Saijo

Makoto Arita

Hidehiro Fukuyama

2011.12.8	**Hidehiro Fukuyama**	Institute of Molecular and Cellular Biology, CNRS	France	**Drosophila models to study chronic inflammation**
2011.12.8	**Kosuke Yusa**	Wellcome Trust Sanger Institute	Japan	**Targeted gene correction of α1-antitrypsin deficiency in induced pluripotent stem cells**
2011.12.12	**Takeshi Nitta**	National Center for Global Health and Medicine	Japan	**Revisiting 'Thymic Nurse Cell': Unique lympho-epithelial complexes in the thymic cortex**
2011.12.16	**Kohsuke Imai**	Department of Community Pediatrics, Perinatal and Maternal Medicine Tokyo Medical and Dental University	Japan	**Primary immunodeficiency in Japan**
2011.12.22	**Kenya Honda**	Department of Immunology, Graduate School of Medicine, The University of Tokyo	Japan	**Intestinal microbiota shapes the immune system**
2011.12.26	**Takashi Yamamura**	Department of Immunology, National Institute of Neuroscience, NCNP	Japan	**Central nervous system autoimmunity and gut flora**
2012.1.19	**Matthias Hebrok**	UCSF Diabetes Center	USA	**Deciphering pancreas development and diseases**
2012.1.25	**Mark Shlomchik**	Yale University	USA	**Germinal Center Selection and the Development of Memory B and Plasma Cells**
2012.1.31	**Takako Hirata**	Kyoto University Graduate School of Medicine, Oxford University	Japan	**The role of the ERM protein moesin in lymphocyte homeostasis**
2012.2.13	**Rob Klose**	Department of Biochemistry, University of Oxford	UK	**Interpreting the CpG island signal**
2012.2.14	**Tomohiro Sawa**	Department of Microbiology, Graduate School of Medical Sciences, Kumamoto University	Japan	**Signaling functions of reactive oxygen species and electrophiles: regulatory mechanisms and implications in chronic inflammation-associated diseases**
2012.2.20	**Sudhir Gupta**	University of California, Irvine	USA	**Paradox of Immunodeficiency and Inflammation & Autoimmunity in Human Aging**
2012.2.20	**Jun Kunisawa**	Division of Mucosal Immunology, Institute of Medical Science, The University of Tokyo	Japan	**Lipid, vitamin, and nucleotide in the regulation of gut immunity**
2012.2.23	**Rudi Balling**	Director, Luxembourg Centre for systems Biomedicine (LCSB), University of Luxembourg	Germany	**A systems biology approach to Parkinson´s disease: Research at the new Luxembourg Centre of Systems Biomedicine**
2012.2.23	**Yoko Hamazaki**	Department of Immunology and Cell Biology, Graduate School of Medicine, Kyoto University	Japan	**Identification of claudin-expressing medullary thymic epithelial stem cells that maintain the functional medulla during life**
2012.2.28	**Kumar Selvarajoo**	Institute for Advanced Biosciences, Keio University	Japan	**Unraveling governing principles from immune cell dynamics**
2012.2.28	**Yoshinori Fukui**	Medical Institute of Bioregulation, Kyushu University	Japan	**Immune regulatory functions of DOCK family proteins in health and disease**
2012.3.8	**Yoshiko Mito**	Massachusetts General Hospital/Harvard Medical School	USA	**Allele-specific profiling of genome-wide epigenetic marks and sequence polymorphisms**
2012.3.23	**Toshiyuki Takai**	Institute of Development, Aging and Cancer, Tohoku University	Japan	**Regulatory effects of IgG on B cell functions**

Kosuke Yusa

Kenya Honda

Masahiro Yamamoto

Jun Seita

Kazuyo Moro

Multidisciplinary Research Projects

With the aim of developing new paradigms in immunology, RCAI launched a program in 2009 to support a limited number of multidisciplinary collaborative projects led by RCAI researchers. The program provides 5-10 million JPY/year to each collaborative research project for up to five years. In FY2011, eight projects were selected to receive this support.

Table : Awardees of RCAI Multidisciplinary Research Projects 2011

Year	Name	Project Title	Collaborators
2009-	**Hiroshi Ohno**	Genomic, transcriptomic and metabolomic analysis of human ulcerous colitis	**Mamoru Watanabe** (Tokyo Medical and Dental Univ.) **Masahira Hattori** (The Univ. of Tokyo) **Jun Kikuchi** (RIKEN Plant Science Center)
2009-	**Hiroshi Kawamoto** **Haruhiko Koseki** **Fumihiko Ishikawa** **Masato Tanaka** **Ichiro Taniuchi** **Osamu Ohara** **Toshitada Takemori** **Masaru Taniguchi**	Creation of artificial immune cells	**Harukazu Suzuki** **Jun Kawai** **Yoshihide Hayashizaki** **Carsten O. Daub** **Piero Carninci** (RIKEN Omics Science Center)
2009-	**Satoshi Ishido**	Modeling of antigen presentation	**Yuji Sugita** (RIKEN Advanced Science Institute)
2009-	**Hisaaki Shinohara**	Modeling of NF-κB activation signals	**Mariko Hatakeyama** (RIKEN Advanced Science Institute)
2009-	**Ichiro Taniuchi**	Regulation of gene expressions for thymic development	**Atsushi Mochizuki** (RIKEN Advanced Science Institute)
2010-	**Tsuneyasu Kaisho**	Generation of antibodies against chemokine receptor XCR1 and elucidation of its crystal structure	**Shigeyuki Yokoyama** **Tomomi Someya** (RIKEN Systems and Structural Biology Center)
2010-	**Haruhiko Koseki**	Elucidation of the lymphocyte differentiation mediated by alternative splicing	**Yoshinori Naoe** (National Center for Geriatrics and Gerontology)
2010-	**Takashi Saito**	Elucidation of the structure and function of the immune receptor complex	**Shigeyuki Yokoyama** **Mikako Shirouzu** (RIKEN Systems and Structural Biology Center)

Student Exchange Programs

In 2011, RCAI's International Summer Program (RISP) started two student exchange programs with immunology schools in Germany; Spring School held by Deutsche Gesellschaft für Immunologie (DGFI) and Summer School held by Zentrum für Infektioinsbiologie und Immunität (ZIBI). Although RISP 2011 was canceled and RCAI could not accept students from Germany, RCAI sent three students to Germany. Hisashi Wada, a master course student in the Laboratory for Transcriptional Regulation, participated in DGFI Spring School in Ettal (photo 1). "I can never forget the experience. In a small lecture room in a monastery, sixty students and lecturers from all over Europe had so much discussions, even until midnight," said Wada. Two postdocs, Shimpei Kawamoto, Laboratory for Mucosal Immunity and Shinji Fukuda, Laboratory for Epithelial Immunobiology participated in the ZIBI Summer School held in Berlin (photo 2). "There were 40 participants from 16 countries in the world. Although the course was targeting mainly to master course students and was a little too basic for me, I enjoyed hands-on experiments using nematode," said Fukuda. RCAI is planning to accept students from Germany in RISP 2012.

photo 1 : Monastery in Ettal where the DGFI Spring School was held

photo 2 : Group photo of ZIBI Summer School

International Research Collaboration Award

The RCAI International Research Collaboration Award is a unique program supporting researchers outside of Japan in setting up semi-independent research units within the laboratory of their collaboration partner at the Center. The program provides up to 10 million JPY/year to each collaborative research project for up to three years. (Until 2007, the program awarded 15 million JPY/year but the amount was reduced in 2008.)

Since the program began in 2004, 14 projects have been funded (Table) and the collaborations have resulted in several important papers. Drs. Vidal and Koseki's collaborative project on Ring1 genes resulted in 5 papers, *Dev. Cell* (2004), *Development* (2006), *Nat. Cell. Biol.* (2007), *Mol. Cell. Biol.* (2008) and *Development* (2008). Drs. Dustin and Saito published their work on T cell microclusters in *Nat. Immunol.* (2005) and *Immunity* (2006). Drs. Ewijk and Kawamoto's work on thymic progenitor cells resulted in three papers in *Development* (2006) and *Mol. Immunol.* (2009 and 2010). Drs. Bix and Kubo published their work on the IL4 repressor in *Nat. Immunol* (2009), and Drs. Ellemeier and Taniuchi published their studies on CD8 gene regulation in *Proc. Natl. Acad. Sci* (2011) and on CD4/CD8 lineage choice in *Nat. Immunol.* (2010).

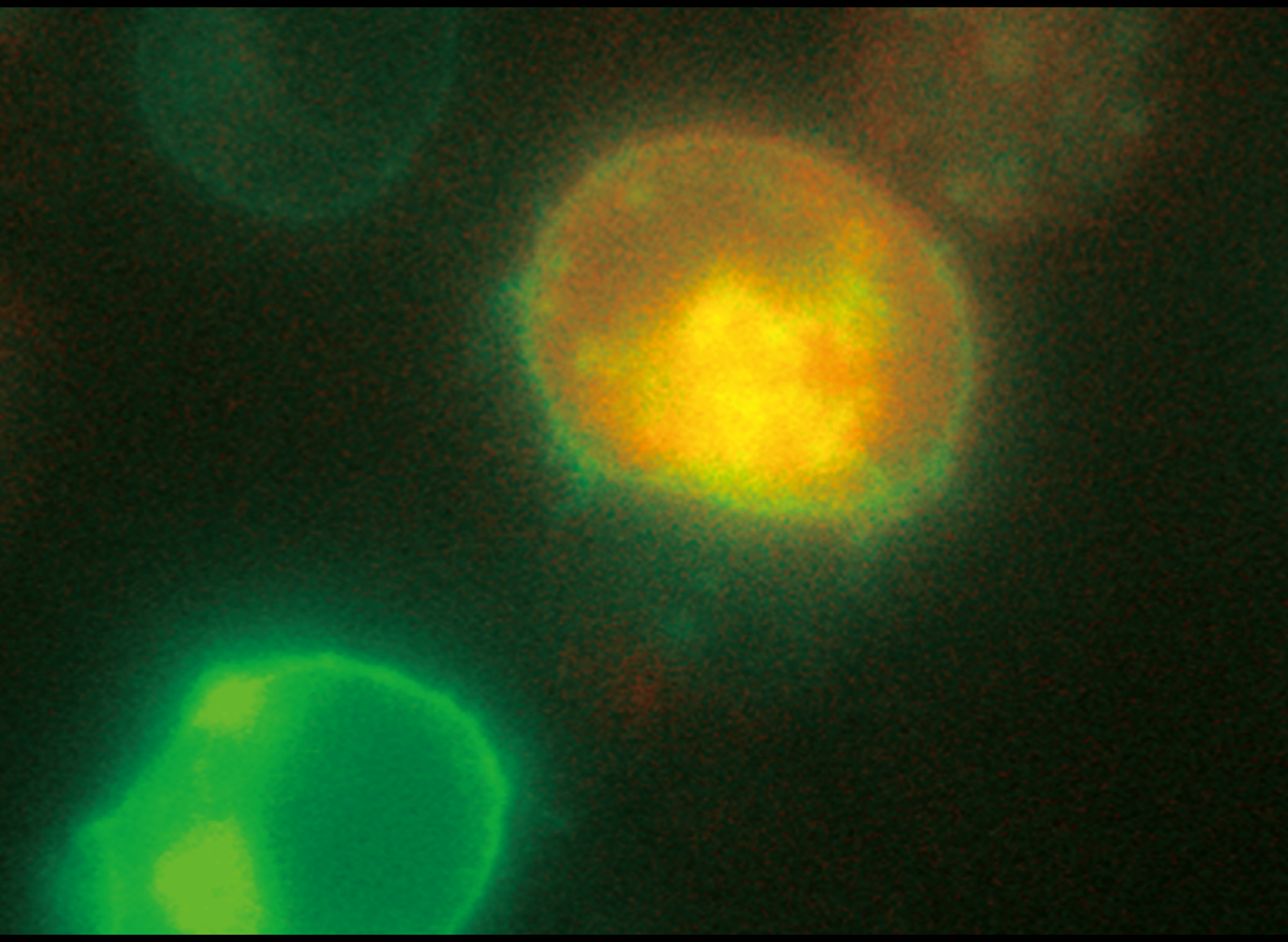
Table : Awardees of RCAI International Collaboration Award Program

Year	Host Lab.	Title of Research	Awardee
2004-2006	**Takashi Saito**	Analysis of dynamism and function of immunological synapse using planar membrane and knock-in T cells	**Dr. Michael DUSTIN** New York University School of Medicine
2004-2006	**Hiroshi Kawamoto**	Regulatory role of lymphoid progenitors during development of thymic microenvironments	**Dr. Willem van EWIJK** Leiden University Medical Center
2004-2006	**Haruhiko Koseki**	Genomic and functional analysis of the role of the Polycomb Ring1 genes in B-cell development	**Dr. Miguel VIDAL** Centro de Investigaciones Biologicas, CSIC
2004-2005	**Masaru Taniguchi**	Role of NKT cells in TSLP-mediated allergic inflammation	**Dr. Steven ZIEGLER** Benaroya Research Institute at Virginia Mason Medical Center
2004-2006	**Ji-Yang Wang**	Expression and function of *FcRY*-a novel Fc receptor-related gene expressed in B cells	**Dr. Peter BURROWS** University of Alabama at Birmingham
2005-2007	**Ichiro Taniuchi**	Study of T cell differentiation mediated by regulated expression of CD8 genes	**Dr. Wilfried ELLEMEIER** Institute of Immunology, Medical University Vienna **Dr. Hilde CHEROUTRE** La Jolla Institute for Allergy and Immunology
2005-2007	**Masato Kubo**	Understanding genetic regulation of interleukin 4 production by a CD4(+) T cell-intrinsic mechanism.	**Dr. Mark BIX** University of Washington, Seattle, Washington
2005-2006	**Yasuyuki Ishii**	Gene-array analysis and proteomics of Th2 tolerance	**Dr. Yun-Cai LIU** La Jolla Institute for Allergy and Immunology
2005-2007	**Osami Kanagawa**	Visualization of STAT protein in the cytokine mediated signaling at a single molecular level.	**Dr. Kenneth MURPHY** Howard Hughes Medical Institute Washington University School of Medicine
2005-2007	**Tomohiro Kurosaki**	Role of signaling molecules in B cell synapse formation and its maintenance	**Dr. Facundo BATISTA** Cancer Research UK London
2006-2008	**Masato Tanaka**	Identification of Novel Necrotic Molecules from Necrotic Hepatocytes and Examination of Its Effect on the Inflammatory Response	**Dr. Sunhwa KIM and Dr. Michael KARIN** Department of Pharmacology, Univ. of California, San Diego, USA
2007-2008	**Takeshi Watanabe**	A study on the spleen and lymph nodes mesenchymal cells that participate in the assembly of artificial secondary lymphoid organs	**Dr. Andrea BRENDOLAN** Cornell University Medical Center, Department of Cell and Developmental Biology
2007-2009	**Ichiro Taniuchi**	Understanding of tumor suppressive mechanism of Runx complexes against leukemia and gastrointestinal cancer	**Dr. Motomi OSATO and Dr. Yoshiaki ITO** Institute of Molecular and Cell Biology, National University of Singapore
2007-2009	**Sidonia Fagarasan**	Nuclear reprogramming of terminally differentiated plasma cells to study the specific role of IgA in mucosal and systemic immunity and B cell development	**Dr. Stefano CASOLA** IFOM-The FIRC Institute of Molecular Oncology Foundation, Milano, Italy
2010-	**Mariko Okada-Hatakeyama**	Proteomics based-quantitative analysis of signal-transcriptional network	**Dr. Boris KHOLODENKO** University College Dublin, Ireland **Dr. Richard JONES** University of Chicago, USA
2011-	**Ichiro Taniuchi**	Understanding and Engineering of Dendritic Epidermal T Cells (DETC) development	**Dr. Florent GINHOUX** Singapore Immunology Network (SIgN), Singapore

2011

Part 5

Outreach Activities



RIKEN Yokohama Open Campus

Oct 8, 2011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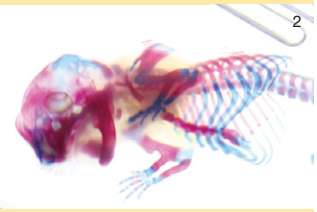
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The RIKEN Yokohama Institute Open Campus was held on Oct. 8, 2011. There were 1,900 visitors ranging in age from children to retired folks.

On Oct. 3, only 5 days before the event, there was an announcement of this year's Nobel Prize for Physiology and Medicine, given to three immunologists, Drs. Bruce A. Beutler, Jules A. Hoffman and Ralph Steinman. There was great excitement that Dr. Steinman, an RCAI Advisory Council member, had received the prize for his discovery of the dendritic cell, but it soon turned to great sorrow after we learned that he had passed away three days previously. RCAI's Hiroshi Kawamoto, together with Shinichiro Fujii, Tsuneyasu Kaisho and Takashi Tanaka prepared posters, leaflets and a model of the dendritic cell to introduce the discoveries of the Nobel laureates: activation of innate immunity and the role of the dendritic cell in adaptive immunity.

Twenty RCAI teams exhibited posters or movies. The highlight of the Open Campus was hands-on learning. Kawamoto and the team members arranged an experiment to isolate $CD4^+CD8^+$ T cells from mice (photo 1). "For this rather precise experiment, we can only accept 30 people who are high school students or above. Still, I think it offers various experiences in one opportunity; a brief immunology lecture, mouse dissection, sophisticated cell sorting, laboratory atmosphere, communication with researchers... participants can feel what we actually do everyday," said Kawamoto.

Yoshida and his team prepared stained samples of fetal mice and newborn mice at various developmental stages (photo 2). Two elementary school girls had lined up in front of the microscopes before the samples weren't even ready. "We were waiting for this for a year. When we came last year, they were amazing, so we came back to take photos," they said.

This annual event, originally planned in July, was rescheduled due to the urgent need to save electricity this summer because of the massive East Japan Earthquake. When the summer heat passed by and the government lifted its electricity saving policy on September 9, various events were begun in many places. "This Open Campus ultimately overlapped with a Sports Festival of the local school, but still many people visited us. We have to appreciate that people maintain their interest in our science," a RIKEN staff member commented.

Immunology Workshop for High School Students

November 1, 2011


1


2

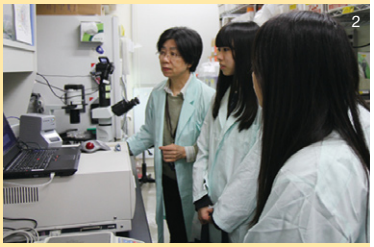
Seventeen students of Kanagawa Sohgoh High School visited RCAI on November 1, 2011. The workshop started with lectures by Dr. Ishikawa and Dr. Kawamoto. In his lecture, Dr. Ishikawa (photo 1) told of his own experiences in high school, medical school, and hospitals in Japan and the US; what he thought and why he chose to be a leukemia researcher. The young students felt empathy with his story and his strong passion for leukemia research (photo 2). "I am very impressed by his message, 'being pure' to myself. I can think more positively now about my future," one student said. "I was almost giving up my dream to go to medical school, but I will rethink about it. This was a valuable opportunity to listen to someone from a clinical field." Then, Dr. Kawamoto explained some very basic immunology using his original cartoons, and took the students to his own laboratory for hands-on practice (photo 3). With the help of his laboratory members, students dissected mice and compared CD4/CD8 pattern of lymphocytes from spleen and thymus using FACS. "It was my first time to do a dissection. It looked the same as the anatomy figures in textbooks," one student said. "I liked the cartoons. They were helpful to understand the principles of immunology. I wish our biology classes were like this," another said. The high school teacher said this kind of opportunity is valuable for young students and he hoped RIKEN and RCAI would continue to organize the immunology workshop for high school students.


3

Lab visit by junior high school and high school students

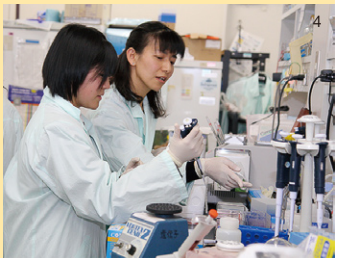
December 16, 2011


1


2

Twenty-six students of Shinagawa Joshi Gakuin, a women's junior and senior high school, visited RIKEN Yokohama Institute. Ms. Hanae Fujimoto, a technical scientist in the FACS laboratory explained the mechanisms of the cell sorter and demonstrated the lymphocyte isolation process (photo 1), and then three female RCAI researchers, Drs. Kumiko Sakata-Sogawa (photo 2), Mari Hoshino (photo 3) and Reiko Onishi (photo 4) took 3-4 students each for laboratory visits and discussions. Taking students to their own laboratories, they explained what kind of work immunology researchers actually do, what the environment of a research institute is like, why they decided to become a researcher, and women's work-life balance. "I am interested in biology, so it was a good opportunity to visit a research institute, although I still don't have a clear vision for the future," one high school student said.


3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Science Café

"Regulation of the cell fate"

March 3, 2012

On March 3, 2012, Ichiro Taniuchi, Group Director of Laboratory for Transcriptional Regulation, talked at the Science Café held at Kanagawa Prefectural Kawasaki Library. Among 170 applications, 50 participants were selected by a random drawing. Taniuchi's talk started with various sensory organs. We are familiar with the eye, skin, nose or ear, but "What about immunity? Where is it? What does it sense?" Dr. Taniuchi explained various immune cells, how they sense pathogenic organisms, memorize and prevent. Then he explained how immune cells are developed. Using various illustrations, he talked about T cell selection in thymus, where functional selection (positive selection) and autoreactive selection (negative selection) occur. Finally he talked about transcriptional regulation of cell fate during their development. Using Yoda in Star Wars to illustrate the concept, he explained how a master transcription factor, ThPOK, regulates the cell fate decision to become a helper or a killer T cell. "My image of researchers was a kind of isolated people, but I am surprised that he was frank but respectful to anyone. He was trying to explain easily to any of the questions," a participant said. "There were many good questions from the participants. Still so many questions exist in the world that even researchers cannot answer. I hope this can be one of the occasions that general people start to find and think of scientific questions in their everyday life," said Dr. Taniuchi.

Science Café

"Is calico cat's pattern inheritable?"

March 14, 2012

On March 14, Dr. Haruhiko Koseki, Group Director of the Laboratory for Developmental Genetics, talked at RIKEN Yokohama Science Café held at Yokohama City Central Library. Thirty-four people, including two junior high school students attended the event. The talk started with the genetics of calico cats. In cats, each of the genes encoding orange fur or black fur is linked with X chromosome. Only when the cat has two X chromosomes (XX), one with the orange fur gene and the other with the black fur gene, the cat becomes calico by mosaicism. Thus, 99.9% of calico cats are female, Dr. Koseki explained. Then, will the pattern be the same if the genes are identical? Dr. Koseki introduced a result of the cloning of a calico cat. Because one of the X chromosomes was already inactivated in the somatic cell used for cloning, the fur color turned out to be different; the cloned cat was a brown tabby. This kind of functionally relevant modification of the genome is called epigenetics. "Definitely, the experience you had in your life, starving or life habit for example, is imprinted into your genome, and it can be inherited," said Dr. Koseki. After his talk, participants wrote down questions on Post-it notes and Dr. Koseki answered them. "I like cats and that was why I came here, but realized that genomic and epigenetic systems control the cat's fur coat pattern," a participant said.

Science Café

"Development of a pollinosis vaccine and a novel cancer therapy"

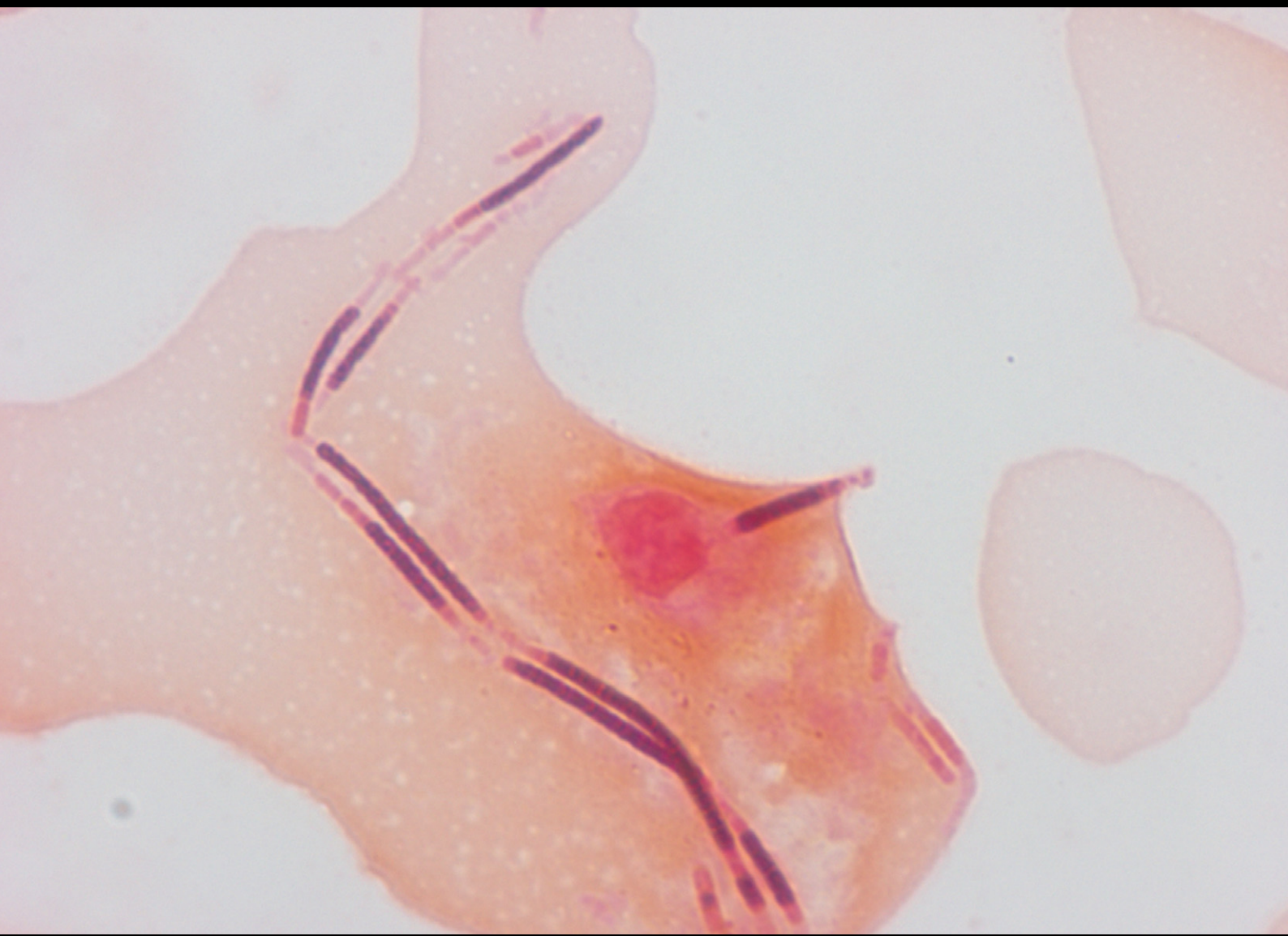
March 24, 2012

Dr. Taniguchi, Director of RCAI and Group Director of the Laboratory for Immune Regulation, talked at Science Café held at Yokohama City Library on March 24. His talk consisted of two parts, development of a pollinosis vaccine and development of a novel cancer therapy. As many as 1/3 of the Japanese population suffers from allergy, and because March is the cedar pollinosis season in Japan, various people from teenagers to retired folks aged over 70 participated in the event. Dr. Taniguchi started his talk from an epidemiologic perspective of pollinosis. It is said that multiple factors such as hygienic environment, use of antibiotics, smog emission, diesel fumes, house dust, etc. correlate with induction of allergy. Then he explained immunological mechanisms and the discovery of IgE by Dr. Kimishige Ishizaka. The vaccine being developed at RCAI is designed to promote the suppression of Th2 and B cells by activation of NKT and Th1 cells. He emphasized the importance of safety for the development of allergy vaccines. In the second half, he talked about NKT cell targeted therapy for lung cancer patients, and generation of NKT cells from iPS cells for therapeutic purposes. During the event, questions from the audience were at a surprisingly high level. One man mentioned a recent newspaper article on Histamine Releasing Factor by Dr. Kawakami of LIAI and the structural analysis of a histamine receptor by Dr. Iwata of Kyoto University. He asked if these results would be useful for the development of therapies. "I am impressed by the audience. They must have been studying a lot, although they might not be researchers," said Dr. Taniguchi.

2011

Part 6

Laboratory Activities



Laboratory for Developmental Genetics

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Technical Staff : **Tamie Endo**, **Naoko Ônaga**, **Rie Suzuki**, **Kayoko Katsuyama**, **Yôko Koseki**, **Kaoru Kondô**, **Mami Kumon**, **Fuyuko Kezuka**, **Raul Eduardo Vizcardo Sakoda**

The Developmental Genetics Research Group fulfills a dual role within RCAI. A large proportion of the manpower and financial resources of the group is devoted to the maintenance of a high-standard mouse facility. Through the Animal Core Facility, the group is also responsible for the generation of knock-out and transgenic animals for the various research laboratories at the center. At the same time, the laboratory is pursuing a research program to elucidate the molecular mechanisms underlying organ development and stem cell functions. Particular emphasis has been put on epigenetic regulation mediated by combinatorial actions of Polycomb group (PcG) gene products and DNA methylation mechanisms in development and stem cell functions.

The role of epigenetic regulators during development and differentiation

Two distinct Polycomb complexes, PRC1 and PRC2, collaborate to maintain epigenetic repression of key developmental loci in embryonic stem cells (ESCs). PRC1 and PRC2 have histone modifying activities, catalyzing mono-ubiquitination of histone H2A (H2AK119u1) and trimethylation of H3 lysine 27 (H3K27me3) respectively. However, the interplay of these modifications and their contribution to Polycomb repression remains not fully understood. We could show that high levels of H2AK119u1 deposition occur at a subset of target loci that are critical for ESC maintenance. We further demonstrated that H2AK119u1 deposition occurs at these targets in PRC2-deficient ESCs, albeit at lower levels, suggesting the presence of H3K27me3-independent compensatory mechanisms for recruitment of PRC1 function. Finally, we showed that the H2A ubiquitination activity of PRC1 is essential for target gene repression and ESC maintenance and that distinct PRC1 functions mediate chromatin compaction and contribute to silencing activity, most notably at *Hox* loci. Based on these results, we have proposed that PRC1 utilizes these diverse sensing and effector mechanisms, which provide a means to maintain a repressive state that is robust yet highly responsive to developmental cues during ES cell self-renewal and differentiation.

Although mechanistic aspects of PcG proteins in ESCs are being elucidated, it is still unknown how PcG proteins regulate differentiation and patterning processes in embryonic tissues or differentiating cells. To tackle this issue, we tested the role of PRC1 on limb organogenesis by depleting Ring1A and/or Ring1B, which are essential E3 ligases for H2A monoubiquitination, in a limb bud-specific manner. In

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2. Watarai H, Fujii S, Yamada D, Rybouchkin A, Sakata S, Nagata Y, Iida-Kobayashi M, Sekine-Kondo E, Shimizu K, Shozaki Y, Sharif J, Matsuda M, Mochiduki S, Hasegawa T, Kitahara G, Endo TA, Toyoda T, Ohara O, Harigaya K, Koseki H, Taniguchi M. Murine induced pluripotent stem cells can be derived from and differentiate into natural killer T cells. ***J Clin Invest.***120, 2610-2618 (2010)

3. Li X, Isono KI, Yamada D, Endo TA, Endoh M, Shinga J, Mizutani-Koseki Y, Otte AP, Casanova M, Kitamura H, Kamijo T, Sharif J, Ohara O, Toyada T, Bernstein BE, Brockdorff N, Koseki H. Mammalian Polycomblike Pcl2/Mtf2 is a novel regulatory component of PRC2 that can differentially modulate Polycomb activity at both the Hox gene cluster and at Cdkn2a genes. ***Mol Cell Biol.*** 31, 351-364 (2011)

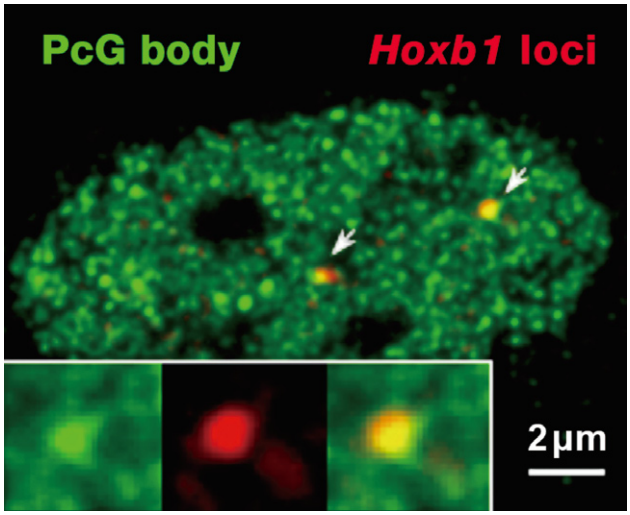

Figure: **PcG bodies in the *Hoxb1* regions.** PcG bodies (*green*) are immunostained in a speckled pattern. The *Hoxb1* loci (*red*) are detected by DNA-FISH.

Ring1B single KO mice, we found shortening of the radius and ulna, suggesting that PRC1 defects predominantly affect distal limb development. *Ring1A/B* double KO mice exhibited severe limb truncation. To identify target genes for PRC1, we used microarray analysis for *Ring1A/B* double KO limbs and ChIP-Chip analysis for binding of Ring1B in developing limb buds. We found the involvement of PRC1 in repressing the expression of transcription factors that demarcate the proximal region of the limb in the distal regions. Interestingly, the expression of these genes is also under the control of retinoic acid (RA) signals suggesting functional antagonism between RA signals and PRC1 for specification of the distal region of limb buds. Consistent with this hypothesis, RA significantly affected Ring1B binding to these target genes and exaggerated distal limb defects in *Ring1B* single KO mice. We thus propose a role for PRC1 in mediating RA signals to generate the distal part of limbs.

Regulation of large scale chromatin structures by epigenetic regulators

Accumulating evidence documents a role for PcG proteins in regulating higher order chromatin structures, but the mechanisms and impact of such structures on transcriptional regulation remain obscure. In this study, we identified PcG bodies in mouse primary fibroblasts as distinct foci at which PRC1 and H3K27me3 are colocalized and canonical PcG target genes are condensed (Figure). We found that PcG body formation requires Phc2-SAM polymerization, which critically contributes to condensation and repression of PcG target genes. We further show that Phc2-SAM polymerization limits the dynamic nature of PRC1, and thereby promotes stable association of PRC1 with PcG target genes. Our findings suggest a novel model by which SAM polymerization of Phc2 modulates the structural organization of PcG complexes to enable robust yet reversible PcG-mediated repression during development. Since *Hox* gene clusters are unusual in retaining a special genomic configuration, we went on to test whether PRC1 also uses this mechanism at solitary target genes. Intriguingly, by re-examining ChIP-seq data for Ring1B distribution in ESCs, we found that PRC1 is distributed not only to promoter regions but also to genomic regions surrounding stop codons of repressed target genes. We hypothesized that promoter regions are associated with 3' regions of their respective targets. We focused on the *Meis2* gene, which occupies a large genomic segment spanning around 200Kb in mammals, and which has a complex expression patterns during development. We used 3C (chromatin conformation capture) and FISH and immuno-FISH histological sections to analyze the interaction of the promoter and the 3'-PcG binding sequence or midbrain specific enhancer region, which was used as a reference. We found that the 3'-PcG binding sequence locates in close proximity to the promoter within cells that do not express *Meis2* and both regions associate with a single PcG body. By using *Ring1A/B* dKO mice, we could also show that this interaction is dependent on PRC1. Here we saw no association of midbrain-specific enhancer with the promoter region. By contrast, the midbrain-specific enhancer comes close to the promoter in the midbrain region, in which *Meis2* is strongly expressed, while the 3'-PcG binding sequence does not. These results suggested that PRC1-mediated regulation of higher chromatin structures is also used to repress solitary target genes, likely by using another PRC1 binding sequence that frequently localizes around stop codons.

4. Takada Y, Naruse C, Costa Y, Shirakawa T, Tachibana M, Sharif J, Kezuka-Shiotani F, Kakiuchi D, Masumoto H, Shinkai Y, Ohbo K, Peters AH, Turner JM, Asano M, Koseki H. HP1γ links histone methylation marks to meiotic synapsis in mice. ***Development*** 138, 4207-17 (2011)

5. Sharif J, Endoh M, Koseki H. Epigenetic memory meets G2/M: to remember or to forget? ***Dev Cell.*** 20, 5-6 (2011)

Laboratory for
Lymphocyte Development

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Student Trainees : **Raul Sakoda**

Visiting Scientists : **Nagahiro Minato, Yoshimoto Katsura, Toshio Kitamura, Takeshi Watanabe**

The major aim of our team is to elucidate the molecular mechanisms that regulate cell fate decisions in the process of lineage restriction from multipotent hematopoietic stem cells to unipotent progenitors. A series of studies from our laboratory on early hematopoiesis have led to a fundamental redefinition of lymphoid progenitors as well as the ontogeny and phylogeny of T- and B-cell development. We thus have proposed our new model of hematopoiesis, the myeloid-based model, in which myeloid potential is retained along the specification pathways towards erythroid, T, and B cell lineages.

Development of an arrest-restart operation system of cell-differentiation capable of manipulating lineage commitment of hematopoietic progenitors

The ideal experimental system for studies on the mechanisms of lineage commitment and differentiation would be one in which commitment and differentiation can be synchronously controlled. We succeeded in establishing several such systems in which the arrest and restart of development of progenitors can be induced at a stage prior to the critical step of early T cell or B cell development. The merits of these systems in terms of basic research are (i) cells are homogenous, (ii) cells are normal (unlike immortalized cell lines), (iii) time course analyses can be performed, (iv) a large number of cells are available, making it possible to analyze genome-wide gene expression profiles and epigenetic status and to do biochemical studies. Another potentially very important advantage is that these systems may become applicable in clinical settings as a method to expand human hematopoietic progenitors.

We recently applied one of such systems for the study of the mechanisms of the T cell lineage determination step, which is where T cell progenitors have terminated all non-T lineage potential and are fully committed to T cell lineage. We have previously determined that the early intrathymic T cell progenitors retain myeloid potential after terminating B cell potential (Wada et al, *Nature*, 2008), and that the T cell lineage determination step takes place at the midst of the so-called double negative (DN) 2 stage of intrathymic T cell development (Masuda et al. *J. Immunol*. 2007). We recently found that when murine hematopoietic progenitors were cultured on immobilized Notch ligand DLL4 protein in the presence of a cocktail of cytokines including interleukin-7, progenitors just prior to the T cell lineage determination step underwent developmental arrest and the arrested progeni-

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2. Kawamoto H, T Ikawa, K Masuda. H Wada, Y Katsura. A map for lineage restriction of progenitors during hematopoiesis: the essence of the myeloid-based model. ***Immunol Reviews***. 238: 23-36, (2010).
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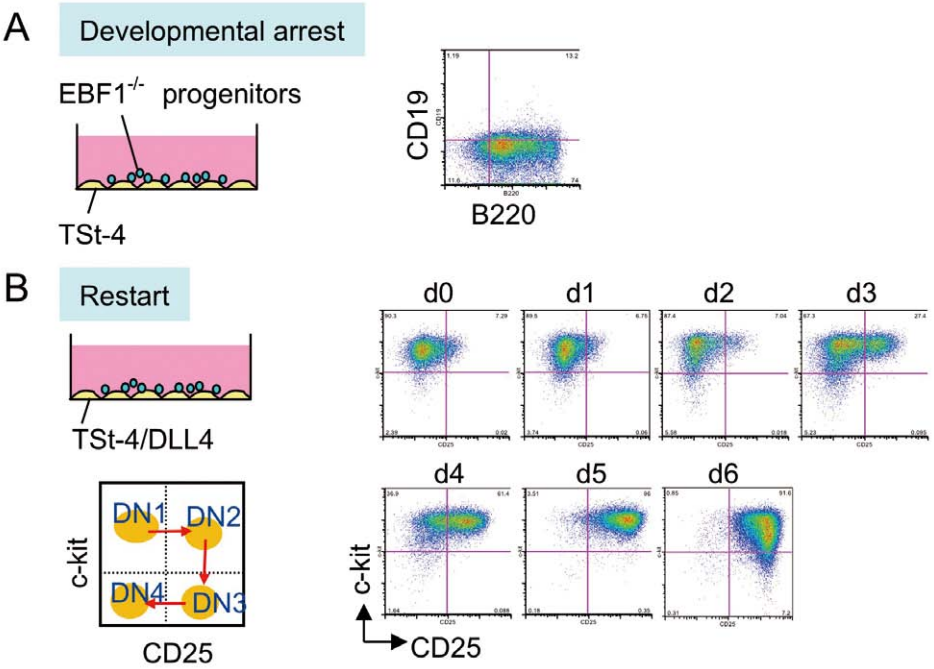

Figure : **Arrest-restart operation system for the study of the T cell lineage specification step**

A. Induction of the developmentally arrested cells at the multipotent progenitor stage. LKS cells from fetal liver of EBF1$^{-/-}$ mice were cultured under B cell inducing conditions. Cells were arrested at the B220^{+}CD19^{-} prepro-B cell stage, which is thought to represent progenitors retaining T, B and myeloid potential.

B. Induction of the T cell lineage specification step. When EBF1$^{-/-}$ progenitors were transferred to the stromal cells expressing Notch ligand DLL4, the cells restart differentiation towards T cells.

tors entered a cycle of self-renewal. Reducing the concentration of interleukin-7 in the cultures promoted T cell lineage determination. A similar arrest and self-renewal of progenitors was observed in thymocytes of mice deficient in the transcription factor Bcl11b. This study thus identifies the earliest checkpoint during T cell development and shows that it is Bcl11b-dependent (Ikawa et al, *Science*, 2010).

The culture system established in the above study allows control of the T-lineage determination step and will be a useful method for further study on the mechanisms of T cell lineage determination.

Dissection of the earliest stage of intrathymic T cell development

We have recently developed another arrest-restart operation culture system in which we can reproduce the earliest intrathymic T cell developmental process. It is known that in the fetus the thymus seeding progenitors are mostly specified to the T cell lineage but still retain residual B cell potential (Kawamoto et al. *Immunol. Rev.*, 2010). Such B cell potential is immediately terminated just after the progenitors encounter the thymic environment where Notch ligand is abundantly expressed, whereas myeloid potential is retained for a while. These early events occur at the so-called double negative (DN) 1 stage. Such DN1 progenitors then begin to express CD25 to enter the DN2 stage, just synchronizing with the upregulation of Bcl11b.

Therefore, in the DN1 stage, two important events take place: namely i) termination of B cell potential, ii) expression of Bcl11b. The step for the termination of B cell potential can alternatively be described as the “T cell lineage specification” step. To recapitulate this process, we used EBF1-deficient progenitors that represent multipotent progenitors retaining T, B and myeloid potential (Figure A). These multipotent progenitors were transferred to stromal cells that highly express Notch ligand DLL4. We found that the transferred cells subsequently differentiate towards the T cell lineage in a very synchronous manner, generating CD25^{+} DN2 cells in four days. Therefore, the first two to three days of culture appear to represent the DN1 stage. We decided to perform a very detailed time course analysis (0h, 1h, 2h, 4h…..and so on) and the cells will be included in the FANTOM5 project conducted by the RIKEN Omics Science Center to analyze genome-wide gene expression profiles. We hope to dissect the precise features of the DN1 stage by using this approach.

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Laboratory for

Transcriptional Regulation

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Hirokazu Tanaka (SPDR)
Wooseok Seo (JSPS)

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Chizuko Miyamoto
Risa Chihara
Mayu Okoshi
Mika Ikegaya

Student Trainees : **Sebastian Nieke** (IPA)
Hisashi Wada

One of the major questions in developmental biology is how the fate of progenitor cells differentiating into opposing lineages is determined. Even as we learn more about cell fate determination, other questions arise, namely how genetic programming after lineage specification functions to establish cell identity and then how cell identity, once established, is maintained in differentiated cells. Research in my laboratory is directed toward understanding (a) how precursor cells sense external or intrinsic stimuli and turn on a genetic program for regulating fate decision and (b) how a lineage-specific gene expression pattern is established during a commitment process to become fully differentiated cells. We are addressing these questions by studying lymphocyte development. In particular, we have been studying the transcriptional regulation of lineage choice by $CD4^+CD8^+$ double-positive (DP) thymocytes differentiating into either $CD4^+$ helper- or $CD8^+$ cytotoxic-lineage T cells. Expression of a functional ThPOK transcription factor is essential for development of helper-lineage cells. Our previous study has identified a transcriptional silencer (the *ThPOK* silencer) in the *ThPOK* locus, and has shown that the *ThPOK* silencer is essential to restrict *ThPOK* gene expression in cells expressing MHC class II-restricted TCR, thereby making it necessary to induce cytotoxic fate in MHC class I-restricted cells. We have also shown that the Runx/Cbfβ transcription factor complexes are essential to activate two distinct silencers embedded on either the *ThPOK* or the *Cd4* locus. We are expanding our findings to understand how the activity of these silencers is regulated at the molecular level and how Runx complexes are involved in immune system development.

Mechanism regulating *ThPOK* gene expression

Both CD4/CD8 co-receptor expression and the specificity of TCRs to MHC molecules correlate well with the outcome of lineage decision by $CD4^+CD8^+$ DP thymocytes. Cells expressing MHC class I-restricted TCR differentiate into the cytotoxic-lineage and terminate *Cd4* gene expression, whereas cells expressing class II-restricted TCR give rise to the $CD4^+$ helper-lineage and loose *Cd8* gene expression. However, it remains unclear how differences in TCR signals are sensed and integrated into developmental programming in the cell nucleus of post-selection thymocytes. Given that helper lineage-specific expression of the *ThPOK* gene is regulated by the activity of the *ThPOK* silencer, we suppose that the *ThPOK* silencer would serve as nuclear sensor and

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3. Sakaguchi S., Hombauer M., Bilic I., Naoe Y., Schebesta A., Taniuchi I. Ellmeier W. The zinc finger protein MAZR is part of the transcription factor network controlling CD4/CD8 cell fate decision of DP thymocytes. ***Nat. Immunol.***11, 42-48 (2010).

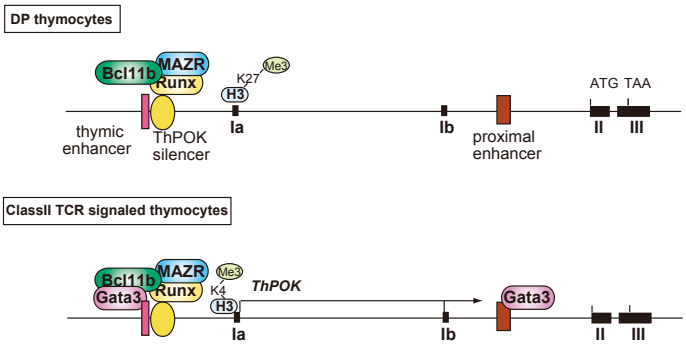


Figure 1: Model for *ThPOK* gene regulation. Expression of the *ThPOK* gene during thymocyte development is regulated by combined activation and inactivation of the two enhancers and the silencer. Binding of Runx, MAZR and Bcl11b is essential to exert silencer activity, which includes, at a minimum, deposition of repressive epigenetic marks such as H3 K27 tri-methylation (H3K27me3). Gata3 functions as an upstream factor for *ThPOK* expression by activating enhancers that remove H3K27me3 and impose active epigenetic marks, H3K4me3.

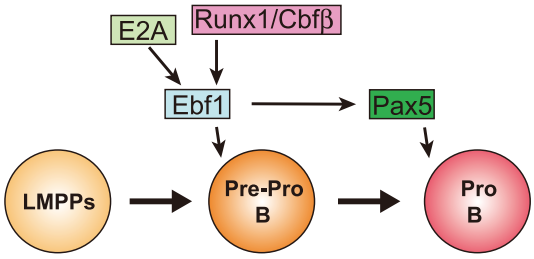


Figure 2: Roles of Runx1/Cbfβ in regulating early B cell development. Runx1/Cbfβ complexes play essential roles in specification to the B lymphocyte lineage in part by epigenetically activating the *Ebf1* gene. LMPPs=Lymphoid-primed multipotent progenitors

converter of TCR signals. Therefore it is crucial to define the molecule(s) or mechanism that acts as a switch to reverse the *ThPOK* silencer activity for inducing *ThPOK* expression upon engagement of class II-restricted TCRs. To this end, recently we have isolated novel proteins that bind to the *ThPOK* silencer and are doing functional characterization using mouse genetics.

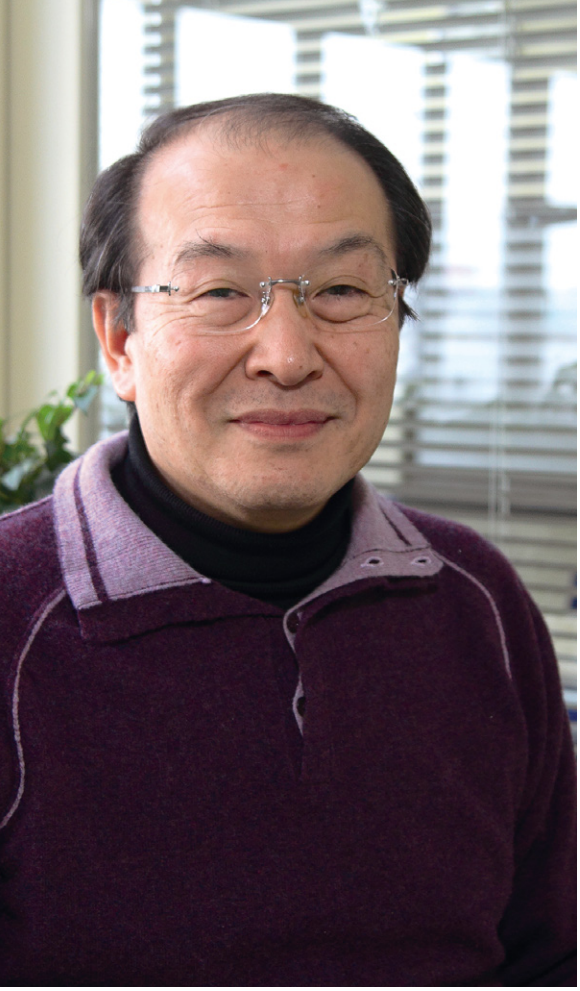
In addition to the reversal of the *ThPOK* silencer, activation of positive regulatory elements, such as enhancers and the promoter, is required for efficient induction of *ThPOK* expression. Previously, we have identified a proximal enhancer that is necessary to maintain *ThPOK* expression at the later developmental stage. Recently we have mapped another enhancer at the 5' end of the gene, in close proximity to the silencer. This enhancer, designated as a thymic enhancer, is necessary for efficient induction of *ThPOK* expression in freshly selected thymocytes, in part via erasing pre-existing repressive epigenetic marks induced by the silencer. Although these enhancers and the silencer are all T-lineage specific, there is another enhancer(s) that allows low level *ThPOK* expression in all mature lymphocytes. Thus appropriate kinetic *ThPOK* expression, which is key in choosing the correct developmental pathway, is orchestrated by the sophisticated interaction between these T cell-specific regulatory regions (Figure 1). Further studies on the molecular control of these regulatory elements as well as epigenetic regulation at the *ThPOK* locus will advance our understanding of how TCR signals are converted into fate determination programs and how the system that generates helper-lineage T cells has evolved and imprinted on our genome.

Role of Runx complexes in early B lymphocyte development.

Since the precise roles of Runx complexes in early B lymphocyte differentiation remain elusive, we examined mouse strains in which the *Runx1*, *Runx3* or *Cbfβ* gene is inactivated in early B lineage progenitors by an *mb1-cre* transgene. We found that loss of Runx1, but not Runx3, function causes a severe developmental block during early B lymphopoiesis, resulting in the lack of IgM$^+$ B cells and V_H to DJ_H recombination. Observing reduced expression of core transcription factors regulating early B cell development, such as E2A, Ebf1 and Pax5, in B cell precursors lacking Runx1, we performed ChIP-on chip approaches and detected binding of Runx complexes to the *Ebf1* proximal promoter, where repressive histone marks, H3K27-tri-methylation, are accumulated in Runx1-deficient B cell progenitors. Interestingly, retroviral transduction of Ebf1, but not Pax5, into Runx1-deficient progenitors restored not only development of B220$^+$ cells that underwent V_H to DJ_H rearrangement but also expression of B-lineage signature genes including the Ebf1 gene itself. Thus Runx1/Cbfβ complexes are essential to initiate B-lineage specification, in part via epigenetic activation of the *Ebf1* gene. Our results place the Runx1/Cbf*β* complex in the right position in the transcription factor network that governs specification to the B-lineage (Figure 2).

4. Seo W., Ikawa, T., Kawamoto H., Taniuchi I. Runx1-Cbfβ facilitates early B lymphocyte development by regulating expression of *Ebf1* gene. ***J. Exp. Med***. 209, 1255-62 (2012).

5. Taniuchi I and Ellmeier W. Transcriptional and Epigenetic Regulation of CD4/CD8 Lineage Choice. ***Adv. Immunol***. 110, 71-110 (2011)



Laboratory for
Cell Signaling

Group Director :
Takashi Saito

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Research Scientists : **Takayuki Imanishi, Reiko Onishi, Yasuo Shikamoto, Arata Takeuchi, Akiko Hashimoto-Tane, Shin-ichi Tsukumo**

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Student Trainees : **Mohamed El Sherif Gadelhaq Gadlehaq Badr (IPA), Minoru Sawaguchi (JRA)**

Central Facility FACS Laboratory : **Hanae Fujimoto, Yukiko Hachiman**

Central Facility Confocal Laboratory : **Yasutaka Wakabayashi** (Leica) **Ikuo Ishige** (BM Equipment)

Central Facility Monoclonal Laboratory : **Tomomi Aoyama, Kazuyo Uchida** (Program for Drug discovery and Medical technology platform)

T cells mediate central roles in immune regulation and are responsible for immune defense against pathogens and cancer. Because of their critical function in immune regulation, impairment in the T cell activation process and function results in immune diseases. The group aims to determine the molecular mechanisms of activation, differentiation and homeostasis of T cells in order to modulate T cell activation/function in immune disorders from the signal transduction perspective. Particularly in studies of T cell activation, the group has been using real-time imaging analysis to elucidate the dynamic regulation of the signaling complex and signal transduction of related downstream pathways. These studies also include the roles of co-stimulation signals, innate-related signals and cytoskeletal regulation. We also analyze regulation at later phases of T cell activation for cell migration, functional differentiation, and establishment of peripheral effector functions and tolerance.

Dynamic regulation of T cell activation and costimulation

We have studied the dynamic movement of signaling molecules in the process of the formation of the immunological synapse (IS) and T cell activation upon antigen recognition at the single-cell level by using a combination of imaging techniques with model planar bilayers and total internal reflection fluorescence microscopy (TIRF). We have identified TCR-microclusters (MCs) as the signalsome to assemble signaling molecules and induce activation signals, which then translocate into the center of the IS and form the cSMAC.

We then analyzed the dynamic features of co-stimulation signals by the positive co-stimulatory receptor CD28 and its negative counterpart CTLA-4 and their relationship with TCR-MCs. CD28 is initially co-localized with TCR-MCs and then accumulates into the cSMAC together with PKCθ for sustained co-stimulation. Later on, CTLA-4 also accumulates in the same area of the cSMAC as CD28, where it inhibits its activation by competing with CD28 for ligand-binding. Furthermore, we analyzed the dynamic regulation of inhibitory signals by PD-1. Unlike CTLA-4 accumulation in the cSMAC, PD-1 accumulates in the TCR-MCs and mediates inhibition of proximal signals within TCR-MCs by recruiting the phosphatase SHP-2. Thus, we have clarified the spatio-temporal dynamic regulation of positive/negative co-stimulation and how these signals exhibit quantitatively fine-tuned regulation.

Recent publications

1. Hashimoto-Tane A., Yokosuka T., Sakata-Sogawa K., Sakuma M., Ishihara C., Tokunaga M., Saito T. Dynein-driven transport of T cell receptor microclusters regulates immune synapse formation and T cell activation. ***Immunity*** 34, 919-931(2011)
2. Kong K-F., Yokosuka T., Canonigo-Balancio A J., Isakov N., Saito T., Altman, A. A motif in the V3 domain of the kinase PKC-θ determines its localization in the immunological synapse and functions in T cells via association with CD28. ***Nat. Immunol***. 12, 1105-12(2011)
3. Yokosuka T., Kobayashi W., Takamatsu M., Sakata-Sogawa K., Zeng H., Hashimoto-Tane A., Yagita H., Tokunaga M., Saito T. Spatiotemporal basis of CTLA-4 costimulatory molecule-mediated negative regulation of T cell activation. ***Immunity*** 33, 326-339(2010)

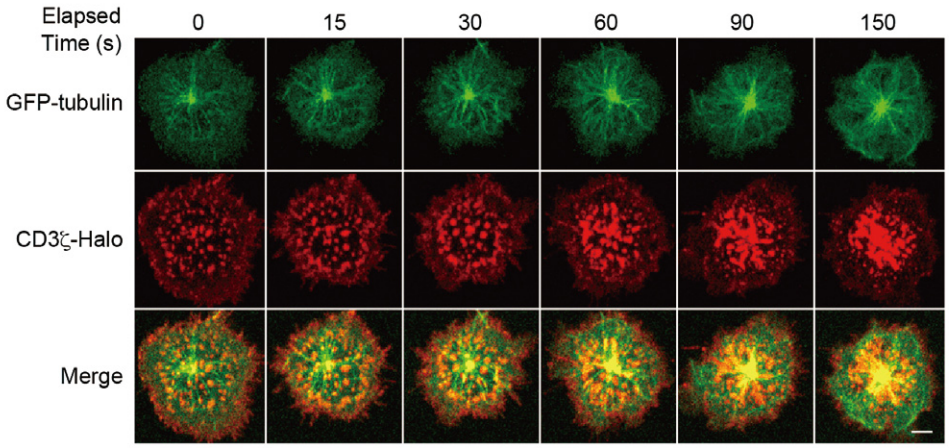


Figure 1 : Dynein-driven translocation of TCR-microclusters along microtubules regulates immune synapse formation and T cell activation. AND-TCR transgenic T cells expressing GFP-tubulin and CD3ζ-Halo were stimulated on a planar bilayer. Real time imaging analysis revealed that TCR-microclusters move along microtubules and ultimately form the cSMAC. Dynein and the components of the dynein complex accumulated within TCR microclusters and moved together. Cells in which dynein or microtubules were disrupted failed to generate the cSMAC and this resulted in enhancement of T cell activation due to the failure to degrade the TCR-microclusters, leading to sustained activation signals such as phosphorylation in the microclusters.

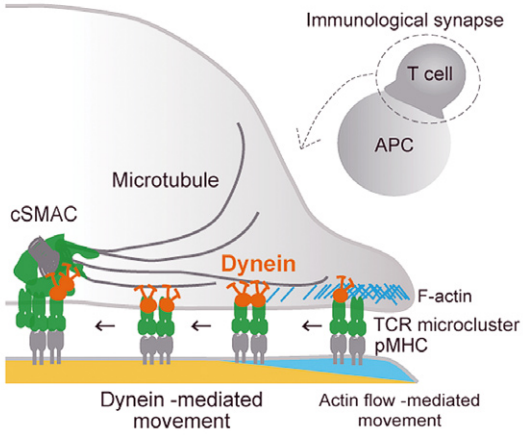


Figure 2 : Model for dynein-mediated translocation of TCR microclusters and T cell activation regulation. TCR-microclusters are generated at the periphery upon antigen recognition by the TCR. They move towards the center of the engaged region, initially by actin-retrograde flow, and this is followed by translocation along microtubules in a dynein-dependent fashion. This pathway is mediated by the association between the TCR complex and the dynein complex upon antigen stimulation. Strong but not weak stimulation induced the translocation of microclusters, thus suggesting that dynein-driven translocation of TCR-microclusters regulates T cell activation.

Cytoskeletal regulation of T cell activation

TCR-MCs generated at the periphery of the IS move to the center to form the cSMAC. This translocation of TCR-MCs thus regulates TCR activation by reducing the number of functional TCR-MCs through their degradation at the cSMAC. We analyzed the mechanism of TCR-MC translocation and found that the minus-end microtubule-associated motor protein dynein and its complex are co-localized with TCR-MCs and co-precipitate with the TCR complex. We have shown that TCR-MCs are translocated along microtubules to form the cSMAC. Depletion of the dynein complex or disruption of microtubule network formation resulted in enhanced T cell signals and activation. These data indicate that signal strength regulates the assembly of the TCR-dynein complex and its translocation to form the cSMAC.

Regulation of T cell responses by innate signals

We have analyzed the interconnected activity of signaling molecules of the innate and acquired immune systems. After we identified the critical roles of IRAK4 in T cell activation, we analyzed the function of TLRs and RLRs expressed in T cells. Initial analysis revealed that TLR2/3/9 mediate co-stimulatory functions whereas TLR2 directly activates Th1 cells to induce cytokine production independently of TCR stimulation. Whereas T cell co-stimulation through TLR2 is MyD88-dependent, surprisingly, the co-stimulation by the ligand of TLR3/9 was found to be independent of MyD88/TRIF. We found that for the optimal T cell co-stimulatory activity, nucleic acids have to be aggregated and this appears to be mediated by an as yet unknown novel T cell-specific sensor of nucleic acids, whose identity is currently under investigation.

4. Hashimoto-Tane A., Yokosuka T., Ishihara C., Sakuma M., Kobayashi W. and Saito T. TCR-microclusters critical for T-cell activation are formed independently of lipid raft clustering. ***Mol. Cel. Biol***. 30, 3421-3429(2010)

5. Takeuchi A., Itoh Y., Takumi A., Ishihara C., Arase N., Yokosuka T., Koseki H., Yamasaki S., Takai Y., Miyoshi J., Ogasawara K., and Saito T. CRTAM confers late-stage activation of CD8+ T cells to regulate retention within lymph node. ***J. Immunol***. 183, 4220-4228(2009)

Research Unit for

Single Molecule Imaging

Unit Leader :

Kumiko Sakata-Sogawa

The goal of our laboratory is the development of new microscopy systems for elucidation of immunological responses. We have been focusing on the study of signaling processes in immune cells using the technique of single molecule imaging and quantification. Single molecule approaches enable us to capture transient intermediates and heterogeneous behavior, thus avoiding ensemble averaging. This capability is powerful for elucidating mechanisms of cellular functions: which molecule interacts with what, when, where, and how it works in living cells. Thus fluorescence imaging and quantitative analysis of single molecules are valuable methods to study the individual behavior of biological systems. For this purpose we developed a novel type of fluorescence microscopy (HILO) for use in single cell/single molecule studies. One of the main technical challenges in single molecule microscopy is to obtain a high signal to noise ratio in order to capture the subtle fluorescent signal of single molecules. HILO has an advantage for observations inside cells with the high signal to noise ratio of 7.6. Our single molecule microscope system has been optimized to allow observation of immunological responses from the cell membrane to the nucleus by introducing a computer system for control of optical devices.

Visualization of cell signaling

Cells are activated by stimuli that usually come from the outside of the cells. The stimulation detected at the cell membrane is transmitted by different signaling proteins to the nucleus, where the transcription of target genes are initiated or terminated depending on the response of the cell to the stimulation. We are applying our single molecule analysis method to the understanding of complex signaling pathways by visualization and quantification of the interaction of signaling proteins. For quantitative analysis of these interactions, it is important to observe multiple molecules simultaneously. It is also necessary to prepare many different cell lines harboring target genes for high throughput data collection. To this end, we have established methods to obtain isogenic cell lines expressing two fluorescence tagged (GFP- or RFP) fusion proteins at a homogenous and low level. In addition to this method, we utilize fluorescence-labeled antibodies specific for physiological protein modifications such as phosphorylation, methylation or acetylation. Together with quantum dot technology and fluorescence-dye labeled protein by the “double-click” technique (Figure), we realize multi-color single molecule imaging and quantitative analysis.

Recent publications

1. Hashimoto-Tane A. ,Yokosuka T., Sakata-Sogawa K., Sakuma M., Ishihara C., Tokunaga M., Saito T.: Dynein-driven transport of T cell receptor microclusters regulates immune synapse formation and T cell activation. ***Immunity*** 34, 919-931 (2011)
2. Yokosuka T., Kobayashi W., Takamatsu M., Sakata-Sogawa K., Zeng H., Hashimoto-Tane A., Yagita H., Tokunaga M., Saito T.: Spatiotemporal basis of CTLA-4-mediated negative regulation of T-cell activation. ***Immunity*** 33, 326-339 (2010)
3. Miletic AV., Graham DB. Sakata-Sogawa K., Hiroshima M., Hamann HJ., Cemerski S., Kloeppel T., Billadearu DD., Kanagawa O., Tokunaga M. and Swat, W: Vav Links the T Cell Antigen Receptor to the Actin Cytoskelton and T Cell Activation Independently of Intrinsic Guanine Nucleotide Exchange Activity. ***PLoS One*** 4, e6599 (2009)

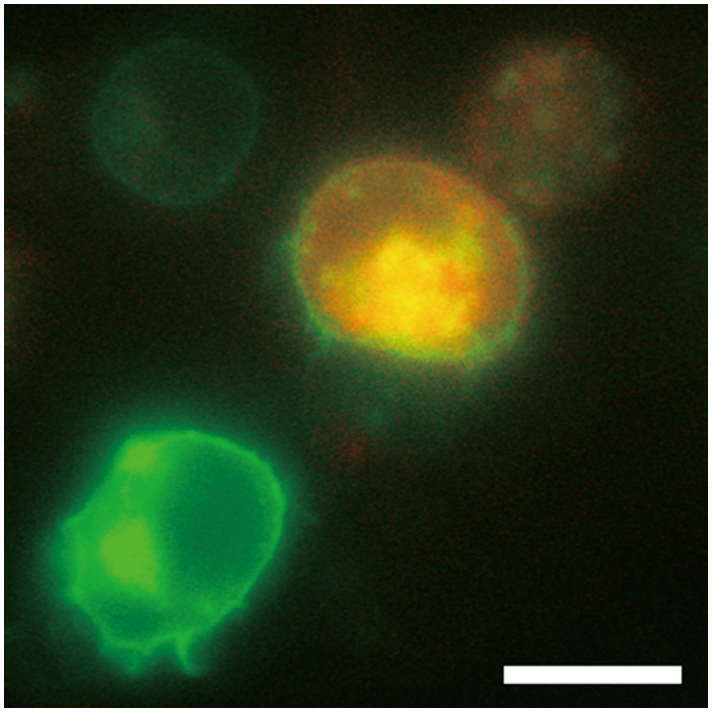


Figure: **Introduction of Cy3-labeled calmodulin in LAT-GFP expressing Jurkat cells.**
Calmodulin was labeled with a single molecule of Cy3 *in vitro* and introduced into LAT-GFP Jurkat cells by electroporation. Co-localization of LAT and calmodulin is indicated by yellow fluorescence. (Collaboration with Dr. Nobuhiro Hayashi at the Tokyo Institute of Technology) Bar: 10 μm.

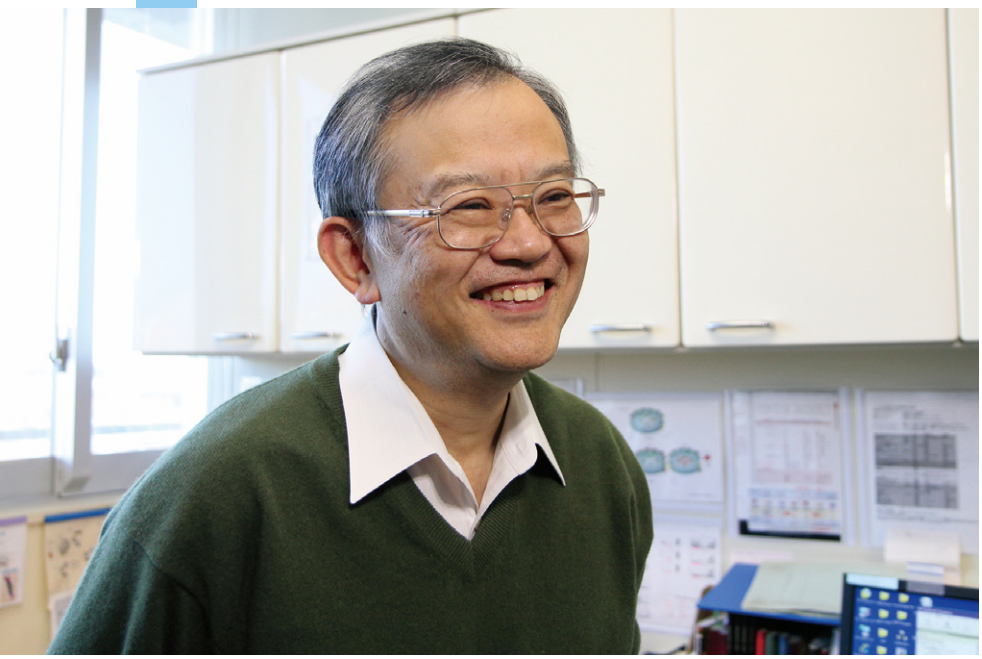
Spatio-temporal regulation of NF-κB inactivation by PDLIM2

NF-κB is an important transcription factor that activates expression of inflammatory genes in response to stimulation. In resting cells, NF-κB binds IκBα, which keeps it in an inactive state sequestered in the cytoplasm. Stimulation leads to phosphorylation of Ser residues on IκBα and its subsequent ubiqutination and degradation by the proteasome. As a result of IκBα degradation, liberated NF-κB translocates to the nucleus and activates expression of target genes including the IκBα gene itself. The nascent IκBα binds to nuclear NF-κB and exports it back into the cytoplasm, resulting in a negative feedback loop. In addition to this canonical pathway, another protein, PDLIM2, was found by RCAI investigators to function as an E3 ubiquitin ligase responsible for the regulation of NF-κB activation (Tanaka T et al. *Nat. Immunol.*, 8, 584-591 2007).

Aiming to elucidate PDLIM2 regulatory mechanisms, we established dual gene expressing cell lines of GFP- and RFP-fusion proteins of PDLIM2 and NF-κB (p65 subunit). Using single molecule fluorescence microscopy, we were able to visualize the dynamics of PDLIM2 function. In resting cells, both proteins localized in the cytoplasm, but upon stimulation, they translocated to the nucleus and colocalized. A PDLIM2 mutant (LIM) that lacks the ubiquitin ligase functional domain also translocated to the nucleus but did not colocalize with p65 in stimulated cells. These results demonstrate that the LIM domain is relevant to the control of NF-κB localization, suggesting that this domain is responsible for regulation of the transcriptional activity of NF-κB.

Laboratory for

Lymphocyte Differentiation



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Student Trainee : **Saya Moriyama**

Memory antibody responses are typically seen to T cell-dependent antigens and are characterized by the rapid production of high-titers of high-affinity antigen-specific antibody. The previous hallmark of T cell-dependent memory B cells is their expression of somatically mutated, isotype-switched B cell antigen receptors, features that are mainly generated in germinal centers (GCs). Indeed, classical studies have focused on isotype-switched memory B cells (mainly the IgG isotype). However, recent advances in monitoring antigen-experienced B cells have revealed the existence of un-switched IgM type memory B cells, demonstrating the considerable heterogeneity of memory B cells. As a first step to understand how such cellular and possibly functional heterogeneity is generated and integrated during humoral memory responses, our laboratory has now focused on clarifying the mechanisms underlying the robustness of memory antibody responses.

Existence of two types of IgM memory B cells

Fate mapping-methods have allowed us to monitor both un-switched (IgM-type) and switched (IgG-type) memory B cells after injection of antigen into mice. When B cells encounter antigens *in vivo*, almost all the B cells induce AID at the transcriptional level. Thus, we can genetically label the antigen-experienced B cells by using AID expression (Figure 1).

Employing this system, we have isolated two subsets of IgM type memory B cells (IgM^+IgD^+ and IgM^+IgD^-) and shown that the IgM^+IgD^+ memory B cells undergo somatic hypermutation, whereas the IgM^+IgD^- cells do not. These observations suggest that IgM type memory B cells are generated through GC-dependent (IgM^+IgD^+) and independent (IgM^+IgD^-) pathways. When the IgM^+IgD^+ or IgM^+IgD^- memory B cells are adoptively transferred together with activated T cells, both types of memory B cells re-initiate the GC reaction. Given that IgG type memory B cells have the propensity to differentiate into plasma cells (described below), these results suggest that IgM type memory B cells have characteristics that are distinct from IgG type memory B cells.

Contribution of transcription factors to efficient differentiation of IgG type memory B cells into plasma cells

Although robust antibody responses of IgG type memory B

Recent publications

1. Yasuda T., Kometani, K., Takahashi, N., Imai, Y., Aiba, Y. and Kurosaki, T. Erk kinases control plasma cell differentiation by regulating expression of Blimp-1. ***Sci. Signal***. 4, ra25 (2011)
2. Matsumoto, M., Fujii, Y., Baba, A., Hikida, M., Kurosaki, T. and Baba, Y. The calcium sensors STIM1 and STIM2 control B cell regulatory function through IL-10 production. ***Immunity*** 34, 703-714 (2011)
3. Limnander, A., Depeille, P., Freedman, T.S., Liou, J., Leitges, M., Kurosaki, T., Roose, J.P. and Weiss, A. Stim1, PKCδ and RasGRP proteins set a threshold for pro-apoptotic Erk signaling during B cell development. ***Nat. Immunol***. 12, 425-433. (2011)

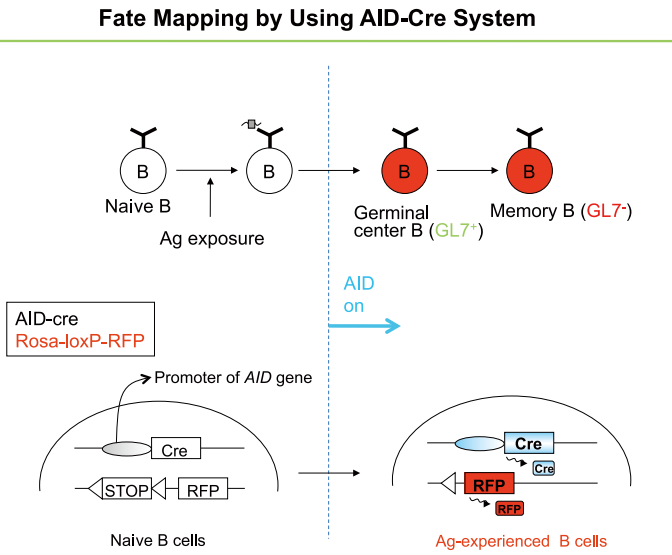

Figure 1: Fate mapping methods for antigen-experienced B cells
In AID-Cre Tg mice, the Cre gene is controlled by the promoter of the AID gene. This Cre gene is expressed in antigen-experienced B cells, since Aid is expressed in activated B cells after the exposure to the antigens. By crossing AID-Cre Tg mice with ROSA-loxP-RFP mice, antigen-experienced GC B cells and memory B cells but not naive B cells were labeled with RFP.

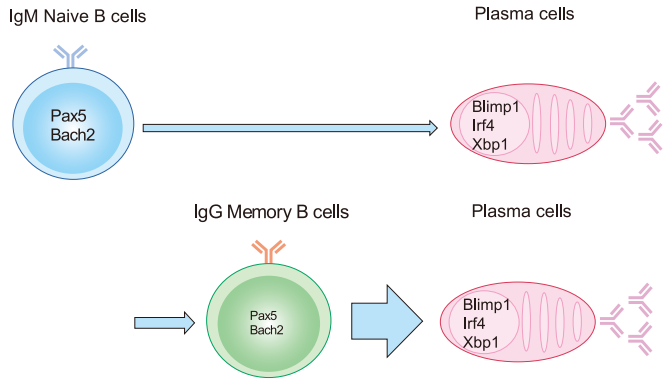

Figure 2: Preferential differentiation to plasma cells by IgG-type memory B cells
IgG-type memory B cells express less Pax5 and Bach2 genes compared to IgM naive B cells. Pax5 and Bach2 suppress Blimp1 expression, which is the essential transcription factor for plasma cell differentiation. This led to the model that IgG-type memory B cells can differentiate to plasma cells preferentially and quickly by reducing Pax5 and Bach2 expression.

cells have been well appreciated, the cellular and molecular basis underlying this phenomenon has been unclear. It has long been suspected that inherent differences in the structure of membrane IgM and IgD on naive B cells versus membrane IgG on memory B cells account for the robust secondary antibody responses (BCR-intrinsic model).

Both membrane IgM and IgD have short, three amino acid cytoplasmic tails that seem not to play a direct role in BCR signaling. By contrast, all membrane IgG subclasses have unique cytoplasmic domain structures of 28 residues that are highly conserved between species. Thus, in addition to BCR signaling executed by the common Igα/Igβ heterodimers, the IgG type BCRs could exert additional BCR signaling functions via the cytoplasmic domains of membrane IgG, thereby contributing to the robust secondary responses.

To directly test this model, we have established NP-specific IgG1 type cloned mice, allowing us to obtain antigen-non-experienced IgG1 B cells. By using these B cells, we have demonstrated that antigen-experienced, but not non-experienced, IgG1$^+$ B cells rapidly differentiated into plasma cells. Furthermore, the heightened differentiation capability of antigen-experienced IgG type memory B cells was associated with low amounts of the transcription factor Bach2, since enforced expression of Bach2 in IgG1 memory B cells reduced this enhanced differentiation capability. Based on these results, we favor a model in which reorganization of transcription factors takes place during generation of IgG1 type memory B cells after primary antigen exposure, and this altered transcription factor profile is critical for rapid responsiveness of memory B cells (BCR-extrinsic model) (Figure 2).

4. Kometani, K., Yamada, T., Sasaki, Y., Yokosuka, T., Saito, T., Rajewsky, K., Ishiai, M., Hikida, M. and Kurosaki, T. CIN85 drives B cell responses by linking BCR signals to the canonical NF-κB pathway. ***J. Exp. Med***. 208, 1447-1457 (2011)

5. Kitano, M., Moriyama, S., Ando, Y., Hikida, M., Mori, Y., Kurosaki, T, and Okada, T. Bcl6 Protein Expression Shapes Pre-Germinal Center B Cell Dynamics and Follicular Helper T Cell Heterogeneity. ***Immunity*** 34, 961-72 (2011)

Research Unit for

Immunodynamics

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Research Scientists : **Masahiro Kitano** (SPDR)
Yoshikazu Ando

Technical Staff : **Noriko Takahashi**

The goal of our research is to understand the mechanisms regulating cell migration and interactions in the tissues that shape immune responses. For this purpose we use real time imaging, in particular two-photon microscopy, to analyze *in vivo* cellular migration and interactions. This microscopy method, which was introduced recently to the field of immunology, has been revealing striking dynamics of immune cells in the lymphoid organs, underlining the importance of this approach to resolve the complexity of the immune system. Last year, we were focusing on clarifying dynamics of antigen-engaged B cells during the formation of germinal centers (GCs), a process that ultimately leads to long-term, high affinity antibody production. This year, we have started new projects to understand the mechanisms controlling the dynamics of two different T cell types, follicular helper T (Tfh) cells and cytotoxic T cells, which play pivotal roles in humoral and cellular immunity, respectively.

Dynamics of GC Tfh cells.

Tfh cells are essential for initiation and maintenance of T-dependent B cell responses, particularly germinal center (GC) reactions. A subpopulation of Tfh cells that has physical access to the GC is believed to be responsible for controlling GC reactions. However, little has been known about mechanisms that regulate the dynamics of GC Tfh cells. We have performed two-photon imaging analysis of antigen-specific Tfh cells within GC-containing B cell follicles. By simultaneously visualizing GC B cell clusters, we have been able to analyze the migration of Tfh cells into the GC area and the surrounding follicular mantle (FM). The analysis clearly showed that Tfh cells in the GC and those in the FM tended to be retained in the respective regions, suggesting differential expression of molecules responsible for their localization. Our previous imaging analysis of Tfh cells harboring hypomorphic mutations in the gene encoding Bcl6, which is an essential transcription factor for Tfh cells, suggested that formation of GC Tfh cells was more severely impaired than that of FM Tfh cells by the partial deficiency of Bcl6 (Figure). This observation led us to perform gene expression analysis of Tfh cells with various surface phenotypes and Bcl6 genotypes to search for genes differentially expressed between GC Tfh cells and FM Tfh cells. We have

Recent publications

1. Baumjohann D, Okada T, Ansel KM. Cutting Edge: Distinct waves of BCL6 expression during T follicular helper cell development. ***J. Immunol***. 187, 2089-2092 (2011)
2. Kitano M, Moriyama S, Ando Y, Hikida M, Mori Y, Kurosaki T, Okada T. Bcl6 protein expression shapes pre-germinal center B cell dynamics and follicular helper T cell heterogeneity. ***Immunity*** 34, 961-972 (2011)
3. Tanizaki H, Egawa G, Inaba K, Honda T, Nakajima S, Moniaga CS, Otsuka A, Ishizaki T, Tomura M, Watanabe T, Miyachi Y, Narumiya S, Okada T, Kabashima K. Rho-mDia1 pathway is required for adhesion, migration, and T-cell stimulation in dedritic cells. ***Blood*** 116, 5875-5884 (2010)

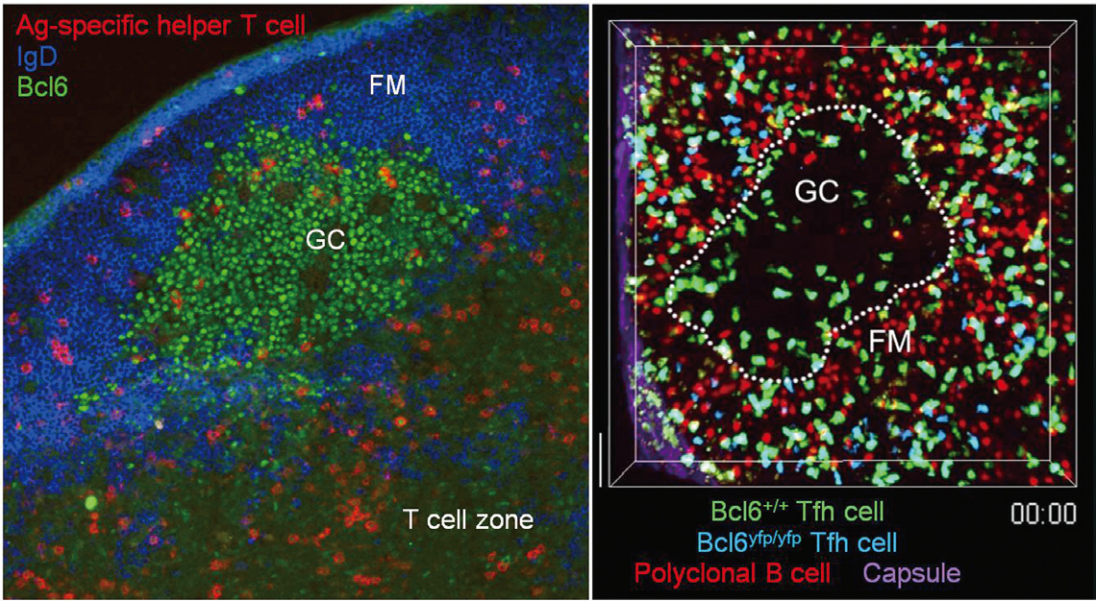


Figure: **Bcl6 expression and function in GC Tfh cells.**
The left image shows a lymph node section stained for antigen-specific helper T cells (adoptively transferred, T cell receptor transgenic $CD4^+$ T cells), IgD, and Bcl6. The GC region is demarcated by the cluster of Bcl6-expressing GC B cells, which are surrounded by IgD positive B cells mostly in the FM. Many of antigen-specific helper T cells in the GC also express Bcl6. The image on the right shows a 105 μm z-projection image of a GC-containing follicle in an intact lymph node. This two-photon image is a part of a time-lapse imaging data set that shows impaired GC accessibility of Tfh cells harboring hypomorphic mutations in the *Bcl6* gene ($Bcl6^{yfp/yfp}$) compared to $Bcl6^{+/+}$ Tfh cells. The GC and FM regions are demarcated based on the density of polyclonal B cells.

identified several candidate genes responsible for localization of these Tfh cell subsets. Preliminary results using gene-targeted animals have begun to show a role for one of these candidate molecules in the localization of GC Tfh cells.

Cellular interaction dynamics during cytotoxic T cell differentiation.

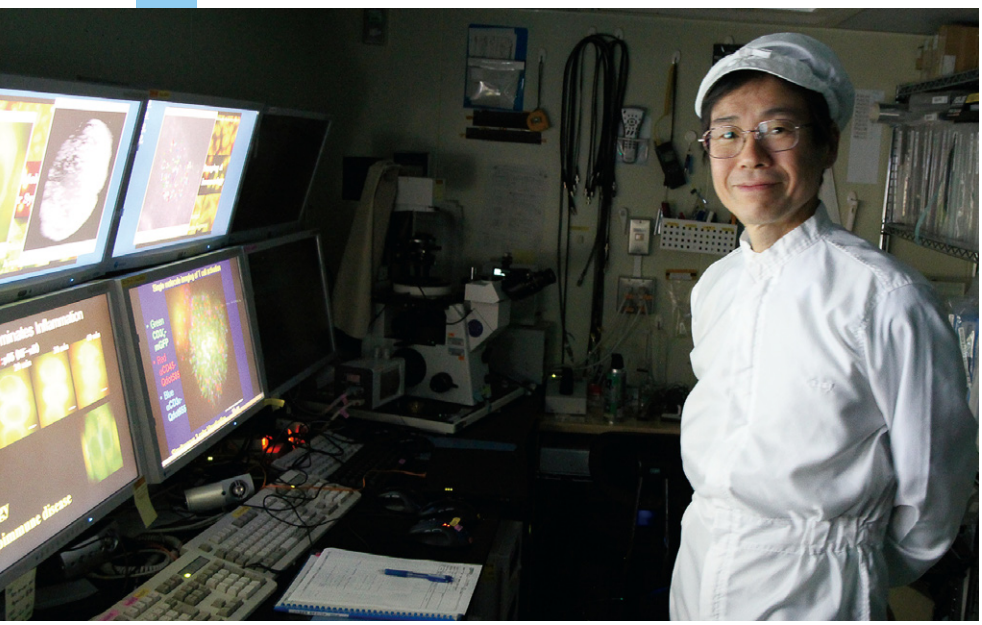
Cytotoxic T cells are known to be generated through interactions of antigen-specific $CD8^+$ T cells with dendritic cells (DCs). Interaction dynamics between $CD8^+$ T cells and DCs have been visualized by two-photon microscopy. However, the previous imaging studies utilized either *in vitro* differentiated DCs or reagents that would visualize all conventional DCs, which consist of numerous subsets with distinct physiological roles. Because of these limitations, there remains much to be learned about interaction dynamics between cytotoxic T cells and native DC subsets in response to various forms of antigen. In collaboration with Dr. Kaisho's group (Lab for Host Defense, RIKEN RCAI; and Immunology Frontier Research Center, Osaka University), we have developed novel methods that allow visualization by two-photon microscopy of specific DC subsets. These methods will provide a basis to study spatiotemporal roles for different DC subsets in generation and maintenance of cytotoxic T cells with effector and/or memory function.

4. Okada T. Two-photon microscopy analysis of leukocyte trafficking and motility. ***Semin. Immunopathol***. 32:215-225 (2010)

5. Katagiri K., Katakai T., Ebisuno Y., Ueda Y., Okada T., Kinashi T.. Mst1 controls lymphocyte trafficking and interstitial motility within lymph nodes. ***EMBO J.*** 28, 1319-1331 (2009)

Research Unit for

Molecular Systems Immunology



Unit Leader :
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Student Trainees : **Yuma Ito**
Jun Takimoto
Katsuo Ichinomiya
Masahiro Shimozawa
Ryuta Okada
Naomichi Inaba
Satoshi Ikeda
Hiroshi Oyama

Our laboratory has developed technologies that allow immune responses and signaling processes to be visualized at the single-molecule level. Single molecule imaging coupled with the ability to simultaneously visualize several different proteins in cells has enabled the quantification of molecular dynamics, interactions, and kinetics. Based on these three-dimensional and temporal parameters, we examine numerical modeling and computer simulations of cell functions. Using the combination of single molecule quantification and "*in silico*" modeling, we aim to open up new frontiers for understanding immune cells as molecular systems.

Single Molecule Imaging and Molecular Quantification in Cells

We have demonstrated that clear visualization of single molecules in cells enables their molecular quantification. Clear single-molecule visualization was achieved using TIRF and HILO microscopy. The main technical challenge of single-molecule fluorescence imaging is increasing the signal/background ratio. We have been involved in the development of total internal reflection fluorescence (TIRF) microscopy, a light-microscopic technique. TIRF is now widely used for single-molecule imaging at cell surfaces, but cannot be used for molecular imaging inside cells.

To overcome this limitation, we have devised a new approach, called highly inclined and laminated optical sheet (HILO) microscopy for single molecule imaging inside cells (Fig. 1a). We have achieved notable success in increasing the signal/background ratio by inclining the illumination beam and by minimizing the illumination area. The incident laser beam is highly inclined by a large refraction, and is laminated as a thin optical sheet at the specimen side. In HILO microscopy, this thin optical sheet is used for illumination.

To evaluate the HILO microscopy technique, we visualized single molecules of the Sp1 transcriptional factor Sp1 in a living cell (Fig. 1b). Clear point-like images of single Sp1 molecules were obtained without the need for deconvolution to remove out-of-focus haze. The fluorescence intensity of the Sp1 point images corresponded well to the theory of image formation. Further, there was much less photobleaching than in conventional confocal microscopy because of the lower intensity and non-focused nature of the illumination.

Reduction of the background intensity of images yields clear images. The background intensity of images depends

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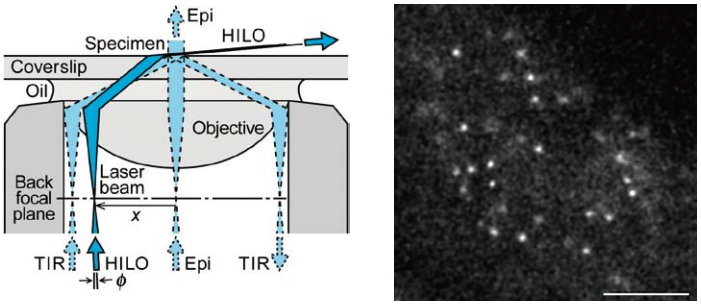

Figure 1: Molecular imaging enables one to visualize and quantify molecular dynamics, interactions, and kinetics in cells for molecular systems biology. (a) HILO microscopy for molecular imaging in cells. Illumination by a highly-inclined and thin beam increases image intensity and decreases background intensity, yielding a signal/background ratio up to about eightfold greater than that of epi-illumination. A high ratio yielded clear single-molecule images and three-dimensional images. (b) To evaluate the HILO microscopy technique, we visualized single molecules of the Sp1 transcription factor in a living cell. Molecular dynamics and interactions can be quantified by image analysis using these single molecule images. Bar, 5 μm.

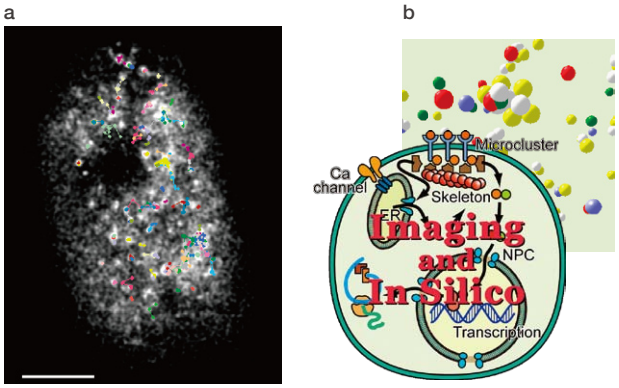

Figure 2: The combination of single molecule quantification and *"in silico"* modeling opens new approaches for developing molecular systems biology. (a) Automatic tracking of single molecules of the Sp1 transcription factor visualized in a living cell. Molecular dynamics and interactions can be quantified by image analysis using these single molecule images. Bar, 5 μm. (b) Aiming at understanding immune cells as molecular systems, we plan to construct *"in silico"* cell models based on single-molecule quantification. Bidirectional research is essential to reconstruct cell functions *in silico*; research from molecules to systems by single molecule analysis, and feedback research from systems to molecules.

on the volume of the illuminated region in specimens. To examine the thickness of the illuminated region, we obtained intensity profiles of illumination along the z-direction, that is, the depth direction in specimens. We devised a method to decrease the illumination thickness by narrowing the illumination area using a field stop. Notably, we have achieved the reduction of the illumination thickness to less than 10 μm (for example, 7 μm of thickness with a field stop for 20 μm-diameter illumination).

Further, we evaluated the signal/background ratio of images in HILO. Inclination of the illumination beam increases intensities of the fluorescence images up to 2.8-fold compared with epi-illumination. The 2.8-fold increase is in excellent agreement with the theory. By contrast, the background intensity is substantially decreased by illumination inclination. As the background is composed of out-of-focus images, the decrease is explained by the reduction of the illuminated range. As a result, illumination inclination increased the ratio of image to background (signal/background) up to 3.1-3.5-fold. Reduction of the illumination diameter further decreased the background intensity. Consequently, reduction of the diameter increased the ratio of signal to background up to 2.2-2.9-fold. Overall, the HILO illumination microscopy approach notably increased the signal/background ratio up to 7.6-fold.

To explore potential new uses of this technology, we performed quantitative analysis of nuclear import to demonstrate its application to kinetic studies. We could visualize single molecules of GFP-importin β mediating the import of cargo through nuclear pores in cells as bright spots on the nuclear envelope. Molecular interactions with the assembled NPC were quantified by single molecule analysis. Retention times, the number of associated molecules, the dissociation constant, and stoichiometry of import were all determined.

"*In silico*" Modeling and Simulation

As shown above, molecular interactions with the assembled NPC were quantified by single molecule analysis. In order to understand the molecular mechanism of nuclear import, a numerical model of import was constructed using these kinetic parameters. Computer simulation was carried out based on the model with two types of binding sites. The simulation fit very well with both the results of single-molecule experiments and the molecular kinetic features in cells.

We are now expanding the simulation studies into whole-cell simulation of single lymphocytes based on single molecule imaging and quantification (Fig. 2). Direct comparison with molecular imaging is indispensable for the simulation, since the values of the parameters have a huge number of degrees of freedom.

We demonstrated that clear visualization of single molecules in cells enabled accurate quantification. The combination of single molecule quantification and "*in silico*" reconstructions of cell functions opens new approaches for developing molecular system biology in immunology and other fields.

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The mucosal epithelium that lines the inner surfaces of the body, especially within the intestine, is exposed to a wide range of antigens, including food-derived macromolecules and microorganisms as well as numerous commensal bacteria, collectively called the intestinal microbiota. Appropriate recognition of these antigens is vital for maintaining immune homeostasis. Epithelial cells that overlay the gut-associated lymphoid tissue (GALT), such as Peyer's patches (PPs) and isolated lymphoid follicles, are distinct from absorptive epithelial cells of the villi and are termed follicle-associated epithelium (FAE). The FAE contains a specialized subset of epithelial cells, the M cells, which are thought to play a pivotal role in immune surveillance by delivering luminal microorganisms to the underlying lymphoid cells via transcytosis. One of the primary aims of our laboratory is to understand the mechanisms that underlie the differentiation and function of FAE and M cells. Our research team is also investigating the interaction of commensal microbiota with the intestinal epithelium and its influence on mucosal and systemic immunity. These studies may lead to the development of new and more efficient mucosal vaccination protocols/drug delivery systems as well as functional foods/preventive medicine based on host-microbiota interactions.

Understanding of M-cell differentiation steps

M cells are atypical epithelial cells specialized for uptake of luminal antigens, including the phagocytosis of viruses and bacteria, to deliver them to dendritic cells that accumulate beneath the FAE. More than three decades after their initial description, however, the molecular mechanisms of M-cell differentiation are not well understood. Recently, Dr. Ifor I. Williams' group identified that RANKL expressed by the stromal cells beneath the FAE is essential for the induction of M cells. They have further shown that systemically administered exogenous recombinant RANKL induces ectopic M-cell differentiation in the small intestinal villi. In collaboration with Dr. Williams, we set up an *in vivo* M-cell differentiation assay to trace the expression kinetics of several M-cell markers we have identified during RANKL-induced ectopic M-cell differentiation. We found that Marcksl1 and Annexin V were expressed as early as 1 day after RANKL injection, whereas CCL9 appeared on day 2, and that GP2 expression was not detected until day 3. This same sequence of expression of the M-cell markers was seen not only in this relatively artificial model of RANKL-induced ectopic M-cell differentiation, but also in PP M cells during normal M-cell differentiation in mouse ontogeny; Marcksl1 was already observed on

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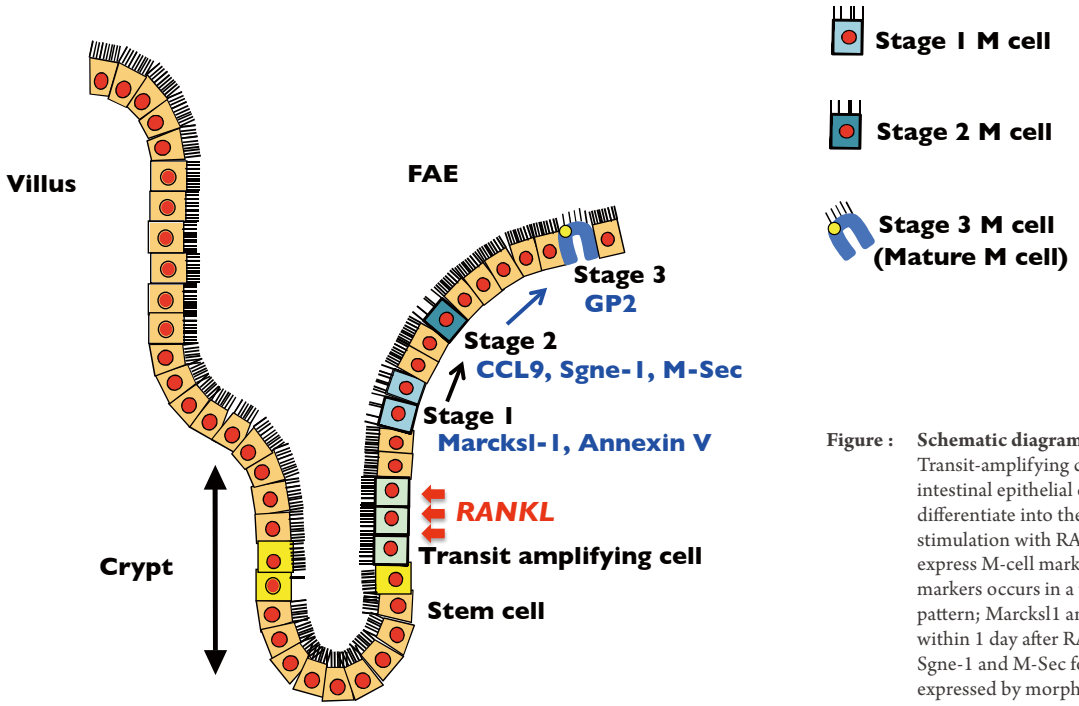

Figure : **Schematic diagram of M-cell differentiation**
Transit-amplifying cells, the immature intestinal epithelial cells in the crypt, can differentiate into the M-cell lineage following stimulation with RANKL and begin to express M-cell markers. Expression of these markers occurs in a temporally distinct pattern; Marcksl1 and Annexin V appear within 1 day after RANKL treatment, CCL9, Sgne-1 and M-Sec follow, and GP2 is finally expressed by morphologically mature M cells.

embryonic day 18, CCL9 in the day 2 neonate, and GP2 was finally detected 7 days after birth. These observations indicate that the process of M-cell differentiation can be practically divided into a series of distinct stages defined by the expression profile of M-cell markers, and that *in vivo* RANKL treatment is a powerful experimental tool for tracing the individual steps of M-cell differentiation. Taking advantage of this strategy, we are now investigating M-cell specific transcription factors expressed early during RANKL-induced M-cell differentiation, since they should regulate and serve as a key to elucidating the molecular mechanisms of M-cell differentiation.

Elucidation of host-intestinal microbiota interactions important for host immunity and biological defense mechanisms

Inside our gut dwell a very large number of bacteria. This intestinal microbiota impacts on both human physiology and pathology. Certain of the commensal microbiota, such as those belonging to the bacterial genus *Bifidobacterium*, have beneficial effects on our health. Among the most distinctive benefits of these bacteria are the modulation of host defense responses and protection against infectious diseases. Nevertheless, the molecular mechanisms underlying these beneficial effects have barely been elucidated. To address this important but complex question, we have developed a comprehensive 'multi-omics' approach, where exhaustive analyses, including (meta)genomics, (meta)transcriptomics and metabolomics, are combined. We employed a simplified model of lethal infection with enterohaemorrhagic *Escherichia coli* O157:H7 (O157) of mice associated with certain bifidobacterial strains to prove that our multi-omics approach is useful for analyzing host-microbial interactions. We showed that genes encoding ATP-binding-cassette (ABC)-type carbohydrate transporters present in certain bifidobacteria contribute to protecting mice against death induced by O157. The bacteria that possess these transporters were able to produce acetate from fructose. Our data strongly suggest that the acetate produced in large amounts by these bifidobacteria exerts its action on the colonic epithelium by inducing anti-inflammatory and/or anti-apoptotic effects, which prevent translocation of the O157 Shiga toxin from the gut lumen into the blood. We therefore propose that acetate produced by protective bifidobacteria improves intestinal defense mediated by epithelial cells and thereby protects the host against lethal infection.

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Laboratory for
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Adaptive coevolution of mammals and bacteria has led to the establishment of mutualistic and symbiotic relationships that have contributed to the development of our immune system and maintenance of homeostasis. In spite of having available a wealth of immune sensing and effector mechanisms capable of triggering inflammation in response to microbial intrusion, we can live together with our body's bacteria without any adverse effects. This is made possible by a continuous dialog between bacteria and host cells that generates finely tuned signaling programs ensuring a state of 'hypo-responsiveness' against dietary antigens and commensal bacteria. At the same time and somewhat paradoxically, these signaling programs generate a state of active readiness that allows efficient and prompt immune responses against pathogens.

Our long-term goal is to understand the host-microbial relationship in the gut and to apply this knowledge for health and disease. Current vaccine strategies and immune therapies could be vastly improved through further advances in mucosal immunology.

PD-1 regulates bacterial communities in the gut

PD-1 is a co-inhibitory receptor expressed on activated T cells. The germinal center (GC) T cells, generally known as T follicular helper (T_{FH}) cells, express high amounts of PD-1. PD-1-deficiency leads to species-specific, antibody-mediated autoimmune diseases. Interestingly, the incidence of disease in PD-1-deficient mice varies among mouse colonies, depends on AID and is nil in germ-free conditions. These observations suggest that autoreactive antibodies in PD-1-deficient mice may arise after AID-induced genetic alterations in GCs, which are driven by stimulation from the gut microflora. Indeed, we found that PD-1 regulates the gut microbiota. The total numbers of 'healthy' bacteria such as *Bifidobacterium* and *Bacteroides* were undetectable or markedly reduced in PD-$1^{-/-}$ mice. By contrast, bacteria of the *Enterobacteriaceae* family, which are minor representatives in the small intestine of WT mice, were significantly increased in PD-$1^{-/-}$ mice. These results obtained by 16S rRNA gene pyrosequencing of cecal contents are shown in Figure 1A. At the Phylum level, PD-$1^{-/-}$ mice had increased percentages of Firmicutes and Proteobacteria and a decreased frequency of the Bacteroidetes compared with WT mice. Importantly, the skewed microbiota in the gut induces hyperactivation of the systemic immune system in PD-$1^{-/-}$ mice. Indeed, along with T cell and GC B cell hyper-

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3. Maruya M., Suzuki K., Fujimoto H., Miyajima M., Kanagawa O., Wakayama T., Fagarasan S. Vitamin A-dependent transcriptional activation of the nuclear factor of activated T cells c1 (NFATc1) is critical for the development and survival of B1 cells. ***Proc Natl Acad Sci U S A***. 108, 722-7 (2011).

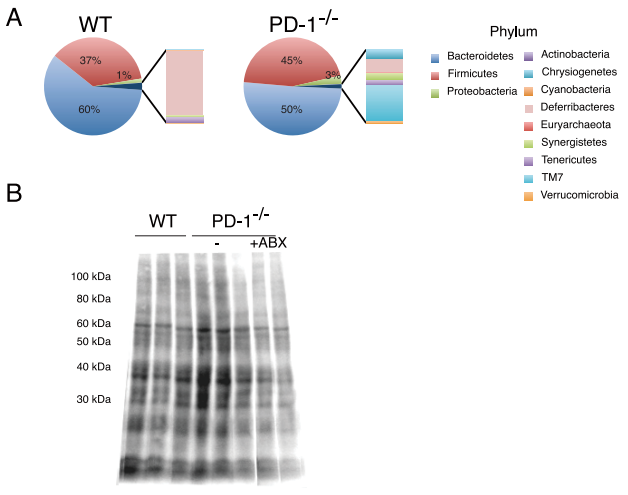

Figure 1: Skewed gut microbiota and a defective mucosal barrier in PD-1-deficient mice.
A. Phylogenetic classification of 16S rRNA frequencies in the cecal contents from WT and PD-1$^{-/-}$ mice.
B. Anti-commensal serum IgGs in PD-1$^{-/-}$ mice. Lysed commensal bacteria from cecal contents of SPF WT mice were used as the target antigens in a Western blot, using serum from WT and PD-1$^{-/-}$ mice with or without antibiotic treatment. Note the decrease of commensal specific serum IgG in PD-1$^{-/-}$ mice after antibiotic treatment.

plasia in PPs and peripheral lymph nodes, we found that serum from PD-1$^{-/-}$ mice contained antibodies specific for components of commensal bacteria, indicating a breach in the normal mucosal-systemic compartmentalization (Figure 1B). Importantly, administration of broad spectrum antibiotics led to normalization of the phenotype of PD-1$^{-/-}$ mice. On the basis of our results, we think that the skewed gut microbial communities that result from the dysregulated selection of IgAs (see below), drive the expansion of auto-reactive B and T cells and production of auto-antibodies. This is the subject for future studies.

PD-1 regulates selection of IgA in germinal centers of Peyer's patches

Intestinal IgA production occurs via both T helper cell-dependent and independent pathways. Diversification of the IgA repertoire by somatic hypermutation (SHM), however, takes place mostly in specialized microenvironments called germinal centers (GC), in which B cell interaction with T_{FH} cells induces the expression of activation-induced cytidine deaminase (AID). PD-1-deficiency generates an excess number of T_{FH} cells (Figure 2) with altered phenotypes (less IL-21, more IFN-γ production), resulting in dysregulated selection of IgA precursor cells in the GCs of Peyer's patches. In short, a large number of IgA B cells, selected and non-selected, receive T_{FH} help and differentiate into IgA plasmablasts and plasma cells.

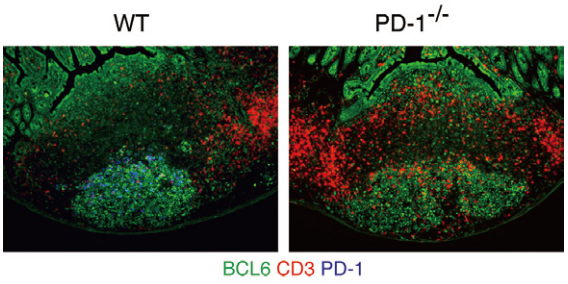

Figure 2: Enlarged germinal centers and excessive number of TFH cells in PPs of PD-1$^{-/-}$ mice. Representative sections of the PPs stained as indicated to reveal the structure and characteristics of GCs.

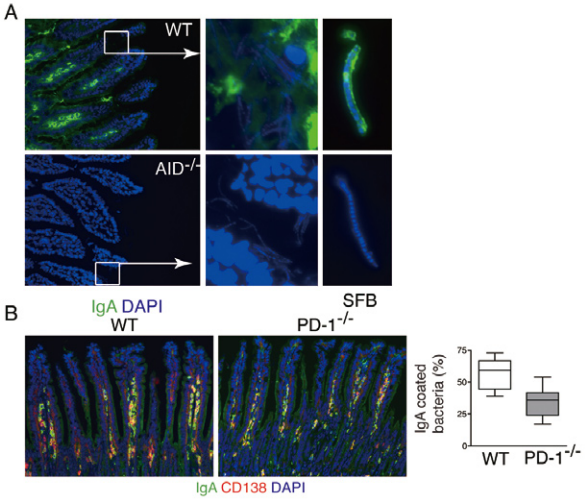

Figure 3: Bacteria shielding by IgAs in the gut.
A. Pictures showing in green IgA plasma cells in lamina propria or secretory IgAs coating bacteria in the intestinal lumen. Blue stains the DNA of eukaryotic and prokaryotic cells. Note the absence of IgAs and the SFB attached directly to the epithelium in AID$^{-/-}$ mice.
B. Representative sections of the small intestine stained as indicated to reveal IgA plasma cells in the lamina propria and the frequency of IgA-coated bacteria as determined by flow cytometry.

The production of IgA by plasma cells in the gut is critical for the containment of the gut commensal microflora, partly through bacterial shielding, as shown in Figure 3A. In the absence of IgA coating, such as in AID$^{-/-}$ mice, we observed expansion of segmented filamentous bacteria (SFB) that attach to the epithelial cells. In PD-1-deficient mice the IgAs, even though produced in large amounts by normal numbers of IgA plasma cells sitting in the lamina propria, have reduced bacteria-binding capacity as shown in Figure 3B. Our results indicate that PD-1 plays a critical role in regulation of antibody diversification required for the maintenance of intact mucosal barrier. Ongoing experiments aim at understanding the nature of T_{FH} cells in PD-1$^{-/-}$ mice and how they impact on selection of IgA-producing cells in the gut.

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Antibodies are crucial effectors for host defense mediated by neutralization and opsonization of pathogens and by activation of the complement system. Antibodies are also important regulators of immune responses, acting through binding to the Fc receptors expressed by various immune cells. Insufficient or excess production of antibodies can lead to immunodeficiencies or autoimmune diseases, respectively. The goal of our current research is to understand how B cell activation and differentiation are positively and negatively regulated to allow appropriate production of diversified antibodies of different classes and to achieve effective humoral immunity.

Uncover new pathways that regulate B cell activation and differentiation through analysis of mice deficient in B cell-specific genes

With the tools provided by completion of human and mouse genome projects, the availability of microarray data, and the technical advancement in generation of gene-manipulated mice, we have taken a molecular genetic approach to study B cell activation and differentiation. We performed comprehensive searches of the RCAI RefDIC and public microarray databases, and have identified several uncharacterized genes that are exclusively expressed in B cells, including the germinal center (GC) subset of B cells and other subsets. We have established knockout mice for these genes and obtained some interesting preliminary results. Deficiency of an ELL-associated molecule resulted in increased survival of the GC B cells and enlarged GC after immunization with a protein antigen (Figure 1). Further analysis of these and additional mice deficient in the other B cell-specific genes should allow us to uncover new pathways that regulate various aspects of B cell activation, differentiation and antibody production.

Explore the mechanism of A/T mutations in GC B cells

High-affinity antibodies are central to humoral immunity. Somatic hypermutation (SHM) of Ig genes in GC B cells is an essential process for generating high-affinity B cells. SHM is initiated by the activation-induced cytidine deaminase (AID), which is thought to convert cytosine (C) to uracil (U) and generate U:G lesions on DNA. Consistent with its substrate specificity, ectopic expression of AID in fibroblasts induces

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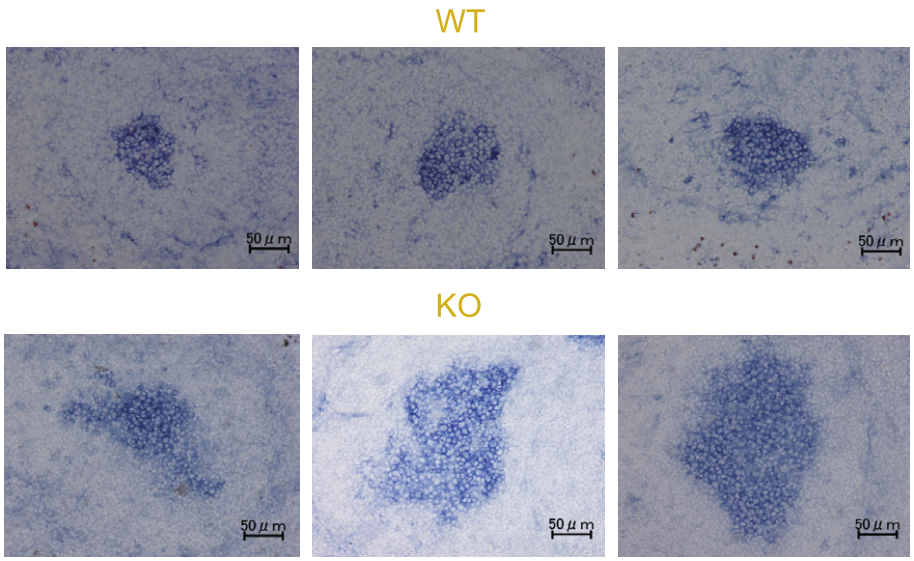

Figure 1 : Enhanced GC formation in mice deficient in an ELL-associated molecule. Three pairs of WT and KO mice were immunized with 100 μg of NP-chicken gamma globulin in alum and two weeks later spleen sections were stained with peanut agglutinin (PNA) to detect GCs. Representative staining from each mouse is shown.

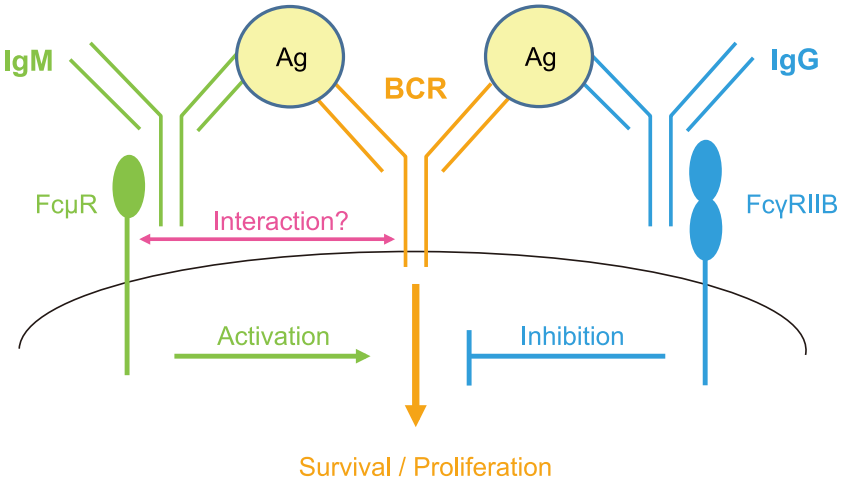

Figure 2 : B cells express two types of Fc receptors with opposing functions. FcγRIIB is known to inhibit B cell activation upon IgG/antigen binding. We found that FcμR is a positive regulator of B cell activation. Therefore, B cell activation can be positively and negatively regulated by IgM and IgG, respectively. It remains to be investigated whether FcμR can directly interact with the IgM B cell antigen receptor (BCR) and provide a survival and proliferative signal.

mutations predominantly at C/G bases. Remarkably, half of the mutations in Ig genes of GC B cells are induced at non-targeted A/T bases. We found that induction of A/T mutations is dependent on the GC B cell environment but independent of the target gene. Experiments are in progress to address how AID-triggered U:G lesions lead to mutations at A/T bases in GC B cells but not fibroblasts.

Elucidate the function of the IgM Fc receptor in the humoral immune response

The existence of a receptor for IgM (FcμR) was first suggested 40 years ago, but the gene encoding FcμR was identified only recently. FcμR is expressed exclusively in B cells in mice and in B and T cells in humans. In collaboration with the Laboratory for Epithelial Immunobiology of RCAI and the University of Alabama at Birmingham, we found that FcμR positively regulates B cell activation and the humoral immune response (Figure 2).

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Memory B cells are antigen (Ag) experienced cells in a resting state, have a unique morphology and phenotype and acquire several intrinsic properties that differ from other B cell stages, such as longevity and rapid response to antigen reexposure by differentiating into plasma cells. However, how and when memory B cells are established in the immune system and the mechanisms underlying their maintenance and prompt differentiation remain obscure. Our aim is to understand the origin and developmental pathways of memory B cells and the regulatory networks responsible for the acquisition of memory B cell properties and functions. Furthermore, we wish to understand the dynamics of human B cells under physiological and pathological conditions in common variable immunodeficiency (CVID), which is characterized by a reduced number of memory B cells in the peripheral blood.

While it is known that mutated, high-affinity memory B cells originate in GCs, the memory compartment consists of both mutated and non-mutated cells and little is known about the cell population dynamics generating this heterogeneity. Our studies indicate that within the first week post-immunization, IgG1$^+$ non-mutated memory B cells develop with T-cell help, prior to GC formation. This population establishes a long-lived IgG1 memory compartment in which, over time, the non-mutated cells are gradually replaced by mutated GC B cell progeny. Non-mutated memory B cells are capable of inducing an IgG1 secondary response and attain functional maturation to elicit a more robust response, which correlates with specific changes in gene expression. Thus, our results demonstrate that the IgG1 memory B cells are generated by two distinct pathways and that the memory compartment undergoes qualitative changes after its initial establishment early in the immune response (submitted).

Thus, B cell memory sustains two classes of antibody repertoire, V_H genes with germline sequence and those accumulating somatic hypermutations (SHM) as a consequence of selection in the GC. We find that IgG1 memory B cells respond to antigen reexposure and accumulate mutations in their rearranged V_H genes through the GC reaction, regardless of whether they express unmutated or mutated or low-affinity or high-affinity V_H genes. In the secondary response, unmutated B cells generated a large number of siblings that accumulated mutations resulting in high-affinity amino acid substitutions for the reexposed antigen. These results support the view that memory B cells can reestablish

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2. Fujimura T, Yonekura S, Horiguchi S, Taniguchi Y, Saito A, Yasueda H, Inamine A, Nakayama T, Takemori T, Taniguchi M, Sakaguchi M, Okamoto Y. Increase of regulatory T cells and the ratio of specific IgE to total IgE are candidates for response monitoring or prognostic biomarkers in 2-year sublingual immunotherapy (SLIT) for Japanese cedar pollinosis. ***Clin Immunol*** 2011 139(1):65-74.
3. Fujimura T, Yonekura S, Taniguchi Y, Horiguchi S, Saito A, Yasueda H, Nakayama T, Takemori T, Taniguchi M, Sakaguchi M, Okamoto Y. The Induced Regulatory T

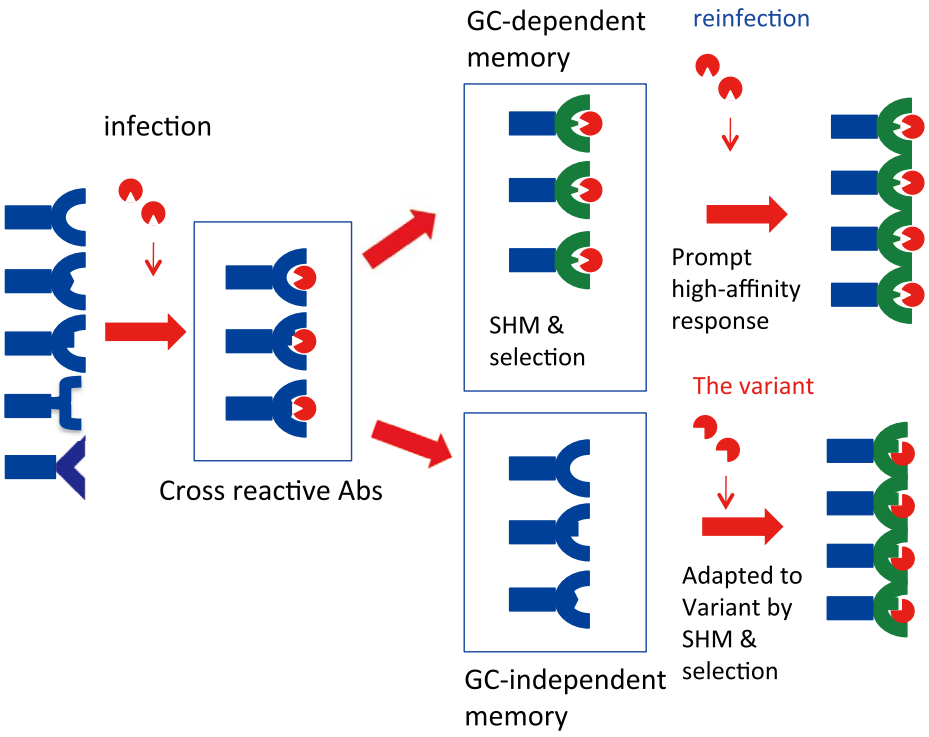

Figure: **Both unmutated and mutated memory B cells are required for protection against pathogens.** After infection several B cells that cross-react with the initiating pathogen are selected and differentiate into memory cells in GCs or prior to GC formation. GC-dependent memory cells express high-affinity antibodies for the initiating pathogen as a consequence of SHM and selection, whereas GC-independent memory cells sustain a cross-reactive Ab repertoire. Upon reinfection with a variant of the initiating pathogen that emerges due to mutations accumulating during the spreading infection, unmutated memory B cells easily recognize the variant by cross-reactivity and undergo adaptation to the variant epitope as a consequence of subsequent SHM and selection, which takes place in the preformed GCs after affinity maturation.

the antibody repertoire that is adapted to the reexposed antigen. The primary infection caused production of long-term and high-affinity antibodies against the initiating pathogens, a mechanism that is critical for protection against reinfection with the same pathogen. The generation of new high-affinity somatic mutants in the memory compartment, in addition to the preexisting antibodies, are advantageous for the system to rapidly and efficiently exclude the initiating pathogen. Of importance, the germ line encoded antibody repertoire in the memory compartment flexibly adapts to the variants of invading pathogens by cross-reactivity and provides a large amount of high-affinity antibodies via SHM. These could be useful as a first-line of defense against common pathogens.

It is known that CXCR5^{high}/PD-1^{high} T follicular helper (Tfh) cells play an important role in GC development. Consistently, our analysis suggests that depletion of Tfh significantly affects the generation and maintenance of high-affinity memory cells and plasma cells. By contrast, development of early memory B cells and plasma cells does not require the help of Tfh, indicating that this B cell response is regulated with the help of different T cell subsets. After antigen encounter, CD4 T cells begin to express BCL6 at the border of the B- and T-cell area after interaction with dendritic cells. This is followed by their development into Tfh cells upon interaction with B cells, which takes outside and inside of GCs. Thus our results support the view that during the process of CD4 T cell differentiation, B cells interact with T cells and differentiate into early memory B cells, which localize to the B cell follicle. We are currently analyzing the features of the non-Tfh cells required for memory B cell generation early after immunization by using CD4 T cells with conditional deletion of *Bcl6*. CD4 memory T cell development is blocked by deletion of *Bcl6* in CD4 T cells, which has prompted us to also analyze how memory T cell development is regulated.

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Laboratory for

Host Defense

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Successful host defense in vertebrates requires both innate and adaptive immunity. The innate system functions as a pathogen sensor and is involved in the initial effort to eradicate an infection. Furthermore, innate immunity also contributes to the establishment of adaptive immunity. Dendritic cells (DCs) are antigen presenting cells critically involved in a sequence of these immune responses. DCs sense various pathogen-derived molecular components and exert their immunostimulatory functions by producing inflammatory cytokines or upregulating expression of costimulatory molecules. Those pathogen components are called immune adjuvants based on these DC activating abilities. Immune adjuvants are recognized by various types of pattern recognition receptors including Toll-like receptors (TLRs). Identification of new types of immune adjuvants and characterization of the mechanisms by which they activate DCs should contribute to development of novel immunoregulatory maneuvers. We are attempting to clarify how DCs are activated through pattern recognition receptors and to obtain critical information for manipulating the immune response effectively. Various immune adjuvants including TLR ligands and gene targeted mice are important tools for this purpose.

Clarifying the molecular mechanisms for type I IFN production by plasmacytoid dendritic cells.

DCs sense nucleic acid adjuvants and produce type I interferon (IFN) in a subset-dependent manner. Among nucleic acid sensors, TLR7 and TLR9 are peculiar in that they recognize not only pathogen- but also host-derived nucleic acids. Accumulating evidence suggests that TLR7/9-induced type I IFN production play important roles in the pathogenesis of autoimmune disorders such as SLE.

The plasmacytoid dendritic cell (pDC) is notable for its ability to produce large amounts of type I IFNs, both IFN-α and IFN-β, in response to signaling through nucleic acid sensors such as TLR7 and TLR9. We have clarified that a serine threonine kinase, IκB kinase α (IKKα), is critical for type I IFN production from TLR7/9-stimulated pDC. IKKα interacts with and phosphorylates a transcription factor, IRF-7, which is critical for type I IFN induction. Critical involvement of IKKα in human pDC was verified with an IKK inhibitor, BAY11-7082. The inhibitor suppressed IFN-α, but not TNF, production by TLR7- or TLR9-stimulated human pDC at less than 1 μM. Nuclear translocation of IRF-7 was also impaired by the inhibitor. Thus, the IKK-IRF-7 axis should also play a critical role in human pDC.

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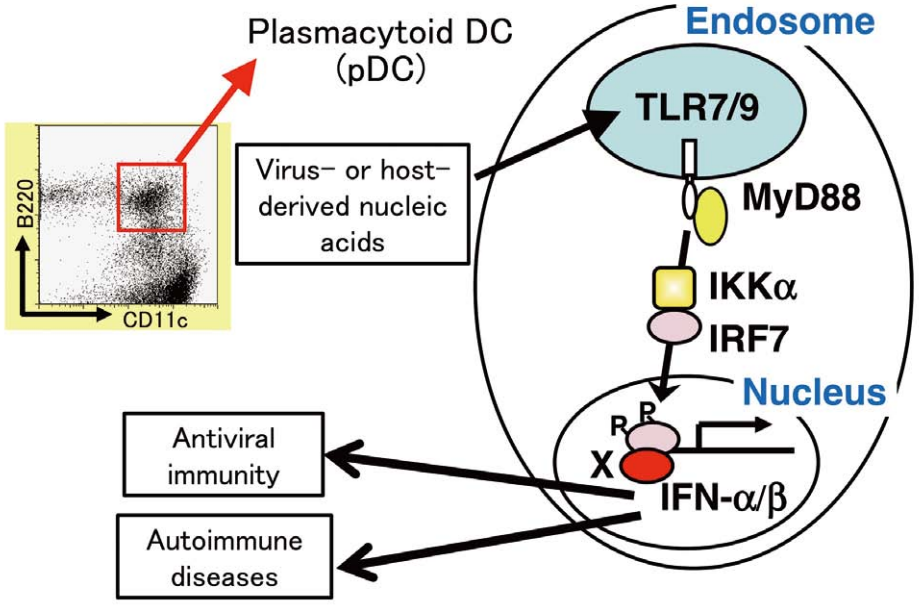

Figure : **Type I interferon production by a TLR7/9-stimulated plasmacytoid dendritic cell.** Plasmacytoid dendritic cells (pDC) belong to a DC subset that is distinguished by the ability to produce large amounts of type I interferon upon TLR7/9 signaling. TLR7/9 can respond not only to virus- but also host-derived nucleic acids and pDC-derived type I IFNs play important roles in both antiviral immunity and pathogenesis of autoimmune diseases. In addition to the IKKα-IRF-7 axis, pDC-specific molecules are also involved in this pDC-specific signal transduction pathway.

Statins are inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase and commonly used for treatment of hypercholesterolemia. Beneficial effects of statins have been reported in autoimmune diseases, but the underlying molecular mechanisms are still unclear. We have found that statins can inhibit IFN-α production and nuclear translocation of IRF-7 in TLR7- or TLR9-stimulated human pDC.

IRF-7 expression is constitutively high in pDC and critical for pDC-specific functions, e.g. the ability to produce large amounts of type I IFNs. However, IRF-7 is abundantly induced also in other DC subsets and IRF-7 alone cannot account for pDC-specific functions. Among pDC-specific genes, we have found a transcription factor that can transactivate the type I IFN promoter in synergy with IRF-7 (Figure).

Clarifying the regulatory mechanisms for a TLR3 expressing DC subset

TLR3 is a sensor for dsRNA and specifically expressed on CD8α⁺ DC among murine splenic DC subsets. The CD8α⁺ DC is distinguished by its high activity to incorporate dead cells and to crosspresent antigens to generate cytotoxic CD8⁺ T cell responses, thereby playing key roles in anti-viral or anti-tumor immunity. TLR3 signaling can augment crosspresentation activity of CD8α⁺ DC. The CD8α⁺ cDC also has a high capacity to produce proinflammatory cytokines in response to signaling by various TLRs. However, it remains unknown whether or how the CD8α⁺ cDC is involved in a variety of immune responses or inflammatory conditions.

We have found that a chemokine receptor, Xcr1, is exclusively expressed by CD8α⁺ cDC. Expression of human Xcr1 was specific to BDCA3⁺ DCs, which are a human counterpart of murine CD8α⁺ cDC (*BBRC* 397:756, 2010). By using a knock-in strategy into the *Xcr1* locus, we have generated gene-manipulated mice in which CD8α⁺ cDC can be traced or deleted. We plan to clarify the *in vivo* behavior and biological roles of CD8α⁺ cDCs in various immune responses and inflammatory conditions. Imaging analysis is ongoing in collaboration with Dr. Takaharu Okada (Research Unit for Immunodynamics).

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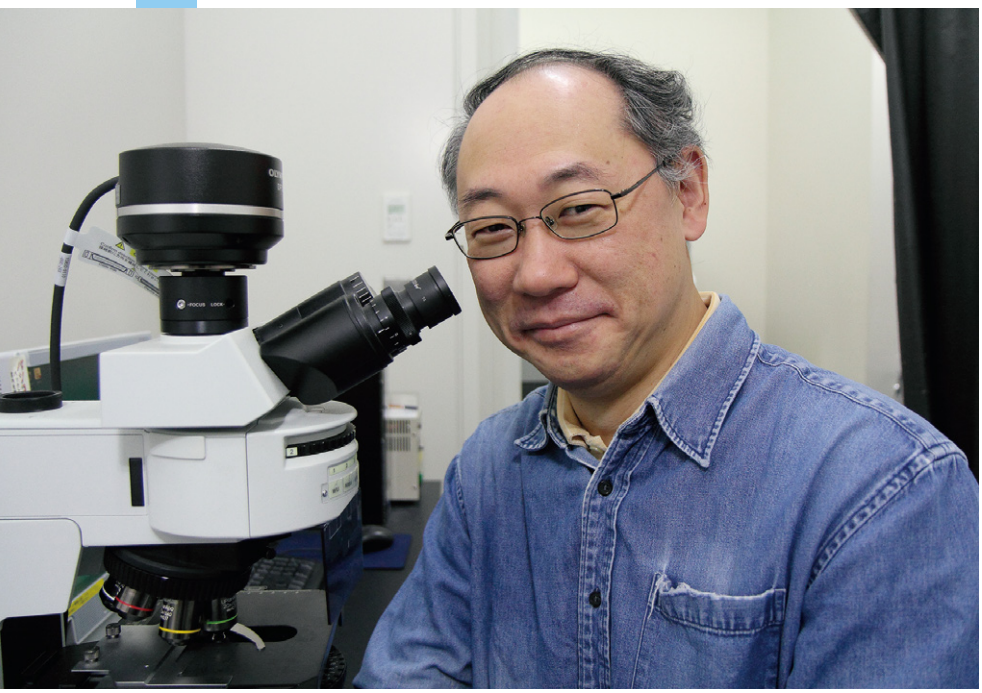
Laboratory for

Infectious Immunity



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MHC class II and CD86 are critical molecules for initiation of immunity and but are also related to immune diseases, therefore, it is important to understand how these molecules are regulated *in vivo*. In this vein, we discovered an E3 ubiquitin ligase referred to as MARCH-I. The expression level of MHC class II-peptide complexes (pMHC II) is regulated through MARCH-I-mediated ubiquitination of the MHC II β chain. In the steady state, MARCH-I is expressed mainly in the antigen-presenting cells (e.g. DCs, B cells and macrophages). In the case of DCs, pMHC II is continuously ubiquitinated by MARCH-I when the cells are in an immature state; however, this ubiquitination is quickly terminated after DC maturation as a result of downregulation of MARCH-I. Thus, loss of pMHC II ubiquitination and downregulation of MARCH-I are thought to be an important step in the DC maturation process. Since the stabilization of pMHC II is increased by loss of pMHC II ubiquitination, regulation of MARCH-I is expected to be a critical process for initiation of immunity and maintenance of tolerance. At present, we are intensively examining this issue by generating suitable genetically modified mice.

MARCH-I belongs to a family of membrane-bound E3 ubiquitin ligases, referred to as MIR (modulator of immune recognition). The MIR family consists of viral MIRs and their mammalian homologues including MARCH-I. Importantly, the different MIR family members recognize different membrane molecules. Viral MIRs encoded by human herpesvirus 8 were shown to down-regulate MHC class I and CD86, suggesting that these MIRs are part of the herpesvirus immune evasion strategy. Therefore, from the clinical perspective, it is important to understand the molecular basis of MIR substrate recognition. However, it is still difficult to uncover how MIRs recognize their substrates in detail because of the difficulty in preparing MIR proteins. To address this issue, we are employing an integrative approach for viral MIRs.

Regulatory T cells induce DC apoptosis by loss of pMHC II in the steady state

Downregulation of MARCH-I and loss of MHC II ubiquitination are DC maturation processes; however, the biological

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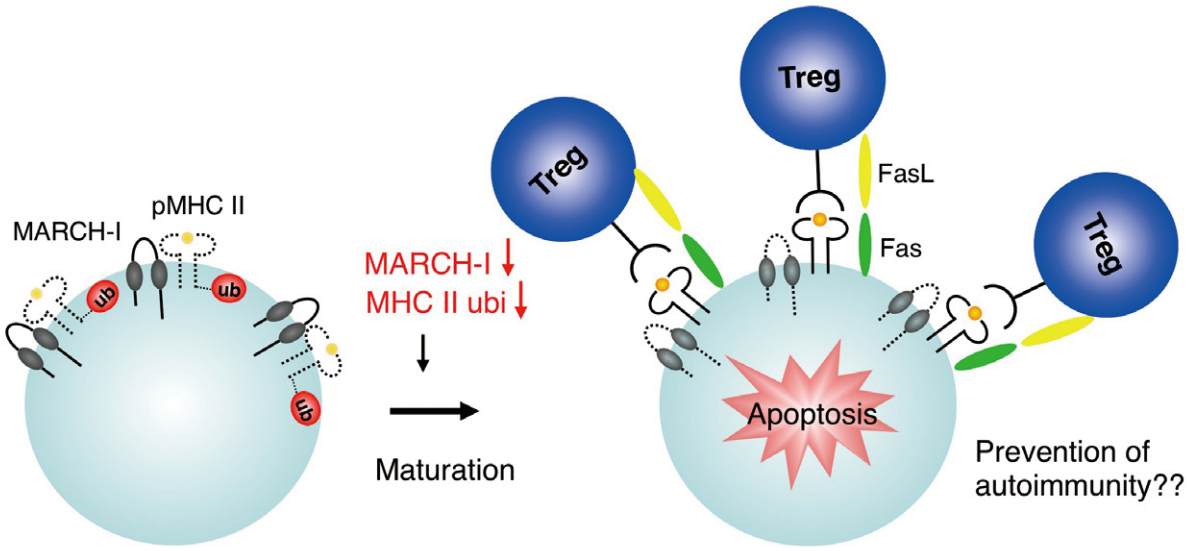

Figure : **Induction of DC apoptosis by natural Tregs through stabilization of pMHC II**
In immature DCs, MHC class II-peptide complexes (pMHC II) are continuously degraded as a result of MARCH-I-mediated ubiquitination (*left*). During the process of maturation, the function of MARCH-I is inhibited and pMHC II is stabilized through loss of ubiquitination. Stabilized pMHC II is effectively recognized by natural Tregs, and the mature DCs undergo apoptosis through interaction between Fas and FasL. This regulation might be critical for prevention of autoimmunity.

consequences of these events *in vivo* are unknown. To address this point, we have generated genetically modified mice in which the *March1* gene can be conditionally deleted by administration of 4-hydroxytamoxifen (OHT). After deletion of MARCH-I, caspase 8-dependent apoptosis was observed in splenic DCs. Given that caspase 8 is involved in Fas or TNF-mediated apoptosis, the contribution of these pathways was examined by using blocking antibodies. This experiment showed the importance of Fas-FasL-mediated signals. By adoptive transfer experiments of BMDCs and the analysis of an MHC II KI in which the ubiquitination site was mutated, stabilization of pMHC II on the cDCs as well as appropriate *in vivo* microenvironments were shown to be required for DC apoptosis. Importantly, deletion of $CD25^+$ CD4 T cells prevented DC apoptosis. Finally, adoptively transferred $FoxP3^+$ CD4 T cells induced apoptosis in BMDCs whose MHC II was stabilized by loss of ubiquitination. These results suggest that in the steady state, maturation might lead to the suicide of DCs in an antigen-dependent manner. Given that DCs must be tightly regulated to prevent excessive immunity, loss of pMHC II should be critical for maintenance of tolerance in the steady state. Right now, we are studying how MARCH-I is regulated in the steady state to test our hypothesis.

Inter-transmembrane region of viral MIR2 contributes to CD86 downregulation

Human herpesvirus 8 (HHV8), a human tumor virus, encodes two MIRs, MIR 1 and 2, to evade host immunity. Both MIR1 and MIR2 downregulate the surface expression of major histocompatibility complex class I (MHC I) molecules through ubiquitin-mediated endocytosis followed by lysosomal degradation. Since MIR2 additionally downregulates a costimulatory molecule CD86 and an integrin ligand CD54, it is thought to be a more important molecule for the immune evasion than MIR1. To address the molecular basis of substrate specificity of MIR2, we examined which regions of B7-2 and MIR2 are required for MIR2-mediated B7-2 downregulation. Experiments using domain-swapping chimeras between human B7-2 and CD8α, a non-MIR2 substrate, and also between MIR1 and MIR2 demonstrated a significant contribution of the juxtamembrane (JM) region of B7-2 and the inter-transmembrane (ITM) region of MIR2 to MIR2-mediated suppression. Computational simulation and mutagenesis analyses suggest that Phe119 and Ser120 of the MIR2 ITM and Asp244 in the B7-2 JM region contribute to the recognition of B7-2 by MIR2. This finding sheds new insight into the molecular basis of substrate recognition by MIR family members, and suggests a novel tactic for drug design to treat HHV8-related cancer.

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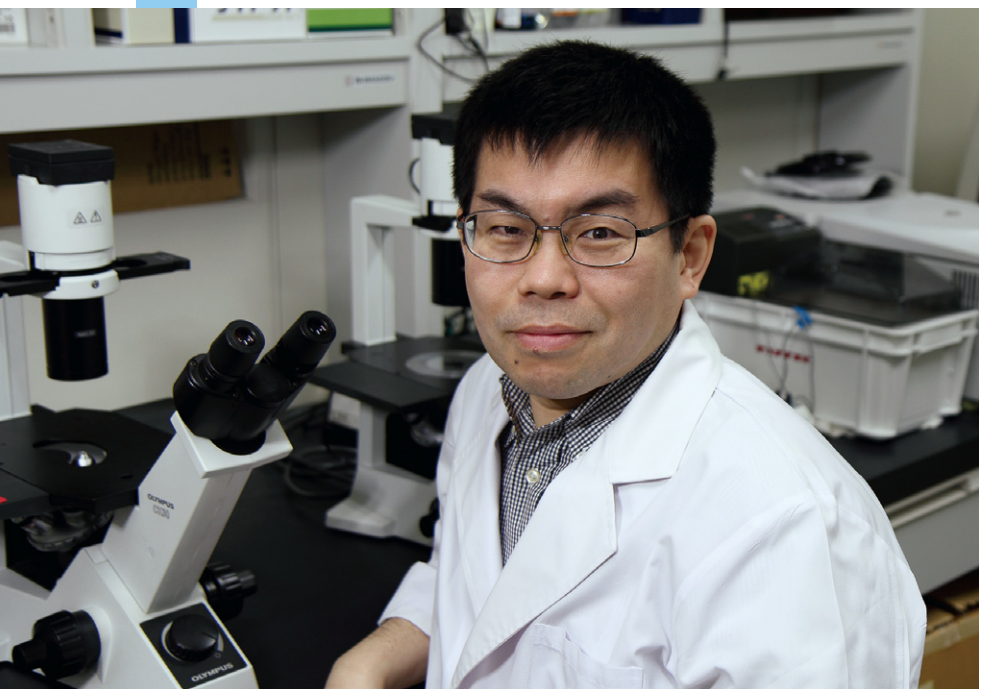
Laboratory for

Innate Cellular Immunity



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Phagocytes, such as macrophages and dendritic cells (DCs), play important roles as a first-line defense for the immune system and exhibit a number of activities. At the site of bacterial infection, macrophages rapidly recognize invading bacteria via a wide variety of surface receptors that bind components found on bacterial surfaces. Phagocytes also have the ability to recognize invading microorganisms through pattern-recognition receptors such as toll-like receptors (TLRs). TLRs recognize microbial components, known as pathogen-associated molecular patterns, including LPS and nucleic acids. Through the recognition by these receptors, phagocytes can distinguish microorganisms, resulting in distinct and appropriate antipathogenic responses including the production of inflammatory cytokines and chemokines. These factors induce inflammation, and the inflammatory response plays a critical role in the effective clearance of invading microorganisms and the induction of appropriate adaptive immunity.

On the other hand, phagocytes also have the ability to recognize dead “self” cells. Apoptotic cell death is an evolutionally conserved physiologic process vital to the elimination of unnecessary cells. Apoptotic cell death also takes place during tissue injury and inflammation, and phagocytes rapidly recognize these cell corpses for removal. The corpse clearance is executed so rapidly by phagocytes that it is extremely difficult to find dying/dead cells that are not associated with phagocytes even in the thymus, where a large number of thymocytes die by apoptosis under physiological conditions because they have failed selection. This rapid clearance of dead cells prevents the release of potentially toxic or immunogenic intracellular materials from the cell corpses. Therefore, the prompt elimination of dying cells and damaged resident cells is thought to be required for the maintenance of tissue integrity, the resolution of inflammation, and normal tissue repair.

The Laboratory for Innate Cellular Immunity is investigating the molecular mechanisms for recognition and phagocytosis of dying cells, and the pathological relevance of impaired phagocytosis to inflammatory disorders including autoimmune diseases.

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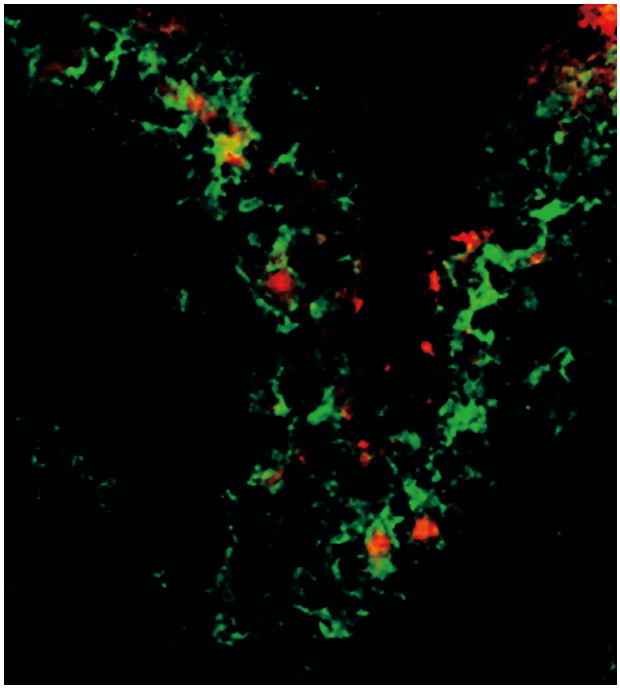

Figure : **CD169-positive macrophages(*Green*) in the lymph node sinus engulf subcutaneously injected dead tumor cells(*red*).**

Critical Roles of Sinus Macrophages in Anti-tumor Immunity

Phagocytes, such as macrophages and DCs, swiftly phagocytose apoptotic cell corpses by recognizing molecules exposed only on the surface of the corpses. Defects in this process result in autoimmune disorders, indicating that apoptotic cell clearance by phagocytes essentially contributes to the maintenance of self-tolerance under physiological conditions. Consistent with these findings, the intravenous injection of apoptotic cells induced antigen-specific immunosuppression or tolerance to cell associated proteins. For such tolerance induction, both rapid corpse clearance by splenic macrophages in the marginal zone (MZ) and also selective phagocytosis and subsequent antigen presentation by the splenic DC subpopulation located in the MZ are required.

On the contrary, subcutaneously injected apoptotic cells are often immunogenic and researchers have taken advantage of immunogenic tumor corpses for tumor vaccination. These findings suggest that apoptotic cells in the periphery are cleared and processed in a different way from blood-borne apoptotic cells in spleen. Antigen presentation in peripheral LNs is thought to be coordinately executed by migratory DCs from peripheral tissues and LN-resident APCs. However, little is known about the role of different APCs in the clearance of dead cells and in the presentation of dead cell-associated antigens in peripheral tissues.

We recently documented how tumor cell-associated antigens derived from dead tumor cells are crosspresented in the draining LNs. We concluded that macrophages that reside in the LN sinus take up dead tumor cells and directly crossprime CTLs. Mice lacking sinus macrophages at the time of dead tumor cell vaccination failed to induce anti-tumor immunity. These findings are expected to improve our understanding of how to optimally generate and activate tumor- specific T cell immunity in response to tumor cell death.

CD169-positive macrophages as first-line defenders

The LN sinus is a highway that links afferent and efferent lymph and is believed to be a filtering zone for lymph-borne molecules. Large particles, including cellular antigens in the lymphatic fluid, make initial contact with $CD169^+$ macrophages that serve as sentinels located along the length of the LN sinus. In the case of spleen, $CD169^+$ marginal metallophilic macrophages as well as marginal zone macrophages are located between the lymphoid compartment and the scavenging red pulp compartment. These macrophages are well equipped to constantly screen the blood for foreign particles and organisms, as well as aberrant molecular debris and dying cells. We can detect the $CD169^+$ macrophage subpopulation in the boundary border between the outer environment and the tissue in several organs. We speculate that this macrophage subpopulation plays a critical role as a sentinel in each organ, and we are studying the physiological and pathological roles of these macrophages.

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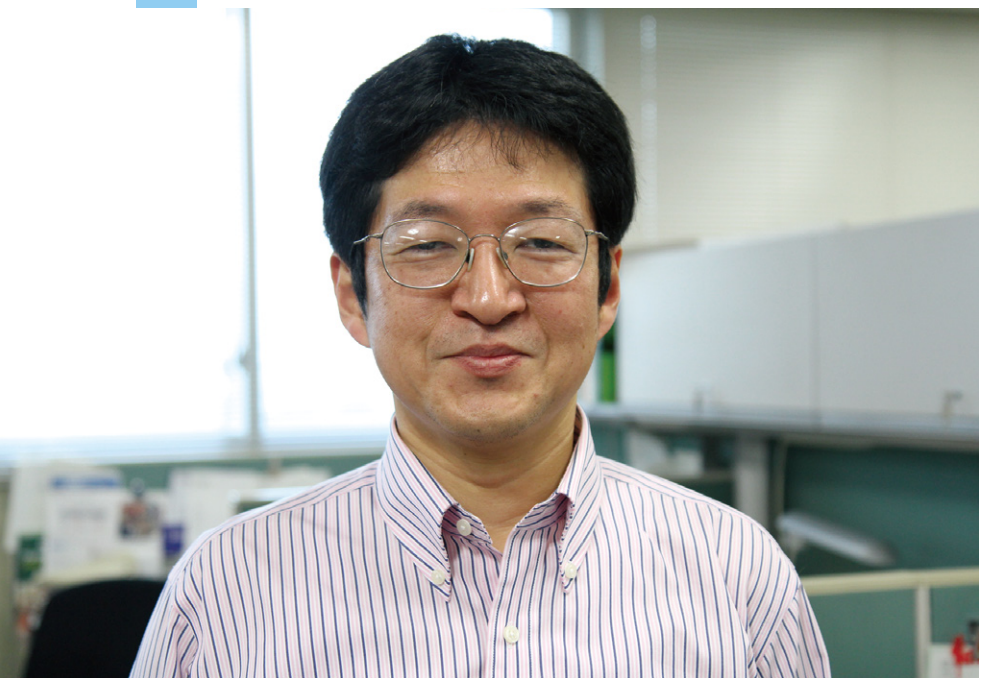
Research Unit for

Inflammatory Regulation



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The inflammatory response is an important host defense mechanism to sense and eliminate invading microbial pathogens. Dendritc cells first detect pathogens by their pathogen sensors (e.g. Toll-like receptors [TLR]). TLR signaling then leads to the activation of the transcription factor NF-κB, which enters the nucleus and induces the expression of a series of inflammation-related genes, including those encoding proinflammatory cytokines such as interleukin-6 (IL-6) and IL-12. These inflammatory responses then direct T-helper (Th) lymphocyte differentiation into distinct effector T cell subsets, such as Th1, Th2 and Th17, to combat various pathogens. These initially helpful inflammatory responses must be terminated at the appropriate time point, however, otherwise excessive responses can damage normal tissue and may cause autoimmune or allergic diseases. Our research goal is to identify key regulators of inflammation-related signal transduction pathways and to clarify the molecular mechanisms for regulating inflammatory responses. These studies should contribute to the development of new therapeutic tools to control the exaggerated inflammation seen in certain human diseases. Our research now focuses on the role of PDLIM2 (PDZ and LIM-domain protein-2) and related LIM proteins in the negative regulation of inflammatory responses.

PDLIM2 inhibits granulomatous inflammation

Granuloma formation is an important host defense mechanism against intracellular bacteria. However, uncontrolled granulomatous responses cause tissue damage and impair normal organ function in several human autoimmune diseases, such as Crohn's disease and sarcoidosis. Recent studies have implicated exaggerated Th17 cell-mediated responses in the development of granulomatous inflammation, suggesting that controlling Th17 cell development will be an important strategy for preventing and treating granulomatous diseases. PDLIM2, also known as SLIM (STAT-interacting protein), is a nuclear protein that contains both PDZ and LIM domains and belongs to a large family of LIM proteins. PDLIM2 was originally isolated as a nuclear ubiquitin E3 ligase for the STAT4 transcription factor in $CD4^+$ T cells, suppressing Th1 cell differentiation (Tanaka T et al, *Immunity*, 2005). We have recently shown that PDLIM2

Recent publications

1. Tanaka, T., Yamamoto, Y., Muromoto, R., Ikeda, O., Sekine, Y., Grusby, M., J., Kaisho, T., Matsuda, T. PDLIM2 inhibits T Helper 17 cell development and granulomatous inflammation through degradation of STAT3. ***Sci. Signal.*** 4(202), ra85 (2011)
2. Yan, P., Fu, J., Qu, Z., Li, S., Tanaka, T., Grusby, M.J., Xiao, G. PDLIM2 suppresses HTLV-I Tax-mediated tumorigenesis by targeting Tax into the nuclear matrix for proteasomal degradation. ***Blood*** 113, 4370-4380 (2009)
3. Kaisho, T. and Tanaka, T. Turning NF-κB and IRFs on and off in DCs. ***Trends Immunol.***, 29, 329-336 (2008)

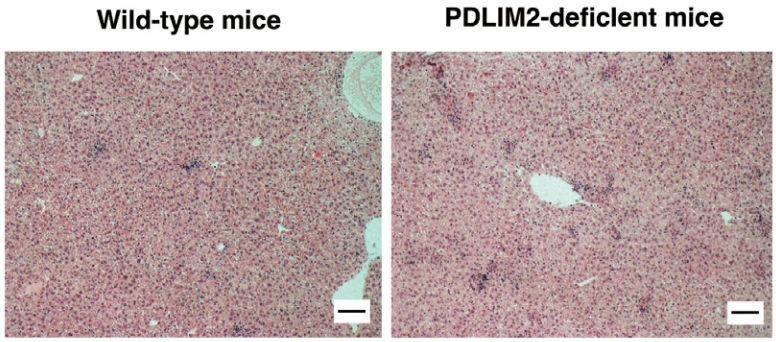
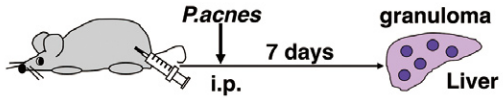


Figure 1: Enhanced *P. acnes*-induced liver granuloma formation in PDLIM2-deficient mice. Hematoxylin and eosin stained liver sections on day 7 after intraperitoneal injection of heat-killed *P. acnes*. Scale bars, 100 μm.



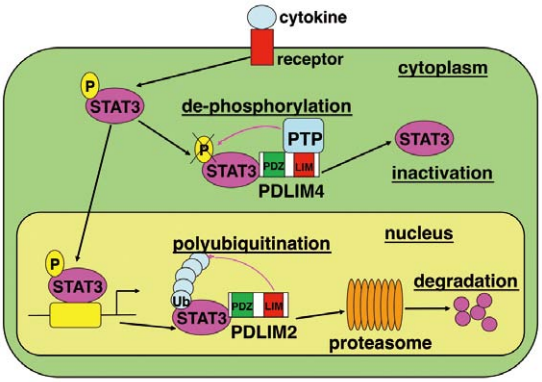


Figure 2: PDLIM2 and PDLIM4 regulate Th17 cell-mediated inflammatory responses through different mechanisms. PDLIM2 interacts with STAT3 and promotes its ubiquitin/proteasome-dependent degradation in the nucleus, whereas PDLIM4 binds to STAT3, recruits PTP and enhances its dephosphorylation in the cytoplasm.

negatively regulates Th17 development and granulomatous responses by acting as a nuclear ubiquitin E3 ligase targeting STAT3, the transcription factor critical for commitment to the Th17 lineage (Tanaka T et al, *Sci. Signal.*, 2011). PDLIM2 promoted polyubiquitination and proteasome-dependent degradation of STAT3, thereby disrupting STAT3-mediated gene activation. PDLIM2 deficiency resulted in an increase in nuclear STAT3 and enhanced Th17 cell differentiation. There was also exacerbated liver granuloma formation induced by *Propionibacterium acnes*, a gram-positive intracellular bacterium that has been suggested to be involved in the pathogenesis of human sarcoidosis. Our findings delineate an essential role of PDLIM2 in negatively regulating Th17-mediated inflammatory responses and provide a potential therapeutic target for autoimmune diseases.

The role of LIM proteins in the regulation of inflammatory responses

The LIM protein family is classified into three groups and PDLIM2 belongs to Group 3. About 10 proteins are included in Group 3, but their functions in the immune system remain unclear. We are in the process of clarifying how these LIM proteins differentially regulate inflammatory responses. STAT transcription factors are critical for T-helper cells to differentiate into particular lineages of effector T cell subsets. We recently found that PDLIM4, another LIM protein family member, is a negative regulator of STAT-mediated signaling. In contrast to PDLIM2, PDLIM4 is located in the cytoplasm. PDLIM4 bound to STAT3, STAT4 and STAT6, and suppressed activation of target genes. Interestingly, PDLIM4 did not promote polyubiquitination and degradation of these STAT molecules, but instead inhibited the phosphorylation of tyrosine residues essential for cytokine-induced STAT activation. We further demonstrated that PDLIM4 bound to and recruited PTP-BL, a protein tyrosine phosphatase, and facilitated dephosphorylation of STAT proteins. PDLIM4-deficiency in $CD4^+$ T cells resulted in augmented tyrosine phosphorylation of these STAT proteins and consequently enhanced Th1, Th2 and Th17 cell differentiation. Our findings delineate an essential role for PDLIM4 in negatively regulating STAT-mediated effector T cell differentiation through a mechanism distinct from PDLIM2. Collectively, LIM proteins may be a new family of adaptors that can negatively regulate signal transduction pathways in the immune system.

4. Tanaka, T. Grusby, M.J. and Kaisho, T. PDLIM2-mediated termination of transcription factor NF-κB activation by intranuclear sequestration and degradation of the p65 subunit. ***Nat. Immunol***. 8, 584-591 (2007)

5. Nakahira, M., Tanaka, T., Robson, B.E., Mizgerd, J.P. and Grusby, M.J. Regulation of Signal Transducer and Activator of Transcription Signaling by the Tyrosine Phosphatase PTP-BL. ***Immunity*** 26, 163-176 (2007)

Research Unit for

Therapeutic Model

Unit Leader :
Kanako Shimizu

Technical staff : **Miki Asakura**

The goal of our laboratory is to develop immunotherapeutic models for cancer. We have been focusing on the biological role of dendritic cells (DCs) *in vivo* as a link between innate and adaptive immunity. NKT cells have unique immunoregulatory features that include the ability to rapidly produce large quantities of cytokines. We have attempted to develop an approach for inducing adaptive immunity based on the adjuvant effect of NKT cell ligands and using *in vivo* DC maturation, which we have found to be more effective than *ex vivo* manipulation of DCs. We have recently established an immunotherapeutic strategy using adjuvant vector cells (AVCs), which are glycolipid-loaded, allogeneic fibroblasts that can be induced to express virtually any antigen by transfecting them with antigen-encoding mRNA. These AVCs enhance both innate (NKT and NK cells) as well as adaptive (T cells) immunity. We are now focusing on the mechanism of DC maturation. Also, to prepare for the launch of clinical studies, we have been performing preclinical studies in collaboration with Dr. Fujii (Cellular Immunotherapy Unit) under the support and training program for translational research of Japan. Second, we have established a method of screening the *i*NKT cell ligand-loading capacity of antigen presenting cells. This approach demonstrated that *in vivo* murine NKT cell responses can be used to quantitate the α-GalCer loading capacity of human APCs.

Antigen mRNA-transfected, allogeneic fibroblasts loaded with NKT cell ligand confer antitumor immunity.

A collaboration with Drs. Fujii (Cellular Immunotherapy Unit) and Ishii (Vaccine Design Team) in RIKEN RCAI, Dr. Mizuno (Yamaguchi Univ.), Dr. Kakimi (Tokyo Univ.) and Dr. Maeda (Iwate Medical Univ.)

We previously demonstrated that the administration of α-GalCer-loaded tumor (tumor/Gal) cells could generate innate and adaptive immunity through the maturation of DCs in a tumor specific manner. In the current study, instead of tumor cells, we used allogeneic fibroblast cells loaded with

Recent publications

1. Fujii S., Shimizu K. (2011) DC-based immunotherapy targeting NKT cells. In Terabe M and Berzofsky JA (eds), ***Natural killer T cells: Balancing the regulation of tumor immunity***. Springer New York Dordrecht Heidelberg London, 95-110.
2. Shimizu K., Asakura M., Fujii S. Prolonged antitumor NK cell reactivity elicited by CXCL-10-expressing dendritic cells licensed by CD40L^{+}CD4^{+} memory T cells. ***J. Immunol***. 186, 5927-37 (2011)
3. Shimizu K, Hidaka M, Bickham K, Moriwaki M, Fujimoto K, Fujii S. Human leukemic cells loaded with α-GalCer activate murine NKT cells in situ. ***Int J Hematol*** 92, 152-60 (2010)

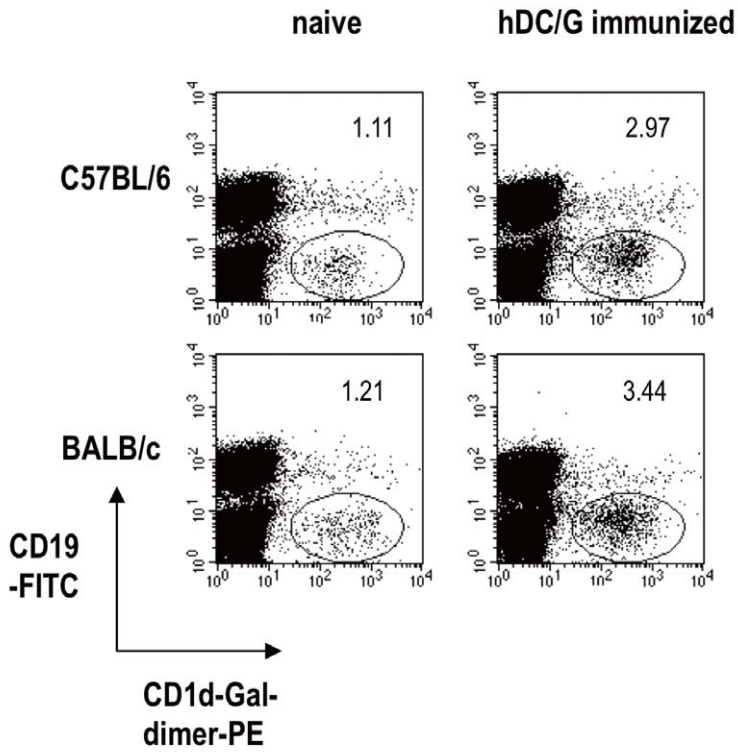


Figure : **Human DC/Gal activate primary NKT cells from mice of different genetic backgrounds**
Murine *i*NKT cell activation following i.v. injection of human DC/Gal was evaluated in C57BL/6 and BALB/c mice. Two days after injection, spleen cells were collected and the number of CD1d/Gal-dimer$^+$CD19$^-$ cells was assessed by flow cytometry. This method should be helpful for evaluation of human antigen presenting cells loaded with *i*NKT cell ligand.

α-GalCer and transfected with antigen-encoding mRNA as adjuvant vector cells (AVCs), thus combining the adjuvant effects of *i*NKT cell activation with delivery of an antigen of choice to DCs *in vivo*. As preclinical studies, we have begun murine and canine experiments to evaluate the safety profile and immune responses. These aAVCs administered to mice and dogs activate invariant NKT (*i*NKT) cells as well as elicit antigen-specific T cell responses with no adverse events. By harnessing the innate immune system and generating an adaptive immune response to a variety of antigens, this unique tool could prove clinically beneficial in the development of immunotherapies for malignant and infectious diseases.

Human leukemic cells loaded with α-galactosylceramide (α-GalCer) activate murine *i*NKT cells *in situ* -a method to quantitate the α-GalCer loading capacity of human APCs.

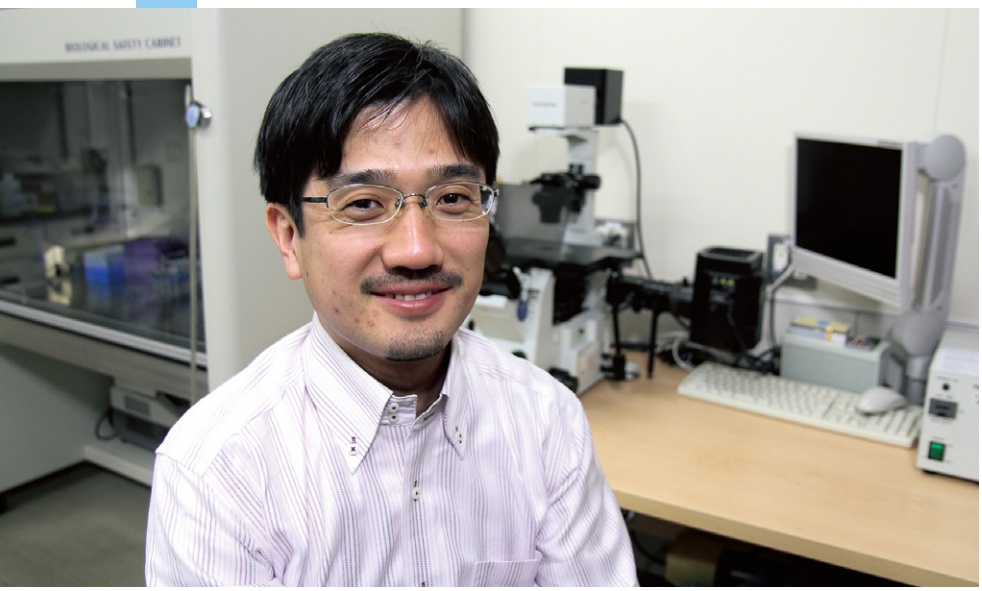
Invariant NKT cells (*i*NKT) cells become activated after stimulation with antigen presenting cells (APCs) loaded with the NKT cell ligand, α-galactosylceramide (α-GalCer). When we investigated whether human APCs loaded with α-GalCer have the ability to activate NKT cells in mice, we found that the human DCs stimulated the secretion of IFN-γ by activated murine NKT cells when injected into C57BL/6 mice. Furthermore, the number of transferred hDC/Gal cells correlated with the number of recovered IFN-γ-producing spleen cells, indicating that the capacity of APCs to load α-GalCer can be measured by IFN-γ release in an ELISPOT assay. Finally, α-GalCer-loaded human leukemic cells, both cell lines and primary leukemic cells, injected into C57BL/6 mice also had the capacity to stimulate murine NKT cells *in vivo*. These results indicate that *in vivo* murine NKT cell responses can be used to quantitate the α-GalCer loading capacity of human APCs. This method could be utilized to develop future immunotherapies in which NKT cells are targeted for activation.

4. Fujii S, Motohashi S, Shimizu K, Nakayama T, Yoshiga Y, Taniguchi M. Adjuvant activity mediated by *i*NKT cells. ***Semin Immunol*** 22, 97-102 (2010)

5. Fujii S, Goto A, Shimizu K. Antigen mRNA-transfected, allogeneic fibroblasts loaded with NKT-cell ligand confer antitumor immunity. ***Blood*** 113, 4262-72 (2009)

Research Unit for

Immune Homeostasis



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Technical Staff : **Takahisa Miyao**
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The ultimate goal of our laboratory is to understand the basic rules of immunological tolerance and homeostasis. While the clonal selection theory has predicted the importance of cell intrinsic mechanisms such as clonal deletion and anergy, their significance in naturally acquired self-tolerance still remains elusive. Over the last two decades, evidence has accumulated that natural self-tolerance may be rather dominant and based on cell-extrinsic regulation of autoaggressive lymphocytes by other functional classes of lymphocytes, in particular by regulatory T (Treg) cells exhibiting suppressive functions. Our studies have demonstrated that a subset of Treg cells characterized by expression of the transcription factor Foxp3 plays an essential role in the establishment and maintenance of natural self-tolerance and immune homeostasis, particularly at the environmental interfaces.

In order to understand the principles of immunological tolerance and homeostasis, it is crucial to understand how the immune system robustly maintains tolerance to "self" yet allows for aggressive responses toward "non-self" in the face of diverse and unpredictable perturbations from the internal as well as external environments. To address this question, we are currently investigating how Foxp3$^+$ Treg cells respond to extrinsic signals and deal with environmental changes to ensure the robustness of self-tolerance and homeostasis.

Plasticity of Foxp3$^+$ T cells: the controversy resolved

The emerging notion of environment-induced Treg cell reprogramming remains highly controversial. We and others have previously shown that at least some of Foxp3$^+$ T cells can differentiate into Foxp3$^-$ helper T (Th) cells in response to environmental changes such as lymphopenia or inflammation, whereas other recent reports argue against the plasticity phenomena. We have recently proposed that this controversy can be resolved by considering the "heterogeneity model" of plasticity, which hypothesizes that the observed plasticity of Foxp3$^+$ T cells does not reflect lineage reprogramming of Treg cells but a minor population of uncommitted Foxp3$^+$ T cells. By genetic fate mapping or adoptive transfers, we have addressed the nature of such uncommitted Foxp3$^+$ T cells and identified them as a minor population of non-regulatory cells exhibiting promiscuous and transient Foxp3 expression. Such cells give rise to Foxp3$^-$ Th cells and selectively accumulate in inflammatory cytokine milieus or in lymphopenic environments including

Recent publications

1. Miyao T., Floess S., Setoguchi R., Luche H., Fehling H.J., Waldmann H., Huehn J., Hori S. Plasticity of Foxp3$^+$ T cells reflects promiscuous Foxp3 expression in conventional T cells but not reprogramming of regulatory T cells. ***Immunity*** 36, 262-275 (2012)
2. Hori S: Stability of regulatory T-cell lineage. ***Adv Immunol***. 112, 1-24 (2011)
3. Hori S: Regulatory T cell plasticity: beyond the controversies. ***Trends Immunol***. 32, 295-300 (2011)

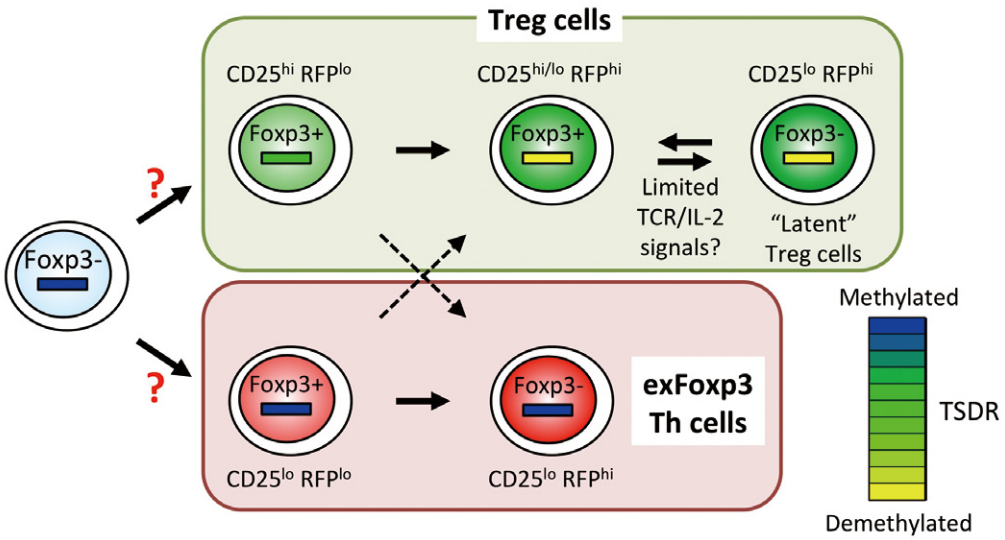


Figure : **A model of Treg cell lineage commitment.** During thymic or peripheral Treg cell differentiation, TCR and other signals lead to Foxp3 up-regulation. (The RFP reporter visualizes Foxp3$^+$ cells and their progeny, independently of continuous or transient Foxp3 expression, see Miyao et al. *Immunity* for details.) However, not all Foxp3$^+$ T cells are committed to the Treg cell fate, because some others transiently up-regulate Foxp3 without acquiring a Treg cell phenotype and function. The latter population eventually loses Foxp3 expression and differentiates into Foxp3$^-$ (exFoxp3) Th cells, while Treg cells eventually undergo complete demethylation of the TSDR to stabilize Foxp3 expression. Nevertheless, Treg cells may transiently lose Foxp3 expression in environments where TCR and/or IL-2 signals are limited but retain an epigenetic memory of Foxp3 expression and suppressive function ("latent" Treg cells). Thus, the TSDR appears to act as a "memory module" that ensures the committed state of Treg cells in a changing environment. The initial signals that lead to the "imprinting" of a stable Treg cell phenotype remain to be uncovered however.

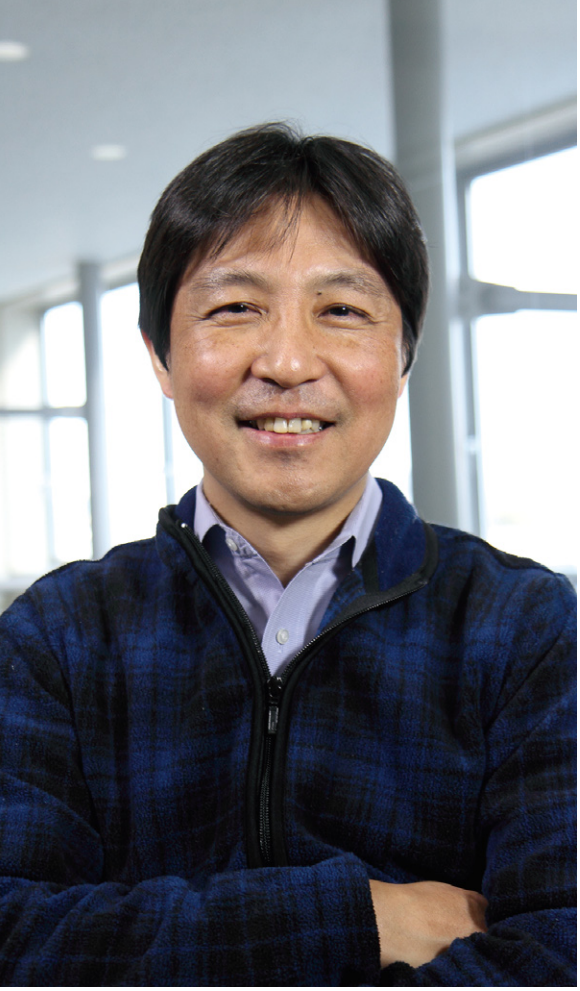
those in early ontogeny. By contrast, Treg cells did not undergo reprogramming under those conditions irrespectively of their thymic or peripheral origins. Moreover, although a few Treg cells transiently lose Foxp3 expression, such "latent" Treg cells retain phenotypic memory and robustly re-express Foxp3 and suppressive function upon activation. The stable differentiated state of Treg cells was associated with complete DNA demethylation of a conserved non-coding region termed Treg-specific demethylation region (TSDR) within the *Foxp3* locus. This study established that Treg cells constitute a stable cell lineage, whose committed state in a changing environment is ensured by an epigenetic mechanism, irrespective of ongoing Foxp3 expression. Our next focus is to discover the signals that lead to irreversible commitment to the Treg cell fate.

Genetic and environmental control of Treg cell fitness in peripheral tissues

In an attempt to understand the molecular mechanisms by which Foxp3 controls Treg cell differentiation and function, we have addressed whether and how *Foxp3* gene mutations that had been identified in human IPEX impinge on Treg cell development and function, using retroviral gene transduction strategies and knock-in mutagenesis in mice. Serendipitously, we have identified one hypomorphic mutation that had no effect on the *in vitro* suppressive activity, thymic differentiation, or global gene expression profile of Foxp3$^+$ T cells. This mutation, however, led to impaired fitness of CCR7^{lo} "tissue-seeking" Foxp3$^+$ T cells. Accordingly, we have found that mutant Foxp3$^+$ T cells are much less competitive than wild-type Foxp3$^+$ T cells in peripheral tissues, although the extent of the reduction of mutant Foxp3$^+$ T cells differed from tissue to tissue, suggesting an environmental control of Treg cell fitness. Importantly, the impaired fitness of mutant Foxp3$^+$ T cells was associated with reduced expression of one transcription factor and was corrected by its retroviral transduction. We suggest that fitness of Treg cells in tissue environments is controlled by both genetic and environmental factors and represents an essential determinant of self-tolerance and immune homeostasis. We are currently investigating i) the molecular nature of such environmental factors; ii) how such extrinsic cues are translated into Foxp3-dependent intracellular control mechanisms, and iii) how "fit" Treg cells in turn ensure the fitness of peripheral tissues.

4. Hori, S. Developmental plasticity of Foxp3$^+$ regulatory T cells. ***Curr Opin Immunol***. 22, 575-582 (2010)

5. Komatsu N., Mariotti-Ferrandiz M.E., Wang Y., Malissen B., Waldmann H., Hori S. Heterogeneity of natural Foxp3$^+$ T cells: a committed regulatory T cell lineage and an uncommitted minor population retaining plasticity. ***Proc Natl Acad Sci USA.*** 106, 1903-1908 (2009)



Laboratory for
Immunochaperones

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Technical Staff : **Chiaki Kajiwara**
Takashi Matsuura
Yu Kato

Student Trainee : **Shizuha Uda**

CD8⁺ T cells recognize naturally processed peptides in the context of MHC class I molecules. During viral infection or malignant transformation, intracellular changes in non-APC (antigen presenting cells) must be reported to CD8⁺ T cells, which are indispensable in fighting virus-infected cells and cancer cells. However, these abnormal cells cannot act by themselves as APC to prime T cells, although they express tumor or viral antigens on their cell surface. In addition, many viruses cannot infect professional APC, such as dendritic cells (DCs), and thus the APC cannot present such viral antigens directly to CD8⁺ T cells. Therefore, DCs must internalize neighboring tumor or infected cells, digest them, and then present antigen peptides to CD8⁺ T cells in the context of MHC class I molecules. This pathway is called cross-presentation and is believed to be specific to DCs and not other APC types such as macrophages or B cells. We have evidence that molecular chaperones such as hsp90 play important roles in this antigen cross-presentation. One of our research goals is to identify the mechanisms of cross-presentation, especially focusing on the role of the molecular chaperone hsp90. During the study of cross-presentation, we developed a mAb that can efficiently detect cell surface hsp90 on DCs and macrophages. Since hsp90 has no transmembrane domain, it must be associated with unknown molecules for anchorage to the cell surface. We are currently examining the physiological significance of cell surface hsp90 in the context of the immune response.

The role of hsp90 in antigen cross-presentation.
In general, the main functions of HSPs are (i) facilitate protein folding/refolding and prevent aggregation of newly synthesized proteins on the ribosome; (ii) maintain the normal function of mature proteins; (iii) target misfolded and/or damaged mature proteins for degradation by the proteasome; (iv) transport proteins between distinct intracellular compartments such as the cytosol and mitochondria. Proteins targeted to the proteasome undergo degradation in order to recycle amino acids and the byproducts of this process (degradation products, peptides) are utilized as antigen peptides, mainly presented by MHC class I molecules. Thus, proteasomal degradation is important at the end of the life cycle of all proteins to prevent waste of raw materials and is used by the immune system to alert T cells of any alterations

Recent publications

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2. Imai T, Kato Y, Kajiwara C, Mizukami S, Ishige I, Ichiyanagi T, Hikida M, Wang JY, Udono H. HSP90 contributes to cytosolic translocation of extracellular antigen for cross-presentation by dendritic cells. ***Proc Natl Acad Sci USA.*** 108, 16363-16368, 2011.
3. Ichiyanagi T, Imai T, Kajiwara C, Mizukami S, Nakai A, Nakayama T, Udono H. Essential role of endogenous HSP90 of dendritic cells in antigen cross-presentation. ***J Immunol.*** 185, 2693-2700, 2010.

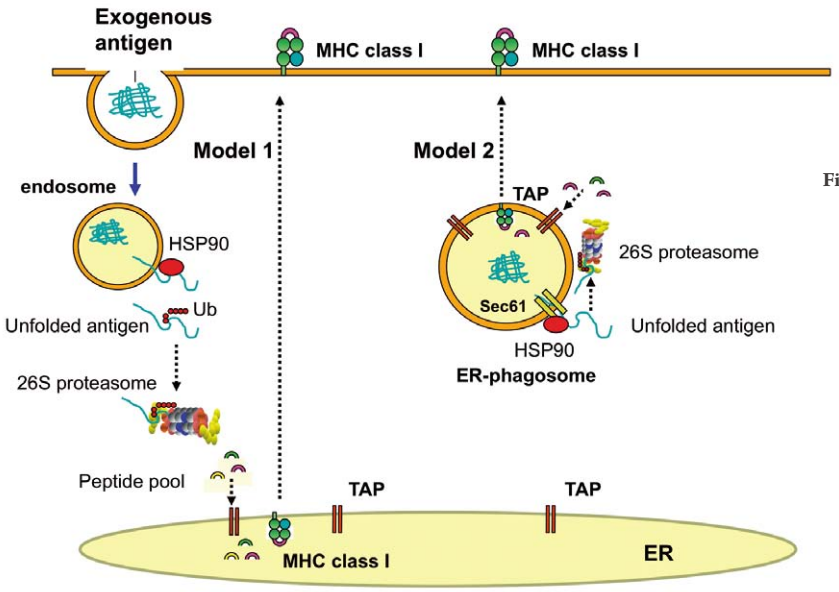


Figure: **Models for cross-presentation of extracellular antigen by DCs after its translocation into the cytosol by HSP90.** DCs take up extracellular antigen through the endosome/phagosome pathway. Cytosolic HSP90 binds to the relatively unfolded antigen as it emerges across the endosomal membranes through a putative translocon (Sec61 complex?) and promotes its translocation into the cytosol. Once the extracellular antigen appears in the cytosol it can be processed by the conventional MHC I pathway: degraded by the proteasome into short peptides that are loaded onto MHC I molecules in the ER. $CD8^+$ T cells then recognize the MHC class I-epitope complex on the cell surface. In model 2, the proteasome is attached on the cytosolic face of the ER-phagosome and immediately degrades translocated antigen into short peptides. The peptides then re-enter the ER-phagosome from which it was translocated.

in the intracellular antigenic milieu. Each step towards proteolysis by the proteasome is regulated, at least in part, by HSPs.

Regarding how HSPs are involved in cross-presentation, we recently demonstrated that HSPs direct retrotranslocation of proteins from the endosome (*PNAS* 2011). Cross-presentation and -priming were decreased in both HSP90α-null-DCs and -mice. $CD8\alpha^+$DC apoptosis mediated by translocation of exogenous cytochrome c to the cytosol was also eliminated in HSP90α-null mice. Ag translocation into the cytosol was diminished in HSP90α-null DCs and in DCs treated with an HSP90 inhibitor. Ag within purified phagosomes was released in an HSP90-dependent manner. Our results demonstrate that cytosolic HSP90 pulls endosomal Ag out into the cytosol during cross-presentation.

The role of cell surface hsp90 on dendritic cells.

With a novel mAb, 6H8, we found that HSP90 is also expressed on the surface of bone marrow derived dendritic cells (BMDCs), macrophages and splenic DCs after heat killed *Propionibacterium acnes* injection. The 6H8 mAb bound to DCs was rapidly internalized. Incubation of 6H8 coupled with OVA (6H8-OVA) with DCs resulted in efficient cross-presentation of the dominant $OVA_{257-264}$epitope, a process that was blocked by free 6H8 mAb, indicating that the cross presentation truly depends on cell surface HSP90. Alexa-labeled 6H8 mAb accumulated mainly on $CD8^+$ and to a lesser extent on $CD8^-$ DCs *in vivo* one hour after intravenous injection. Administration of less than a microgram of a 6H8-OVA conjugate induced vigorous proliferation of adoptively transferred OT-I $CD8^+$ T cells, a response that did not occur in DC-deficient mice or TAP (transporter associated with antigen processing) 1-deficient mice. Furthermore, 6H8-OVA induced CTL in naïve mice protected them from lung metastasis with melanoma MO5 cells expressing OVA. Thus, targeting cell surface HSP90 by 6H8 coupled antigens of interest enables cross-priming but not cross-tolerance of antigen-specific $CD8^+$ T cells, raising the possibility of a novel antibody-based vaccine strategy for induction of cytotoxic T cell immunity.

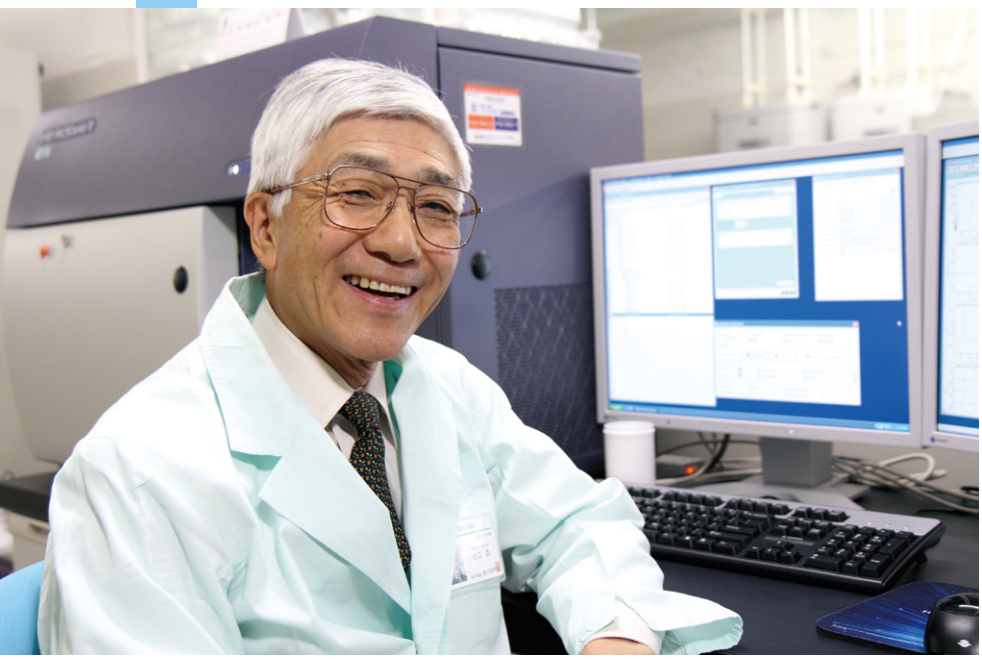
In addition, we are currently investigating more fundamental aspects of this process – the physiological significance of cell surface hsp90 and the mechanism by which hsp90 is localized on the cell surface of DCs and macrophages. A full answer to these questions may open an avenue to a novel role of the molecular chaperone hsp90 in immunity.

4. Ohkusu-Tsukada K, Toda M, Udono H, Kawakami Y, Takahashi K. Targeted inhibition of the p38 MAP kinase of IL-10-secreting $CD25^-$ T regulatory cells in cancer immunotherapy. ***Eur J Immunol.*** 40,1011-1021, 2010.

5. Udono H, Ichiyanagi T, Mizukami S, Imai T. Heat Shock Proteins in Antigen Trafficking -Implications on Antigen Presentation to T cells- ***Intl J Hyperthermia.*** 25, 617-625, 2009.

Laboratory for

Immune Regulation



Group Director :
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Senior Scientist :	**Hiroshi Watarai**
Research Scientists :	**Takuya Tashiro** **Michishige Harada** **Diana Eissens** **Masao Shiozaki**
Visiting Scientist :	**Nyambayar Dashtsoodol**
Technical Staff :	**Sakura Sakata** **Etsuko Sekine-Kondo** (until Aug 2011) **Yuko Nagata** **Tomokuni Shigeura**
Student Trainee :	**Yue Ren (IPA)**
Intern :	**Sabrina Hamouche** (from Jan 2012)

NKT cells act as innate immune cells but also bridge the innate and acquired immune systems. The nature of the NKT cell response is dictated by the initial cytokine environment: interaction with IL-10-producing cells induces negative regulatory T_H2/regulatory T cell-type NKT cells, while that with IL-12-producing cells results in pro-inflammatory T_H1-type responses. A prime example of one such function is adjuvant activity: NKT cells augment anti-tumor responses by their production of IFN-γ, which acts on NK cells to eliminate MHC negative target tumor cells and also on CD8 cytotoxic T cells to kill MHC positive tumor cells. Thus, upon administration of α-GalCer-pulsed DCs, both MHC negative and positive tumor cells can be effectively eliminated. Based on these findings, we have developed NKT cell-targeted adjuvant cell therapies with strong antitumor activity in humans.

A phase I-II clinical study of α-galactosylceramide (α-GalCer)-pulsed IL-2/GM-CSF-cultured peripheral blood mononuclear cells in patients with advanced and recurrent non-small cell lung cancer.

A Phase I/IIa clinical trial has been carried out on 17 patients with advanced lung cancer. 60% of patients with high IFN-γ production had a median survival time (MST) of 31.9 months, and the disease was stable with only with a primary treatment. This is significantly more effective than treatment with molecular target drugs, where the MST was 10 months (p=0.0015). These results are encouraging and warrant further evaluation of the survival benefit of this immunotherapy.

For the next step, we have applied in 2011 for the advanced medical care assessment system established in 2008 by the Japanese Ministry of Health, Labor and Welfare (MHLW), so that the patients' immunotherapy treatment will be covered in part by health insurance. The system was established because advanced medical technologies using medical devices or pharmaceuticals, such as immune cell therapy, that are not yet approved under the Pharmaceutical Affairs Law (PAL), are not covered by health insurance in Japan. However in response to recent rapid progress in medical technology and the patients' need to have safe and lower cost treatment with such technologies, this system was introduced to allow health insurance payments. It is also

Recent publications

1. Watarai H, Sekine-Kondo E, Shigeura T, Motomura Y, Yasuda T, Satoh R, Yoshida H, Kubo M, Kawamoto H, Koseki H, Taniguchi M. Development and Function of Invariant Natural Killer T cells Producing T_H2- and T_H17-cytokines. ***PLoS Biol***. 10, e1001255 (2012).
2. Motomura Y, Kitamura H, Hijikata A, Matsunaga Y, Matsumoto K, Inoue H, Atarashi K, Hori S, Watarai H, Zhu J, Taniguchi M, Kubo M. E4bp4, a mammalian basic leucine zipper transcriptinal factor, is a critical regulator of IL-10 and IL-13 production by CD4 T cells. ***Nat Immunol***. 12, 450-459 (2011).
3. Watarai H, Fujii SI, Yamada D, Rybouchkin A, Sakata S, Nagata Y, Iida-Kobayashi M, Sekine-Kondo E, Shimizu K, Shozaki Y, Sharif J, Matsuda M, Mochiduki S, Hasegawa T, Kitahara G, Endo T, Toyoda T, Ohara O, Harigaya KI, Koseki H, Taniguchi M. Murine induced pluripotent stem cells can be derived from and differentiate into natural killer T cells. ***J Clin Invest***. 120, 2610-2618 (2010).

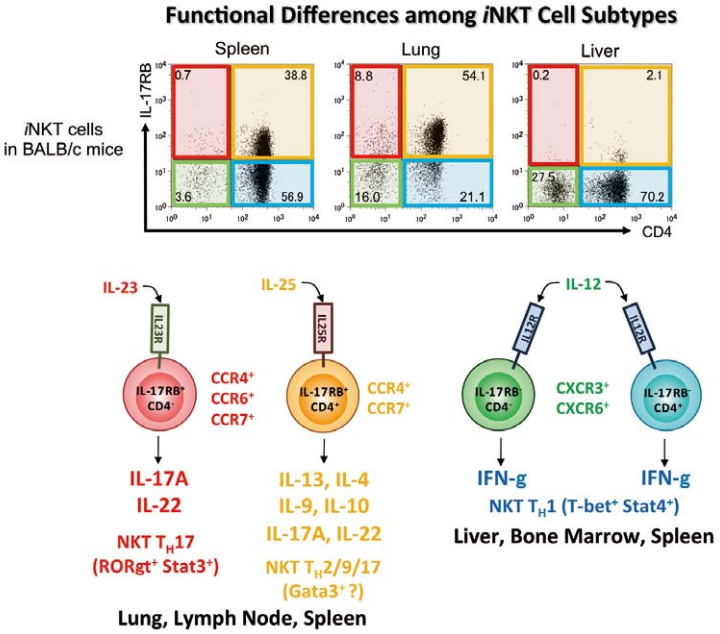

Figure : **The NKT cell subsets.** NKT cells can be divided into four subsets based on the expression of IL-17RB and CD4. The newly identified IL-17RB$^+$ CD4$^-$ and IL-17RB$^+$ CD4$^+$ NKT cells respond to IL-23 and IL-25, respectively, triggering inflammation by the production of TH2/9/17 cytokines, especially in the lung.

the case that the collection of appropriate clinical research data is facilitated by this system, and thus can then lead to approval of the advanced medical technologies under the PAL. The NKT cell-targeted therapy was recently (October, 2011) approved by the government to use the advanced medical care assessment system.

Induced pluripotent stem cells (iPSCs)-derived NKT cells are transplantable without acute graft-vs-host disease (GvHD).

NKT cells show antitumor activity when activated to produce Th1 cytokines by DCs loaded with α-GalCer. However, most cancer patients do not have sufficient numbers of NKT cells to induce an effective immune response in this context, indicating a need for a source of NKT cells that could be used to supplement the endogenous cell population. iPSCs hold tremendous potential for cell-replacement therapy, and we successfully derived iPSCs both from embryonic fibroblasts from mice harboring functional NKT cell-specific rearranged T cell receptor loci in the germline and from splenic NKT cells from adult B6 mice. These iPSCs could be differentiated into NKT cells *in vitro* and secreted large amounts of the T_H1 cytokine IFN-γ. Importantly, iPSC-derived NKT cells recapitulated the known adjuvant effects of natural NKT cells and suppressed tumor growth *in vivo*. Furthermore, iPSC-derived B6 NKT cells, in contrast to B6 CD4 helper T cells, did not induce acute GvHD when transferred into T/B/NKT-deficient Rag1-knockout BALB/c mice. These studies demonstrate the feasibility of expanding functionally competent NKT cells via an iPSC phase, an approach that may be adapted for allogeneic NKT cell-targeted therapy in humans (Patent application).

Development and function of NKT cells producing T_H2- and T_H17-cytokines.

NKT cells are heterogeneous in terms of the expression of CD4 and the IL-17 receptor B (IL-17RB), a receptor for IL-25 and a key factor in T_H2 immunity. However, the development pathway and precise function of these NKT cell subtypes remain unknown. IL-17RB$^+$ NKT cells are present in the thymic CD44$^{+/-}$ NK1.1$^-$ population and develop normally even in the absence of IL-15, which is required for maturation and homeostasis of IL-17RB$^-$ NKT cells producing IFN-γ. These results suggest that NKT cells contain at least two subsets, IL-17RB$^+$ and IL-17RB$^-$. The IL-17RB$^+$ NKT cells can be further divided into two subtypes on the basis of CD4 expression, both in the thymus and in the periphery. CD4$^+$ IL-17RB$^+$ NKT cells produce T_H2 (IL-13), T_H9 (IL-9 and IL-10) and T_H17 (IL-17A and IL-22) cytokines in response to IL-25 in an E4BP4-dependent fashion, whereas CD4- IL-17RB$^+$ NKT cells are a retinoic acid receptor-related orphan receptor (ROR)γt$^+$ subset producing T_H17 cytokines in an E4BP4-independent fashion upon stimulation with IL-23. The IL-17RB$^+$ NKT cells are abundant in the lung in the steady state and mediate the pathogenesis in virus-induced airway hyperreactivity (AHR). In this study we demonstrated that the development of IL-17RB$^+$ NKT cell subsets is distinct from the classical NKT cell developmental stages in the thymus and that these IL-17RB$^+$ NKT cells play important roles in the pathogenesis of airway diseases.

4. Matsuoka N, Itoh T, Watarai H, Sekine-Kondo E, Nagata N, Okamoto K, Mera T, Yamamoto H, Yamada S, Maruyama I, Taniguchi M, Yasunami Y. High-mobility group box 1 is involved in the initial events of early loss of transplanted islets in mice. ***J Clin Invest***. 120, 735-743 (2010).
5. Watarai H, Rybouchkin A, Hongo N, Nagata Y, Sakata S, Sekine E, Dashtsoodol N, Tashiro T, Fujii SI, Shimizu K, Mori K, Masuda K, Kawamoto H, Koseki H, Taniguchi M. Generation of functional NKT cells in vitro from embryonic stem cells bearing rearranged invariant Vα14-Jα18 TCRα gene. ***Blood*** 115, 230-237 (2010).
6. Motohashi S, Nagato K, Kunii N, Yamamoto H, Yamasaki K, Okita K, Hanaoka H, Shimizu N, Suzuki M, Yoshino I, Taniguchi M, Fujisawa T, Nakayama T. A phase I-II study of alpha-galactosylceramide-pulsed IL-2/GM-CSF-cultured peripheral blood mononuclear cells in patients with advanced and recurrent non-small cell lung cancer. ***J Immunol***. 182, 2492-2501 (2009).

Laboratory for

Dendritic Cell Immunobiology

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Dendritic cells (DCs) are essential antigen-presenting cells (APCs) that initiate primary immune responses. DCs are heterogeneous, but the two major subsets are conventional DCs (cDCs) and plasmacytoid DCs (pDCs), which are distinguishable by surface and intracellular phenotypic markers, immunologic function, and anatomic distribution. Immature DCs (iDCs) serve as sentinels, recognizing the presence of invading pathogens or virus-infected cells through various pattern-recognition receptors. Subsequently, they become mature DCs (mDCs) with up-regulated expression of MHC and costimulatory molecules under inflammatory conditions. The mDCs then migrate via the afferent lymphatics into the T-cell areas of secondary lymphoid tissues, where they prime rare antigen-specific naive T cells for differentiation into effector T (T_{eff}) cells, including T helper type (T_H)1 cells, T_H2 cells, and T_H17 cells, depending on environmental cues. DCs thereby play a crucial role in linking innate and adaptive immunity. Conversely, iDCs are also crucial for the induction of immunological tolerance under steady-state conditions. The tolerogenic mechanisms include recessive tolerance mediated by clonal deletion and anergy as well as dominant tolerance involving active immune suppression by $CD4^+Foxp3^+$regulatory T (T_{reg}) cells in the periphery, a function of likely importance in self-tolerance as well as immune disorders and transplant rejection. However, the precise functional role of each DC subset in immune responses remains unclear. Our goal is to clarify the role of DC subsets in the immune system *in vivo* and to identify the molecular basis for the regulation of their function.

Crucial roles of B7-H1 and B7-DC expressed on mesenteric lymph node dendritic cells in the generation of antigen-specific $CD4^+Foxp3^+$ regulatory T cells in the establishment of oral tolerance

Oral tolerance, which generates systemic tolerance to fed antigens, is a key feature of intestinal immunity. However, the molecular mechanism mediating oral tolerance remains unclear. In this study, we examined the role of the B7 family of costimulatory molecules in the establishment of oral tolerance. Deficiencies of B7-H1 and B7-DC abrogated oral tolerance, accompanied by enhanced antigen-specific $CD4^+$T cell-responses and IgG_1 production. Mesenteric lymph node (MLN) dendritic cells (DCs) express higher levels of B7-H1 and B7-DC than systemic DCs, whereas they have similar levels of CD80, CD86, and B7-H2. Owing to their dominant

Recent publications

1. Takagi H., Fukaya T., Eizumi K., Sato Y., Sato K., Shibazaki A., Otsuka H., Hijikata A., Watanabe T., Ohara O., Kaisho T., Malissen B., Sato, K. Crucial role of plasmacytoid dendritic cells in the initiation of inflammation and T cell immunity in vivo. ***Immunity*** 35, 1-14 (2011)
2. Fukaya T., Takagi H., Sato Y., Sato K., Eizumi K., Taya H., Shin T., Chen L., Dong C., Azuma M., Yagita H., Malissen B., Sato K. Crucial roles of B7-H1 and B7-DC expressed on mesenteric lymph node dendritic cells in the generation of antigen-specific $CD4^+Foxp3^+$regulatory T cells in the establishment of oral tolerance. ***Blood*** 116, 2266-2276 (2010)
3. Fukaya T., Takagi H., Taya H., Sato K. DCs in immune tolerance in steady-state conditions. ***Methods Mol. Biol.*** 677, 113-126 (2010)

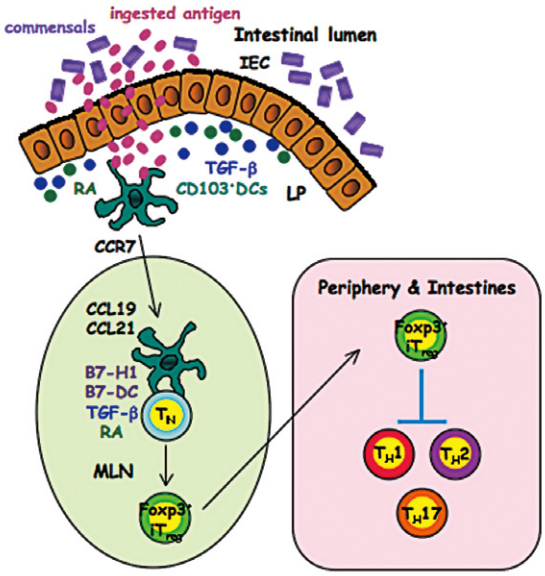

Figure 1: Proposed model for the role of MLN DCs in the establishment of oral tolerance.
In MLNs, $CD103^+$ DCs migrating from the lamina propria (LP) after sampling ingested antigens present them to naïve (n)$CD4^+$ T cells to generate $CD4^+Foxp3^+$ iT_{reg} cells mediated through B7-H1 and B7-DC as well as TGF-β and retinoic acid (RA). These $CD4^+Foxp3^+$ iT_{reg} cells dampen antigen-specific $CD4^+$ T_{eff} cell-responses and that leads to the establishment of oral tolerance.

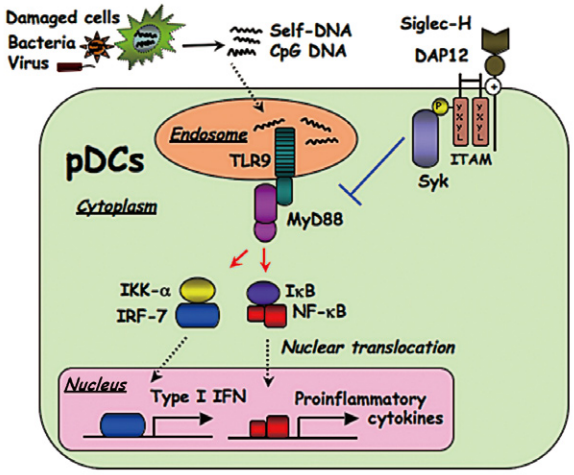

Figure 2: Hypothetical model for the role of Siglec-H in the control of cytokine production by pDCs.
Siglec-H impairs the TLR9-MyD88-mediated activation of the signaling cascades for NF-κB and IKK-α in pDCs after the recognition of self-DNA from host damaged cells or CpG DNA from bacteria and virus, and that leads to the inhibition of the production of type I IFN and proinflammatory cytokines.

expression of B7-H1 and B7-DC, MLN DCs enhanced the antigen-specific generation of $CD4^+Foxp3^+$ inducible regulatory T (iT_{reg}) cells from $CD4^+Foxp3^-$ T cells rather than $CD4^+$ effector T (T_{eff}) cells relative to systemic DCs. Furthermore, the antigen-specific conversion of $CD4^+Foxp3^-$ T cells into $CD4^+Foxp3^+$ iT_{reg} cells occurred to a greater extent in MLNs compared to other peripheral lymphoid organs during oral tolerance under steady-state conditions. Such a conversion was much more dependent on B7-H1 and B7-DC than on other B7 family members, and it was severely impaired under inflammatory conditions. In conclusion, our findings suggest that B7-H1 and B7-DC expressed on MLN DCs are essential for establishing oral tolerance through the *de novo* generation of antigen-specific $CD4^+Foxp3^+$ iT_{reg} cells.

A critical role of Siglec-H in the regulation of inflammation and T cell immunity by plasmacytoid dendritic cells *in vivo*

Plasmacytoid dendritic cells (pDCs) are specialized immune cells that are capable of producing large amounts of type I interferon (IFN) after sensing single-stranded RNA or unmethylated CpG DNA through endosomal toll-like receptors (TLRs). Sialic acid binding Ig-like lectin (Siglec)-H is exclusively expressed on murine pDCs and is unique among Siglec proteins in that it associates with the ITAM-bearing adaptor molecule, DAP12. To understand how Siglec-H might regulate the function of pDCs, we generated Siglec-H-deficient mice. We made three fundamental observations about the role of Siglec-H: 1. By regulating a MyD88-mediated downstream signaling pathway, it controls the threshold of responsiveness to TLR ligand for the production of type I IFN and proinflammatory cytokines. 2. By controlling MHC class II expression it regulates the ability of pDCs to prime antigen-specific $CD4^+$ T cells for the generation of $CD4^+Foxp3^+$ inducible regulatory T (iT_{reg}) cells and $CD4^+$ effector T (T_{eff}) cells. 3. By regulating cross-presentation of soluble and viral antigens it is required for the capacity of pDCs to prime antigen-specific $CD8^+$ T cells for the generation of cytotoxic T lymphocytes (CTLs). Thus, our findings suggest that Siglec-H plays a critical role in the function of pDCs for the regulation of inflammation and T cell immunity *in vivo*.

4. Sato K., Eizumi K., Fukaya T., Fujita S., Sato Y., Takagi H., Yamamoto M., Yamashita N., Hijikata A., Kitamura H., Ohara O., Yamasaki S., Saito T., Sato K. Naturally occurring regulatory dendritic cells regulate murine cutaneous chronic graft-versus-host disease. ***Blood*** 113, 4780-4789 (2009)

5. Candolfi M., Yagiz K., Foulad D., Alzadeh G.E., Tesarfreund M., Muhammad A.K., Puntel M., Kroeger K.M., Liu C., Lee S., Curtin J.F., King G.D., Lerner J., Sato K., Mineharu Y., Xiong W., Lowenstein P.R., Castro M.G. Release of HMGB1 in response to proapoptotic glioma killing strategies: efficacy and neurotoxicity. ***Clin. Cancer Res***. 15, 4401-4414 (2009)

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Despite their increasing prevalence in developed countries, the molecular mechanisms leading to allergy and autoimmune diseases remain poorly understood. The ultimate goal of the Cytokine Signaling Research Group is to help elucidate the underlying molecular and immunological mechanisms of allergy and autoimmune diseases from the viewpoint of signal transduction within the immune system. We have shown that heavy metal cations such as Zinc (Zn) might act as intracellular signaling molecules, i.e., molecules whose intracellular status is altered in response to an extracellular stimulus, and that are capable of transducing the extracellular stimulus into an intracellular signaling event. Zn is known to be important in the immune system, although its precise roles and mechanisms have not been resolved. Therefore, we are focusing our attention on the largely unknown universe of signaling through Zn transporter proteins, to reveal the role of Zn in immune and other physiological systems.

Role of Zn and its transporters in immune and non-immune tissues

We found that the Zn transporter Slc39a6/Zip6/Liv1 is a STAT3 target gene and showed that it has a role in cell migration during early zebrafish development. (Yamashita et al., *Nature*. 2004), and that LPS-induced maturation of DCs is partly mediated through lowering the intracellular concentration of free Zn by down regulating Zn transporters, including Slc39a6 (Kitamura et al., *Nature Immunology*. 2006), suggesting involvement of Zn in MHC class II cell surface expression through regulating endocytosis and membrane trafficking. In order to understand the role and function of Zn and Zn transporters *in vivo*, we have generated mice deficient in the Slc39/Zip family of zinc transporters. We found that Slc39a13/Zip13 knockout mice (*Slc39a13*-KO) show changes in connective tissues reminiscent of the human disease Ehlers-Danlos syndrome (EDS), in which there are defects in the maturation of osteoblasts, chondrocytes, odontoblasts, and fibroblasts. Impairment of bone morphogenic protein (BMP) and TGF-β signaling was observed in the corresponding tissues and cells. Homozygosity for a *SLC39A13* loss of function mutation was identified in sibs affected with a unique variant of EDS that recapitulates the phenotype observed in Slc39a13-KO mice. Hence, our results have revealed a crucial role of SLC39A13 in connective tissue development at least in part due to its involvement in BMP/TGF- β signaling pathways (Fukada et al, *PLoS ONE*, 2008). We have found that the human ZIP13 protein forms homo-dimers (Bin et al, *J. Biol. Chem*, 2011), and its further characterization by crystallography has been under investigation. In addition, we have investigated the role of Slc39a14 by generating genetically deficient mice. The *Slc39a14*-KO mice showed progressive systemic growth defects, and impaired signaling of G-protein-coupled receptors (GPCR) in the growth plate, pituitary grand, and liver

Recent publications

1. Bin, B-H., T. Fukada, T. Hosaka, S. Yamasaki, W. Ohashi, S. Hojyo, T. Miyai, K. Nishida, S. Yokoyama and T. Hirano. Biochemical characterization of human ZIP13 protein: a homo-dimerized zinc transporter involved in the Spondylocheiro dysplastic Ehlers-Danlos syndrome. ***J. Biol. Chem***. 286, 40255-40265 (2011)
2. Nishida K., S. Yamasaki, A. Hasegawa, A. Iwamatsu, H. Koseki, and T. Hirano. Gab2, via PI-3K, Regulates ARF1 in FcεRI-Mediated Granule Translocation and Mast Cell Degranulation. ***J. Immunol***. 187, 932-941 (2011)
3. Murakami, M*., Y. Okuyama*, H. Ogura*, S. Asano, Y. Arima, M. Tsuruoka, M. Harada, M. Kanamoto, Y. Sawa, Y. Iwakura, K. Takatsu, D. Kamimura, T. Hirano. (*equal contribution) Local microbleeding facilitates IL-6– and IL-17–dependent arthritis in the absence of tissue antigen recognition by activated T cells. ***J. Exp. Med***. 208, 103-114 (2011)

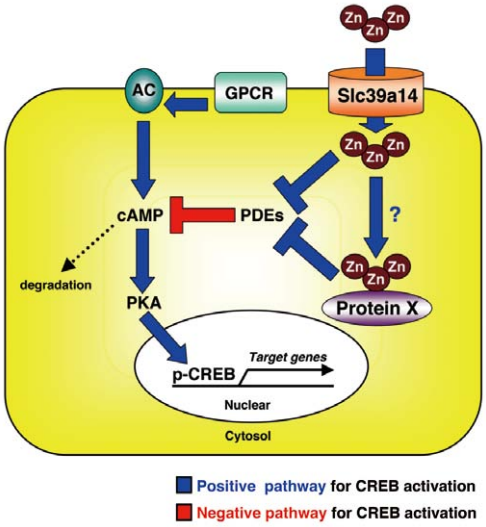

Figure 1: Schematic model of the regulation of GPCR-mediated signaling by Slc39a14. Slc39a14 regulates basal cAMP levels by suppressing PDE activity, which enhances the GPCR-cAMP-CREB pathway to control systemic growth.

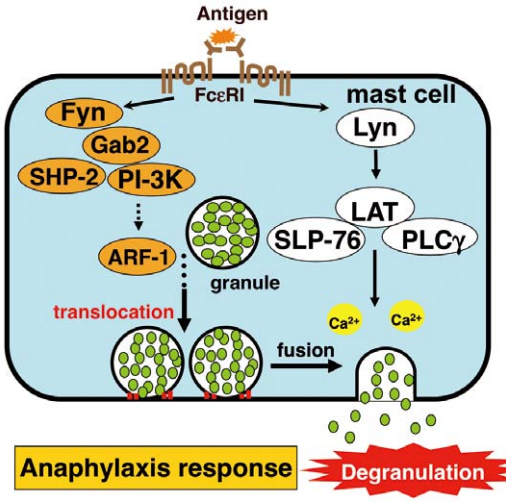

Figure 2: Model of the Fc epsilon RI-mediated Fyn/Gab2/PI-3K/ARF1 signaling pathway involved in granule translocation and mast cell degranulation. Fc epsilon RI activates the Src family kinase Fyn, which phosphorylates/activates Gab2, which then associates with the SH2 domain of PI-3K. PI-3K controls granule translocation, degranulation, and anaphylaxis responses via ARF1 activation.

was observed. We found that Slc39a14-mediated Zn transport plays a role in the control of GPCR-cAMP response element-binding protein (CREB) signaling via regulating cAMP levels by suppressing phosphodiesterase (PDE) activity (Figure 1, Hojyo et al, *PLoS ONE*, 2011), suggesting a crucial role of Slc39a14 in control of mammalian systemic growth.

Recently, we showed that Zn suppresses T_h17-mediated autoimmune diseases by inhibiting the development of T_h17 cells via attenuating STAT3 activation. In mice injected with type II collagen to induce arthritis, Zn treatment inhibited T_h17 cell development. IL-6-mediated activation of STAT3 and *in vitro* T_h17 cell development were all suppressed by Zn. Importantly, Zn binding changed the α-helical secondary structure of STAT3, disrupting the association of STAT3 with JAK2 kinase and with a phospho-peptide that included a STAT3-binding motif from the IL-6 signal transducer gp130. Thus, we conclude that Zn suppresses STAT3 activation, which is a critical step for T_h17 development (Kitabayashi et al, *International Immunology*, 2010).

Together these results support the idea that Zn has roles for mediating and controlling intracellular signaling events. We propose the term "Late Zn signaling" for this type of Zn signaling, in contrast to "Early Zn signaling". The former is dependent on changes in the transcription of Zn transporter genes, whereas the latter is not (Hirano et al, *Adv. Immunol.*, 2008).

Molecular Mechanisms of Mast Cell degranulation

Immunological and molecular mechanisms of the role of mast cells in allergy, inflammation, and autoimmune diseases are being investigated. We have dissected the degranulation process of mast cells. First, Fc epsilon RI stimulation triggers microtubule polymerization and granule translocation to the plasma membrane in a calcium-independent manner. Second, the granules fuse with the plasma membrane in a well-characterized calcium-dependent manner. Furthermore, we showed that the Fyn/Gab2/RhoA, but not the Lyn/SLP-76 signaling pathways play a critical role in the calcium-independent microtubule-dependent pathway. At present, we are especially focused on clarifying the molecular mechanisms of calcium-independent microtubule-dependent granule translocation. In order to clarify how Gab2 mediates granule translocation and degranulation, we established Gab2 knock-in mice that express Gab2 mutated in either the PI-3K- or SHP-2-binding sites. We found that Gab2 knock-in mice that express Gab2 mutated in either of these binding sites had defective mast cell-mediated immediate-type allergic responses, but not delayed-type responses. We also showed that the PI-3K-binding site but not the SHP-2-binding site was involved in Fc epsilon RI-dependent ARF1 activation and granule translocation to the plasma membrane in the degranulation process, further dissecting the signals required for degranulation. Finally, we revealed the molecular framework of the Fyn/Gab2/PI-3K-dependent ARF1-mediated granule translocation and mast cell degranulation (Fig. 2). Thus, these findings provided us with potential therapeutic targets.

4. The Zinc Transporter SLC39A14/ZIP14 Controls G-Protein Coupled Receptor-Mediated Signaling Required for Systemic Growth. Hojyo, S.*, T. Fukada*, S. Shimoda, W. Ohashi, B-H. Bin, H. Koseki, T. Hirano. ***PLoS ONE*** 6(3): e18059 (2011) (*Equally contribution)

5. Kitabayashi C., T. Fukada, M. Kanamoto1, W. Ohashi, S. Hojyo, T. Atsumi, N. Ueda, I. Azuma, H. Hirota, M. Murakami, and T. Hirano. Zinc suppresses Th17 development via inhibition of STAT3 activation. ***Int. Immunol***. 22, 375-386 (2010)

6. Nishida K*., A. Hasegawa*, S. Nakae, K. Oboki, H. Saito, S. Yamasaki, and T. Hirano. (*equal contribution) Zinc transporter Znt5/Slc30a5 is required for the mast cell-mediated delayed-type allergic reaction but not the immediate-type reaction. ***J. Exp. Med***. 206, 1351-1364 (2009)

Laboratory for

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Ayako Kobayashi
Mikiko Sato
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The main activity of our team is the screening of a large ethylnitrosourea (ENU) mutant mouse panel and is a collaborative effort with the RIKEN Genomic Sciences Center. An important goal of this project for RCAI is the development of novel mouse models for various immunological disorders, notably allergic and autoimmune disorders. Since ENU mutagenesis introduces approximately 3,000 point mutations in a genome, we can expect 100 coding region mutations in one pedigree. In parallel, we are screening the mutant mice under environmental bias, using a variety of approaches including immunization with allergens and adjuvants to identify allergy modifier genes.

ENU mutant panel study

In order to identify the genetic basis for immune disorders, we are screening a pool of mutant mice generated by random chemical mutagenesis. ENU induces random single-base pair changes in genomic DNA at approximately 3,000 sites throughout the entire genome, resulting in approximately 100 sites in protein coding regions per first-generation (G1) mutant mouse.

We have analyzed the mutant phenotypes by pathological, cytological and molecular biological evaluations of affected tissues, lymphoid organs and blood cells.

The mutant loci responsible for any abnormal phenotypes identified in candidate lines are mapped by backcrossing mutant individuals with the C3H/HeJ strain for gene detection by single-nucleotide polymorphism analysis. Candidate gene mapping has been done in collaboration with the Phenome Informatic Team and Mouse Mutation Resource Exploration Team in the GSC, RIKEN

Allergic disease model mutant mouse model

Using this approach, we identified and established a mutant mouse line with phenotypic features reminiscent of a typical human allergic disease. The ear skin became thicker and red

Recent publications

1. Sugiyama M, Nakato G, Jinnohara T, Akiba H, Okumura K, Ohno H and Yoshida, H Expression pattern changes and function of RANKL during mouse lymph node microarchitecture development. ***Int Immunol.*** 24, 369-378 (2012)
2. Tachibana M, Tenno M, Tezuka C, Sugiyama M, Yoshida H, Taniuchi I. Runx1/Cbfβ2 complexes are required for lymphoid tissue inducer cell differentiation at two developmental stages. ***J Immunol.*** 186,1450-7 (2011)
3. Matsushima Y, Kikkawa Y, Takada T, Matsuoka K, Seki Y, Yoshida H, Minegishi Y, Karasuyama H, Yonekawa H. An Atopic Dermatitis-Like Skin Disease with Hyper-IgE–Emia Develops in Mice Carrying a Spontaneous Recessive Point Mutation in the *Traf3ip2 (Act1/ CIKS)* Gene. ***J Immunol***. 185,2340-9 (2010)

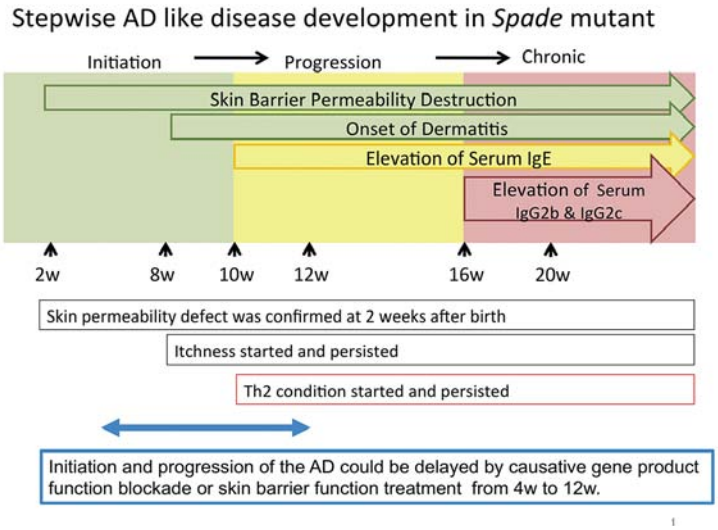


Figure 1: An atopic dermatitis-like mutant mouse was established from the ENU screen.
The diagram illustrates the stepwise progression of AD disease in the mutant animals.

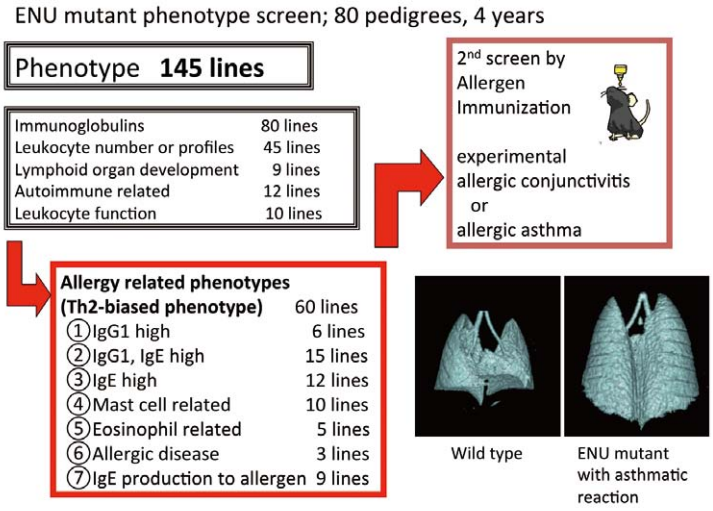


Figure 2: Summary of four years of ENU mutant screening.
In total, approximately 8,000 mutant mice from 80 genomes have been screened and 145 phenotypes related to immunity or allergy have been identified. The images in the bottom right show the 3 dimensional renderings of the lungs of WT and mutant mice after OVA immunization and challenge.

and, as the mice aged, they started to scratch the ear skin or face. Finally, they developed chronic facial and ear skin inflammation and increased levels of not only Th2 serum immunoglobulins but also Th1 serum immunoglobulins. This stepwise development of symptoms and other findings are compatible with the criteria for human infantile atopic dermatitis (AD). We have mapped the phenotype-causing point mutation to a distinct gene and directly confirmed this causality by genetic manipulation to introduce the same mutation into the gene of wild type mice. We have clarified the disease onset mechanism and succeeded in disease onset prevention based on the results of our studies (manuscript under submission). We are now further analyzing the stepwise progression of this AD-like disease in the mutant mouse in combination with various gene-manipulated mice to better understand the allergic disease progression in skin and in the immune system.

By ENU recessive mutant screening, we have identified and established more than 145 mutant lines with immune or blood disease phenotypes. In keeping with our expectation, more than 50 lines had phenotypes related to allergic diseases. From these mutant lines, we are now analyzing a mutant line with features of experimental allergic asthma, rhinitis and conjunctivitis by OVA immunization.

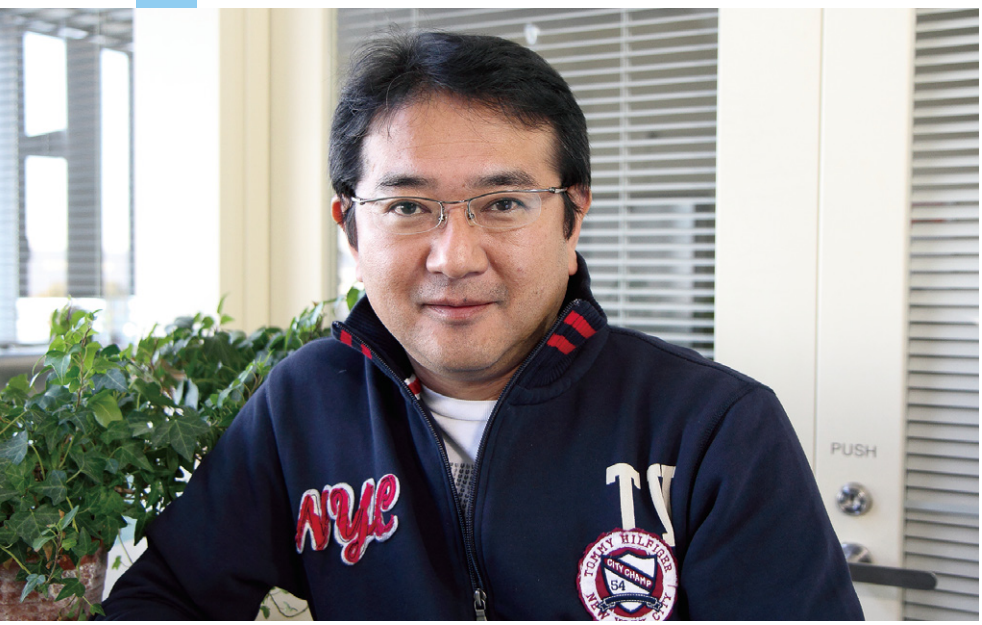
As of December 2011, we have mapped thirty-two independent mutant loci to distinct regions, and nine of them have been identified to be point mutations in independent genes. Along with allergic disease model mutant screening, we have identified and characterized a few other interesting phenotypes in mutant lines with/without allergic defects as listed in the publications section. One is a good model for the human neurodegenerative disease infantile neuroaxonal dystrophy (INAD) (Wada et al., 2009), and another is a point mutation in the *Themis* gene, which we found plays an important role in thymocyte development (Kakugawa et al., 2009).

4. Wada H., Yasuda T., Miura I., Watabe K., Sawa C., Kamijuku H., Kojo S., Taniguchi M., Nishino I., Wakana S., Yoshida H., Seino K. Establishment of an improved mouse model for infantile neuroaxonal dystrophy that shows early disease onset and bears a point mutation in Pla2g6. ***Am J Pathol***. 175, 2257-63 (2009)

5. Kakugawa K., Yasuda T., Miura I., Kobayashi A., Fukiage H., Satoh R., Matsuda M., Koseki H., Wakana S., Kawamoto H., Yoshida H. A novel gene essential for the development of single positive thymocytes. ***Mol Cell Biol***. 29, 5128-35. (2009)

Laboratory for

Vaccine Design



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In the course of research and development for cedar pollen allergy vaccines, we had found that a liposomal form of α-GalCer, but not an aqueous one, could predominantly enhance the immunoregulatory functions of invariant Natural Killer T (*i*NKT) cells, such as IL-10 production. Then, we attempted to suppress IgE antibody formation in a mouse allergy model by the administration of liposomal α-GalCer. In the case of primary IgE antibody formation, a systemic injection of liposomal α-GalCer prior to the immunization with alum-absorbed antigen resulted in the remarkable suppression of not only IgE but also IgG antibody. However, the secondary antibody response of fully antigen-sensitized mice after antigen challenge was not suppressed by even repeated systemic injection of liposomal α-GalCer. Since it had been suggested that regulatory T cells such as Foxp3-positive naturally occurring Treg cells or IL-10-producing Tr1 cells might be involved in the suppression of the secondary antibody response, we tested whether the liposomal α-GalCer encapsulating an antigenic polypeptide would generate antigen-specific Treg cells. We demonstrated that the systemic administration of liposomal α-GalCer encapsulated OVA could induce suppression of the secondary anti-OVA IgE antibody response in OVA-immunized mice but not that in cedar antigen Cry j 2-immunized mice. Moreover, this antigen-specific phenomenon could be reproduced by the adoptive transfer of $CD4^+CD25^+$T cells derived from mice treated with the liposomal α-GalCer encapsulated OVA. We are now trying to elucidate the precise mechanism of antigen-specific IgE suppression by the $CD4^+CD25^+$Treg cells.

Mechanism for generation of $CD4^+CD25^+$regulatory T cells by liposomal α-GalCer -antigen

Last fiscal year, we found that rhodamine-labeled liposomal α-GalCer was incorporated into splenic $B220^+CD1d^{high}$ cells, but not $B220^+CD1d^{low}$ cells, and much more than into $CD11b^+$ macrophages or $CD11c^+$ dendritic cells. Immunohistochemical observation clearly showed that rhodamine-positive dots corresponding to $B220^+CD1d^{high}$ cells were localized at the marginal zone area in spleen and that co-localization of *i*NKT cells after the rhodamine-liposome injection could be detected in the mice transferred NKT-ES-*V* cells in which *IRES-Venus* was inserted downstream of the *TCR Cα* stop codon in NKT-ES cells (1). The expression of CXCL16, a ligand for CXCR6, on rhodamine-positive $B220^+CD1d^{high}$ cells and $CD11c^+$ cells was analyzed since CXCR6 is a major chemokine receptor on *i*NKT cells. The result of cell surface staining with anti-CXCL16 mAb showed that the expression level of CXCL16 on $B220^+CD1d^{high}$ cells and $CD11c^+$ cells

Recent publications

1. Duramad O, Laysang A, Li J, Ishii Y and Namikawa R, Pharmacologic Expansion of Donor-Derived, Naturally Occurring CD4(+)Foxp3(+) Regulatory T Cells Reduces Acute Graft-versus-Host Disease Lethality Without Abrogating the Graft-versus-Leukemia Effect in Murine Models. ***Biol Blood Marrow Transplant*** 17, 1154-1168, 2011
2. Okayama T, Matsuno Y, Yasuda N, Tsukui T, Suzuta Y, Koyanagi M, Sakaguchi M, Ishii Y, Olivry T, Masuda K. Establishment of a quantitative ELISA for the measurement of allergen-specific IgE in dogs using anti-IgE antibody cross-reactive to mouse and dog IgE. ***Vet Immunol Immunopathol***. 139, 99-106, 2011
3. Ishii Y, Motohashi S, Shimizu K, Nakayama T, Taniguchi M, and Fujii S, Application of NKT cells in immunotherapy, ***Curr Immunol. Rev***. 6, 109-115, 2010

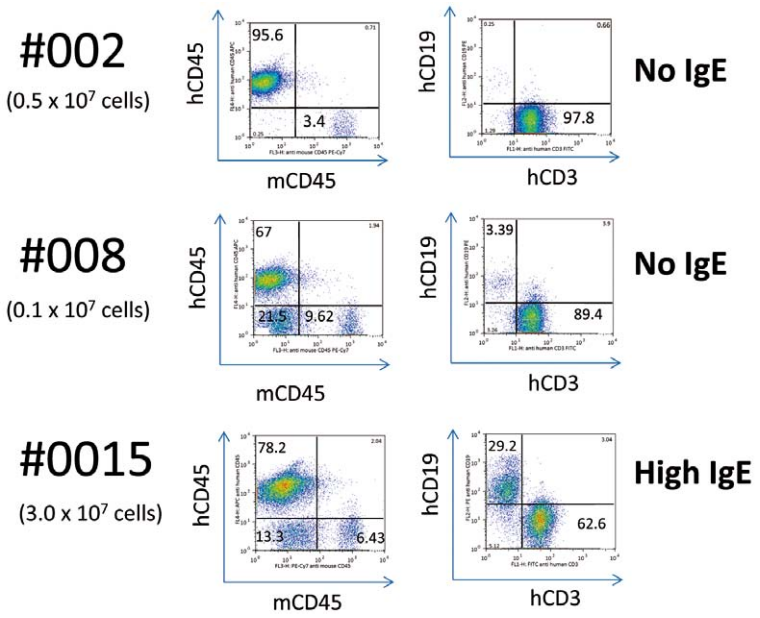

Figure : Correlation between human CD19-positive B cell expansion and human IgE production in NOD/SCID/Il2rγKO mice infused with PBMCs from allergic volunteers.

was quite low in unmanipulated mice, but was augmented after rhodamine-liposome injection, suggesting the involvement of the CXCR6/CXCL16 system. To test whether CXCR6/CXCL16 was involved in the interaction of the *i*NKT cells with the $B220^{+}CD1d^{low}$ cells, neutralizing antibody against CXCL16 and rhodamine-labeled liposomes were co-administered into the NKT-Venus cloned mice. Immunohistochemical analysis showed that *i*NKT cells failed to take up residence in the marginal zone area. To determine the significance of the interaction of *i*NKT cells with marginal zone $B220^{+}CD1d^{high}$ cells, cytokine expression of splenic *i*NKT cells after the injection of liposomal α-GalCer with or without anti-CXCL16 neutralizing mAb was analyzed by Q-PCR. The result showed that IL-10 and IL-21mRNA expression among the tested cytokines was remarkably reduced in *i*NKT cells recovered from the mice treated with anti-CXCL16 neutralizing mAb, suggesting that IL-10 and IL-21 production by *i*NKT cells might be indispensable for the interaction with the liposomal α-GalCer-pulsed $B220^{+}CD1d^{high}$ cells. We are now speculating that this IL-10 production could play a role in the expansion of $CD4^{+}CD25^{+}$Treg cells, while IL-21 production could be involved in the inhibition of IgE class switching. In our previous studies, IL-10 expression by α-GalCer-pulsed $B220^{+}CD1d^{high}$ cells after their interaction with *i*NKT cells was much higher than that of the *i*NKT cells, and this influenced the expansion of $Foxp3^{+}CD4^{+}CD25^{+}$Treg cells. Although IL-21 production by *i*NKT cells may partly explain the IgE suppression mechanism, it may not explain the suppression of the antigen-specific secondary IgE antibody response in mice treated with liposomal α-GalCer encapsulated antigen. In future studies, we will define the characteristics, such as Foxp3, IL-10 and/or IL-21, of $CD4^{+}CD25^{+}$Treg cells expanded by *in vivo* treatment with liposomal α-GalCer encapsulated OVA.

1) Watarai et al. (2010) *Blood* 115(2):230-237.

Establishment of humanized cedar pollen allergy mice

Based on the RCAI mission, we have already produced two types of allergy vaccines for Japanese cedar pollinosis. One is a polyethylene glycol (PEG)-modified recombinant fusion protein of Cry j 1 and Cry j 2, which are major cedar pollen antigens, the other is the same fusion protein encapsulated in α-GalCer liposomes. Though both vaccine projects are still in the pre-clinical study phase, new systems for prediction of human efficacy and safety are needed before going into clinical studies. We started a program for the establishment of humanized cedar pollen allergy mice, in which there would be production of human IgE antibody specific for cedar antigens and a type I allergic reaction including anaphylaxis following antigen challenge. Among cedar pollinosis volunteers, three individuals with the highest CAP-RAST value were selected for a transfer experiment. Their peripheral blood mononuclear cells were infused into NOD/SCID/Il2rγKO mice, which were simultaneously immunized with the cedar antigen Cry j 1. After ten days, the recipient mice were boosted by intraperitoneal injection with antigen. Total IgE and anti-Cry j 1-IgE titers in sera were measured by ELISA 21 days after the first immunization. In mice that had received peripheral blood mononuclear cells of one volunteer (#015), both total human IgE and anti-Cry j 1-IgE antibody were detected even though their levels were much lower than that in the blood of this volunteer. No IgE was detected in recipients of cells from the other two volunteers (#002 & #008). As shown in the figure, human CD19-positive cells were not observed in the spleen of the non-IgE^{+} mice but were found in the high IgE mice, suggesting that the some sort of treatment to maintain survival and induce the IgE class switch of human CD19-positive B cells in NOD/SCID/Il2rγKO recipient mice will be essential. We are now examining the effect of infusing antigen-pulsed peripheral blood mononuclear cells, with or without human IL-4, into NOD/SCID/Il2rγKO mice. We hope to find conditions to extend the survival of the human B cells in the transferred mice for the purpose of long term observation of vaccine safety.

Laboratory for

Human Disease Models

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Since 2006, our lab has been focusing on the investigation of normal and diseased human hematopoiesis and immunity through creation of humanized mouse models. In this fiscal year, we have created a humanized mouse model with expression of membrane-bound human Kit ligand/stem cell factor. Humanization of the mouse bone marrow microenvironment has led to nearly 100% human hematopoietic repopulation and efficient myeloid development. The reconstituted mice also have abundant human mast cells in spleen and gastrointestinal mucosa, which may enable us to apply this new humanized mouse model to the area of allergy research. In addition to the creation of immune-compromised mice with a humanized microenvironment, we have aimed to create *in vivo* models for human diseases such as leukemia, primary immunodeficiency, and virus infection. In the leukemia study, we found distinct mechanisms of leukemogenesis between adult myeloid leukemia (AML) and infant lymphoid leukemia and have started to explore the possibility of drug development in order to eliminate chemotherapy-resistant AML stem cells. Finally, in collaboration with other laboratories within RCAI, we intend to analyze hematopoietic development from human iPS cells *in vitro* and *in vivo*.

Creation of hSCF transgenic humanized mouse model

We have aimed to improve lymphoid-skewed differentiation of transplanted human hematopoietic stem cells (HSCs) in the bone marrow of the humanized mouse. Since inefficient human myeloid development could be attributed to the mouse microenvironment not fully supporting differentiation and maturation of human myeloid lineage cells, we created membrane-bound form of human Kit ligand (KL)/stem cell factor (SCF) transgenic mice in collaboration with Dr. Leonard D. Shultz at The Jackson Laboratory. Transplantation of cord blood-derived human HSCs resulted in significantly

Recent publications

1. Takagi S, Saito Y, Hijikata A, Tanaka S, Watanabe T, Hasegawa T, Mochizuki S, Kunisawa J, Kiyono H, Koseki H, Ohara O, Saito T, Taniguchi S, Shultz LD, Ishikawa F. Membrane-bound human SCF/KL promotes in vivo human hematopoietic engraftment and myeloid differentiation. ***Blood***, 119, 2768-2777, 2012
2. Shultz LD, Saito Y, Najima Y, Tanaka S, Ochi T, Tomizawa M, Doi T, Sone A, Suzuki N, Fujiwara H, Yasukawa M, Ishikawa F. Generation of functional human T-cell subsets with HLA-restricted immune responses in HLA class I expressing NOD/SCID/IL2rγ^{null} humanized mice. ***Proc. Natl. Acad. Sci. U.S.A.*** 107, 13022-13027, 2010
3. Saito Y, Uchida N, Tanaka S, Suzuki N, Tomizawa-Murasawa M, Sone A, Najima Y, Takagi S, Aoki Y, Wake A, Taniguchi S, Shultz LD, Ishikawa F. Induction of cell cycle entry eliminates human leukemia stem cells in a mouse model of AML. ***Nat. Biotech.*** 28, 275-280, 2010.

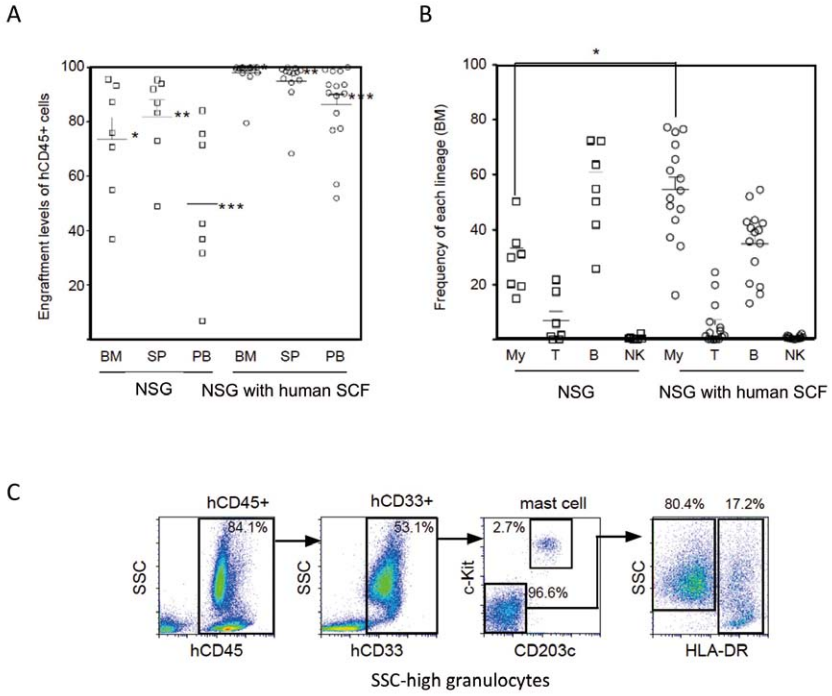

Figure : **Creating a novel NSG strain with a humanized BM microenvironment.** (A) To humanize the bone marrow microenvironment, we created a new strain of NSG mice expressing human membrane-bound stem cell factor. Transplantation of human cord blood-derived HSCs resulted in higher levels of human hematopoietic engraftment in hSCF TG NSG recipients compared with non-TG NSG controls. (B, C) In particular, the development of human myeloid lineage cells became more efficient in the human SCF-expressing humanized mice.

higher long-term engraftment of human leukocytes in the bone marrow, spleen, and peripheral blood of hSCF Tg NSG recipients compared with those of non-transgenic NSG recipients. In the bone marrow, the frequency of human CD33$^+$ myeloid cells within the total human CD45$^+$ population was significantly higher in the hSCF Tg NSG recipients than in the non-Tg NSG recipients and constituted the majority of human hematopoietic cells. With expression of human SCF in the recipient microenvironment, human mast cells efficiently developed in the bone marrow, spleen, and gastrointestinal tract of the hSCF Tg NSG recipients. Taken together, the data from our new *in vivo* humanized mouse model demonstrate the essential role of membrane-bound SCF in human myeloid development, and moreover the hSCF Tg NSG humanized mice may be useful in studying human allergic responses and innate immunity *in vivo*.

Development of an immune-therapy model using HLA class I transgenic humanized mice

Last year, we tried to overcome one of the major limitations of the current humanized mouse models, the lack of an HLA-restricted human immune response, through creation of HLA class I transgenic humanized mice. When we infected these humanized mice with EBV, we consistently detected tetramer$^+$ CD8$^+$ T cells in the recipient lymphoid organs, and IFN-γ production by humanized mouse-derived CD8$^+$ T cells in an HLA-restricted manner. In addition to the HLA-restricted human CTL response against virus-derived antigens, we have sought to evaluate whether the HLA-class I transgenic humanized mouse can be utilized as an *in vivo* model for human immune therapy against malignancies. To this end, we chose as a target antigen WT1, a molecule that we and others have previously reported as being expressed in a wide variety of tumors and tumor stem cells,. We immunized HLA class I transgenic humanized mice with WT1 peptide and TLR adjuvant or with WT1 peptide-loaded dendritic cells three times, with 10-day intervals between injections. Compared with the human CTL response against EBV-associated antigens such as LMP1 or EBNA1, the human HLA-restricted immune response against WT1 peptide was weaker but was readily detectable. Human CD8$^+$ T cells harvested from HLA class I TG humanized mouse spleen and lymph nodes were able to secrete IFNγ in response to WT-1 expressing cells. We are currently trying to evaluate cytotoxic activity of humanized mouse-derived CD8$^+$ T cells against WT1-expressing AML stem cells *in vitro* and *in vivo*. Through this analysis, we hope to address whether immune-therapy against WT1 could be a successful therapeutic strategy targeting chemotherapy-resistant AML stem cells.

4. Saito Y, Kitamura H, Hijikata A, Tomizawa-Murasawa M, Tanaka S, Takagi S, Uchida N, Suzuki N, Sone A, Najima Y, Ozawa H, Wake A, Taniguchi S, Shultz LD, Ohara O, Ishikawa F. Identification of therapeutic targets for quiescent, chemotherapy-resistant human leukemia stem cells. ***Sci. Transl. Med.*** 2, 17ra9, 2010.

Research Unit for

Cellular Immunotherapy

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Technical staff : **Hidetoshi Sugahara**
Jun Shinga

Student Trainee : **Yusuke Sato**

Human Vα24⁺ NKT cells bearing an invariant Vα24Jα18 antigen receptor can be activated by a specific ligand, α-GalCer, in a CD1d-dependent manner. We previously showed that circulating Vα24⁺ NKT cells present in lung cancer patients were functional. Therefore, to evaluate the immunological and clinical responses to NKT cell therapy in advanced non-small cell lung cancer patients we have been developing a joint clinical study with Chiba University using α-galactosylceramide (α-GalCer)-pulsed autologous dendritic cells (DCs). Based on our initial analyses, the immunological and clinical results of the phase I/IIa trials are encouraging.

We have also studied the role of DCs *in situ* for tumor immunity by focusing on the link between innate and adaptive immunity. Using a mouse model, we successfully developed a strategy for the induction of antigen-specific T cell responses using tumor-associated antigen expressing, α-GalCer-loaded allogeneic fibroblasts to stimulate *in situ* DC maturation. Based on these preclinical findings, we are attempting to launch clinical studies of such human artificial adjuvant vector cells (aAVCs), in collaboration with Dr. Shimizu, (Therapeutic Model Research Unit) and with the support and training program for translational research of Japan.

Increased number of NKT cells in the lung after α-GalCer-loaded dendritic cell (DC/Gal) therapy in translational research (A collaboration with Drs. Nakayama and Motohashi in Chiba University and Dr. Taniguchi in RCAI)

We have been developing a Phase I/IIa clinical study of the application of NKT cell therapy for advanced non-small cell lung cancer (NSCLC) patients refractory to standard treatment as a post second line therapy (stage IIIB, IV or recurrence). α-GalCer-pulsed APCs (PBMC-derived DCs) were intravenously administered four times. In this study, we demonstrated that the increased IFN-γ production by NKT cells upon α-GalCer stimulation was significantly associated with clinical outcome. Also, we detected a larger number of NKT cells capable of producing IFN-γ in the lung tumor rather than peripheral blood. To develop this therapy, we have started to use a bronchoscope for trans-bronchial injection

Recent publications

1. Fujii S., Shimizu K.(2011) DC-based immunotherapy targeting NKT cells. In Terabe M and Berzofsky JA(eds), ***Natural killer T cells: Balancing the regulation of tumor immunity.*** Springer New York Dordrecht Heidelberg London, 95-110.
2. Shimizu K., Asakura M., Fujii S. Prolonged antitumor NK cell reactivity elicited by CXCL-10-expressing dendritic cells licensed by CD40L⁺CD4⁺ memory T cells. ***J. Immunol***. 186, 5927-37(2011)
3. Asano K., Nabeyama A., Miyake Y., Qiu CH., Kurita A., Tomura M., Kanagawa O., Fujii S., Tanaka M. CD169-Positive Macrophages Dominate Antitumor Immunity by Crosspresenting Dead Cell-Associated Antigens. ***Immunity*** 34, 85-95(2011)

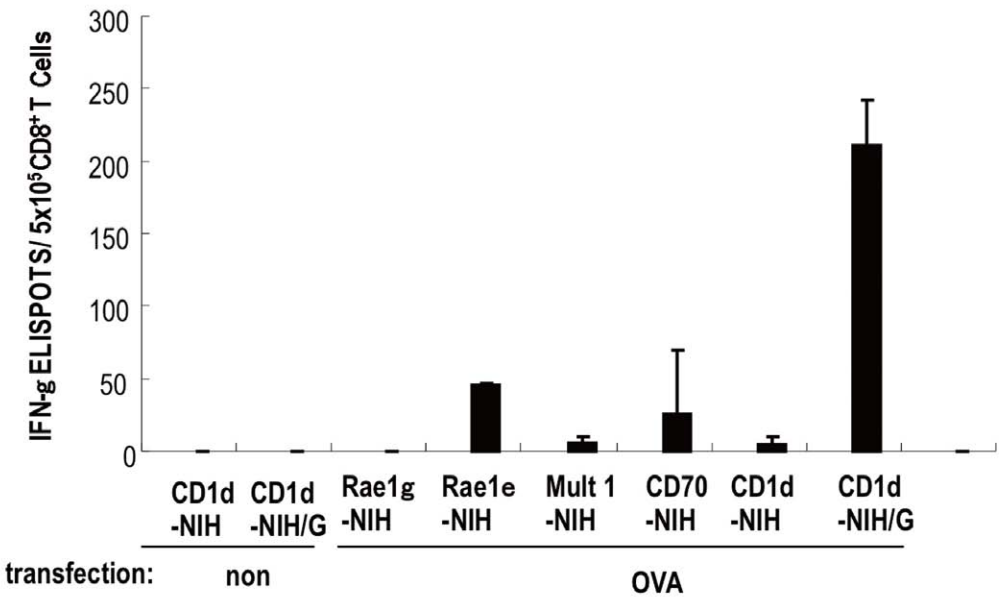

Figure: **Efficacy of aAVCs for induction of Innate Immunity and Adaptive immunity**
C57BL/6 mice were immunized with CD1d^{hi}-NIH3T3/Gal-ova, Rae-1ε-NIH3T3-ova, Rae-1γ-NIH3T3-ova, Mult1-NIH3T3-ova or CD70-NIH3T3-ova. Spleen cells from different groups of immunized mice were collected one week after immunization. CD8$^+$T cells were positively selected from spleens in these mice and cocultured with $OVA_{257-264}$ peptide-pulsed CD11c$^+$ cells for 36 hours. The number of IFN-γ secretion from CD8$^+$ T cells (/5x10^5 cells) in response to $OVA_{257-264}$ peptide was evaluated by ELISPOT.

of DC/Gal, that is, via the bronchial instead of intravenous route. Since this study includes a novel technique, we started it as new phase I study to confirm the safety profile.

Preclinical study with antigen mRNA-transfected, allogeneic fibroblasts loaded with NKT cell ligand
(A collaboration with Drs. Shimizu (Therapeutic Unit) and Ishii (Vaccine Design Team) in RIKEN RCAI, Dr. Mizuno (Yamaguchi Univ.), Dr. Kakimi (Tokyo Univ.) and Dr. Maeda (Iwate Medical Univ.)

We previously utilized allogeneic fibroblast cells loaded with α-GalCer and transfected with antigen-encoding mRNA, thus combining the adjuvant effects of *i*NKT cell activation with delivery of antigen to DCs *in vivo* (Blood 2009). We found that these cells produce antigen protein and activate NK and *i*NKT cells. When injected into mice, they elicited antigen-specific T cell responses and provided tumor protection. Thus, glycolipid-loaded, mRNA-transfected allogeneic fibroblasts act as adjuvant vector cells (aAVCs) to promote *i*NKT cell activation, leading to DC maturation and T cell immunity. When we compared the magnitude of T cell responses after priming with the *i*NKT cell ligand α-GalCer versus ligands of NK cells, the number of IFN-γ-producing T cells was much higher in mice given CD1d^{hi}-NIH3T3/Gal-ova than in other groups (Figure). As preclinical studies, we have set up canine studies to observe the safety profile and immune response. These aAVCs administered to dogs activate *i*NKT cells as well as elicit antigen-specific T cell responses with no adverse events. This unique tool could prove clinically beneficial in the development of immunotherapies for malignant and infectious diseases.

4. Watarai H, Fujii S, Yamada D, Rybouchkin A, Sakata S, Nagata Y, Iida-Kobayashi M, Sekine-Kondo E, Shimizu K, Shozaki Y, Sharif J, Matsuda M, Mochiduki S, Hasegawa T, Kitahara G, Endo TA, Toyoda T, Ohara O, Harigaya K, Koseki H, Taniguchi M. Murine induced pluripotent stem cells can be derived from and differentiate into natural killer T cells. ***J. Clin. Invest.*** 120, 2610-2618 (2010)

5. Fujii S, Goto A, Shimizu K. Antigen mRNA-transfected, allogeneic fibroblasts loaded with NKT-cell ligand confer antitumor immunity. ***Blood*** 113, 4262-72 (2009)

Laboratory for
Immunogenomics

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Student Trainees : **Nanako Shimura** (JRA), **Asahi Nakahara, Masayuki Ishii**

The very basic mission of the immunogenomics group has been to function as a “Gateway” to genomics for immunologists. For this mission, we established a three-pronged approach to our research activities since our group first launched: (1) central support activities; (2) strategic and collaborative research activities; and (3) exploratory research activities aimed at new technology development. New in 2011, we have introduced two next-generation DNA sequencers (Roche GS Junior and Illumina HiSeq1000) to make it possible to exploit recent advances in DNA sequencing technology at RCAI. Next-generation DNA sequencing (NGS) will drastically change the genomic approaches that will now become practical for use by immunologists, and the current technology is just the beginning of the change. In parallel, we have also placed a significant emphasis on development of new technology to ultimately enhance strategic and collaborative research activities as well as the central support activities. Our current emphasis is on the development of technology for single-cell analyses and for efficient genetic testing using next-generation DNA sequencing technology. Examples of our recent achievements along these directions are described below.

Development of a highly accurate method to detect low-frequency mosaicisms in the *NLRP3* gene using next generation DNA sequencing

NGS results in a great reduction in the cost of DNA sequencing and offers new applications including detecting somatic mosaicism with low allele frequencies. However, one of the major concerns with NGS is how to discriminate real changes from sequencing errors. We tackled this issue in collaboration with clinicians in Kyoto University to make NGS the method of choice for practical genetic tests in clinical situations.

Chronic infantile neurological cutaneous and articular syndrome (CINCA), also known as neonatal-onset multisystem inflammatory disease (NOMID), is a dominantly-inherited systemic autoinflammatory disease caused by a heterozygous germline gain-of-function mutation in the *NLRP3* gene. The recent finding of a high incidence of *NLRP3* somatic mosaicism in apparently mutation-negative CINCA/NOMID patients suggests that a rapid diagnosis of somatic *NLRP3* mosaicism is crucial to ensure proper treatment. However, conventional DNA sequencing approaches require large investments of time, cost, and labor, making it difficult to routinely diagnose low-level somatic *NLRP3* mosaicism by this approach. To address this problem, we used NGS to

Recent publications

1. Detection of Base Substitution-Type Somatic Mosaicism of the NLRP3 Gene with >99.9% Statistical Confidence by Massively Parallel Sequencing: Izawa K, Hijikata A, Tanaka N, Kawai T, Saito KM, Goldbach-Mansky R, Aksentijevich I, Yasumi T, Nakahata T, Heike T, Nishikomori R, **Ohara O**. ***DNA Res***. 2012 in press.
2. XPS and NEXAFS studies of VUV/O(3)-treated aromatic polyurea and its application to microchip electrophoresis: Shinohara H, Nakahara A, Kitagawa F, Takahashi Y, Otsuka K, Shoji S, Ohara O, Mizuno ***J. IET Nanobiotechnol***. 2011 5(4):136.
3. Plasmacytoid dendritic cells are crucial for the initiation of inflammation and T cell immunity in vivo: Takagi H, Fukaya T, Eizumi K, Sato Y, Sato K, Shibazaki A, Otsuka H, Hijikata A, Watanabe T, Ohara O, Kaisho T, Malissen B, Sato K ***Immunity***. 2011 35(6):958-71.

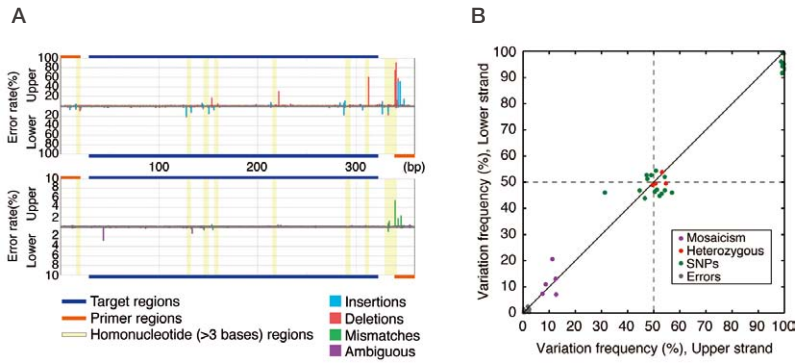


Figure 1: Analysis of NLRP3 somatic mosaicism by NGS
(A) The reference error rate map for the amplicon of NLRP3 exon 1 on the upper and lower strands. The colors of bars represent the error category (insertions: cyan; deletions: *red*; mismatches: *green*; ambiguous base calls: *purple*). The *orange* and *blue* lines depict the primer and target regions, respectively. The *yellow* shaded area depicts the homonucleotide stretches (n>3) region.
(B) Scatter plot of the observed variation frequency on both strands. The colors depict mosaic mutations (*magenta*), heterozygous mutations (*orange*), single-nucleotide polymorphisms (SNPs, *green*) and sequencing errors (*gray*), respectively.

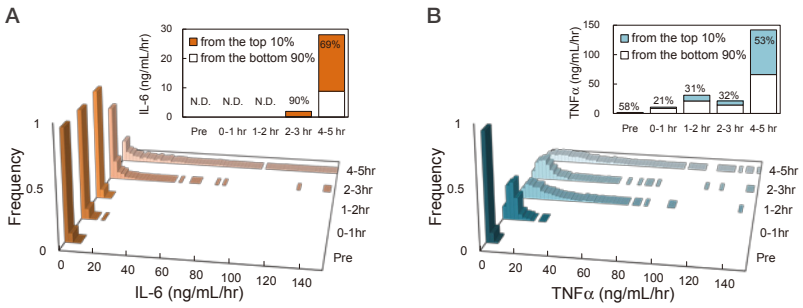


Figure 2: Single-cell measurements reveal a large variation in cytokine secretion activities of J774.1 cells after LPS stimulation.
IL-6 (panel A) and TNFα (panel B) secretion by single cells was measured by a microengraving method before and after stimulation with LPS. These histograms show the frequency (vertical axis) of J774.1 cells with each secretion activity (ng/mL/hr, horizontal axis). Inset bar graphs show the secretion activity of the whole cell population. Each colored part of bar indicates the contribution from the higher secretor cells in the top 10%.

develop a new pipeline to detect even a low-frequency *NLRP3* allele with statistical significance. To solve the problem of how to discriminate a low-level allele from sequencing errors, we first constructed position- and strand-specific error rate maps of 14 PCR products covering the entire coding *NLRP3* exons from 50 control samples without mosaicism on a Roche 454 GS-FLX sequencer (Fig. 1). Based on these results, we formulated a statistical confidence value for each sequence variation on each strand to discriminate sequencing errors from real genetic variation even in a low-frequency allele, and thereby realized detection of base substitutions at an allele frequency as low as 1% with a 99.9% or higher significance level. This new method can easily be applied to other cases of somatic mosaicism, e.g., various types of cancers to detect disease-causing mutations.

Monitoring of secretion of cytokines from single macrophages measured by a "Microengraving" method

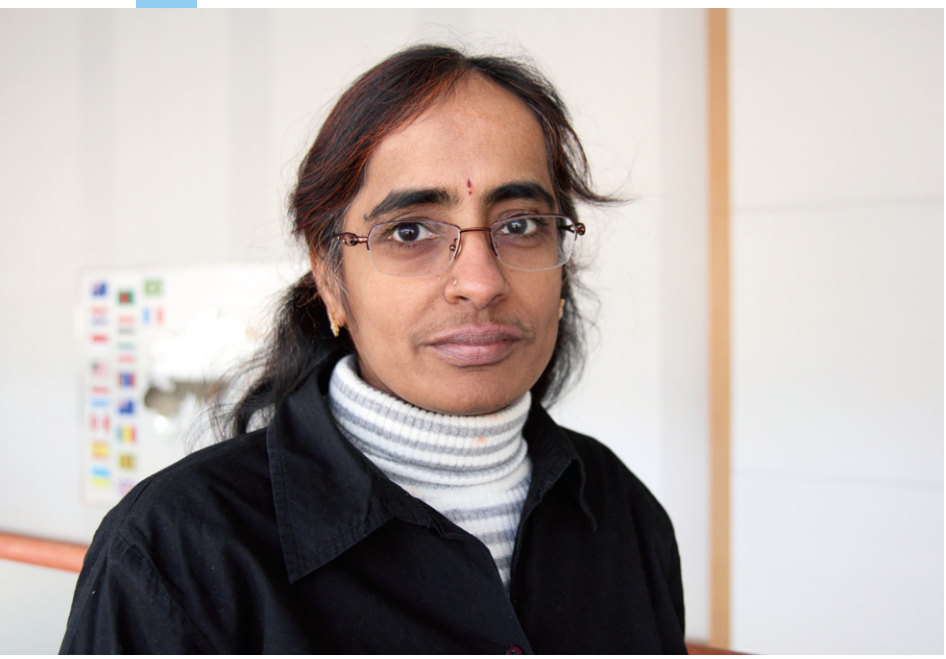
To maintain a dynamic and integrated immune system, immune cells closely communicate with each other by means of humoral factors and cell surface molecules. Traditionally, secreted humoral factors are quantified to estimate the functions of the cells based on their concentrations in culture supernatants from a population of purified immune cells. However, recent single cell analyses are revealing great heterogeneity even within an isogenic cell population. Therefore, it is interesting to discover just how heterogeneous immune cells are, even among a clonal population, in terms of responses to external stimuli. Here, we introduced a new method named "microengraving", which allows simultaneous measuring of secretion activity of thousands of single cells, to establish an approach to describe a secretion activity distribution measurement at the single-cell level in a cell population. In this experiment, we used a macrophage-like cell line, J774.1. After the J774.1 cells were activated with bacterial lipopolysaccharide (LPS), the secretion activity distribution was measured for IL-6 and TNFα by the "microengraving" method. The IL-6 response to LPS stimulation was observed with a short delay of 2 hrs while TNFα was secreted immediately after LPS stimulation. The secretion activity of IL-6 and TNFα 4 hrs after stimulation varied widely from cell to cell (Fig. 2A). For IL-6, this heterogeneity meant that the high secretor cells in the top 10% were able to account for the majority of total secreted cytokine. On the other hand, secretion activity of TNFα after a short period of stimulation showed a positively skewed bell-shaped distribution (Fig. 2B). The more limited heterogeneity of TNFα secretion among the cells was reflected in the lower contribution of the secreted TNFα from the top 10% cells to the total secreted TNFα, especially at early time points. Such large cell-to-cell variations in cytokine secretion could never have been detected by a bulk cytokine measurement in culture medium and thus the microengraving method provides us with unexplored lines of information to build a systems view of a multicellular ensemble.

4. High incidence of NLRP3 somatic mosaicism in patients with chronic infantile neurologic, cutaneous, articular syndrome: results of an International Multicenter Collaborative Study: Tanaka N, Izawa K, Saito MK, Sakuma M, Oshima K, Ohara O, et. al. ***Arthritis Rheum***. 2011 63(11):3625-32.

5. Quantification of κ-deleting recombination excision circles in Guthrie cards for the identification of early B-cell maturation defects: Nakagawa N, Imai K, Kanegane H, Sato H, Yamada M, Kondoh K, Okada S, Kobayashi M, Agematsu K, Takada H, Mitsuiki N, Oshima K, Ohara O, Suri D, Rawat A, Singh S, Pan-Hammarström Q, Hammarström L, Reichenbach J, Seger R, Ariga T, Hara T, Miyawaki T, Nonoyama S. ***J Allergy Clin Immunol***. 2011 128(1):223-225.e2.

Research Unit for

Immunoinformatics



Unit Leader :
S. Sujatha Mohan

Research Associate : **Suresh Kumar Ramadoss**

An important mission of our research unit is to develop and maintain an open access bioinformatics platform and data resource in order to gain insights into primary immunodeficiency diseases (PID) through genomic, transcriptomic and proteomic data. In this pursuit, we launched a web-based compendium of molecular alterations in PID, Resource of Asian Primary Immunodeficiency diseases (RAPID) at http://rapid.rcai.riken.jp in 2008. RAPID also hosts other pertinent information such as expression profiles, interaction networks, mouse studies and standardized DNA sequencing protocols for recognized PIDs. All mutation data have been linked with a graphical user interface (GUI) enabled tool, named Mutation@A Glance, to visualize and evaluate reported mutations. Using RAPID data, we have used Support Vector Machine (SVM) based parameter classification to predict candidate PID genes by scanning genes in the whole human genome. Furthermore, RAPID has been updated with a standardized PID expert page, a terms search option for mouse and human phenotypes and a mutation data submission tool.

Our ultimate goal is to provide relevant, up-to-date and validated information on PID as per global community standards in an easily decipherable and usable format.

RAPID statistics

At present, RAPID includes a total of 234 genes that are confirmed to cause over 250 PIDs. There are over 4700 unique mutations annotated from 1704 citations as of January 2012. The data growth of RAPID since its launch is depicted in Fig. 1.

PhenomeR: A Genotype – Phenotype integrated approach for mining Primary Immunodeficiency Disease causal genes using semantic web technology

The main challenge for *in silico* genotype-phenotype correlation for any genetic disease is to standardize phenotype ontology terms and the genotype. We have standardized PID specific phenotype terms using NCBO's BioPortal (http://bioportal.bioontology.org/) for mapping and standardizing terms from other ontology resources. We are following a unique semi-automated method to assign literature collected phenotype terms to specific PIDs. This kind of analysis should bridge a gap between genotype and phenotype correlation, thereby improving phenotype-based genetic analy-

Recent publications

1. Raju, R., V. Nanjappa, *et al.* "NetSlim: high-confidence curated signaling maps." ***Database (Oxford)*** bar032 (2011).
2. Suresh Kumar Ramadoss and Sujatha Mohan, In Silico Identification of Prioritized Interacting Domains in Primary Immunodeficiency Disease Causing Genes. ***Intl J Biosci, Biochem Bioinformatics***, 1 (2) : 84-88 (2011).
3. Hiroshi Masuya, Yuko Makita, Norio Kobayashi, *et al.* The RIKEN integrated database of mammals. ***Nucleic Acids Res***, 39 (Database issue) : D861-70 (2011).

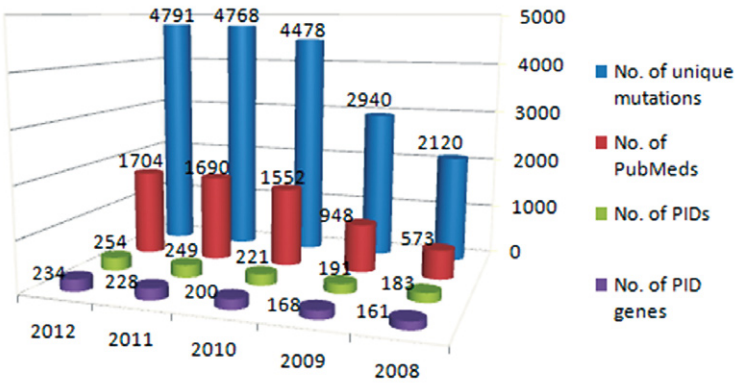


Figure 1: RAPID data growth as of January 2012
RAPID data growth as of January, 2012. No. of PubMeds refers to the number of annotated reference papers with mutation data, No. of mutations indicates non-redundant entries of mutation data annotated and No. of PIDs represents unique primary immunodeficiency disease names included in RAPID.

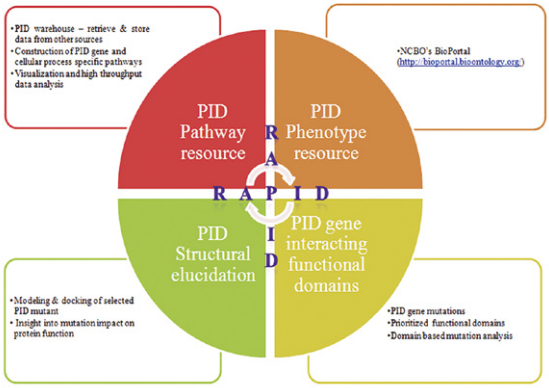


Figure 2: RAPID - Integrated PID KnowledgeBase
Overview of RAPID integral features and its elements in the PID integrated KnowledgeBase

sis of PID genes. Moreover, it should aid clinicians in confirming early PID diagnosis and also be helpful in implementing appropriate therapeutic interventions.

Road map towards RAPID - integrated PID KnowledgeBase

RAPID's ongoing and future tasks are identified as four major components and their salient features are highlighted as follows:

i) PID Pathway resource - Generation of a web-based integrated PID-specific immune signaling and regulatory pathway framework that would aid biomedical investigators in understanding the molecular mechanisms of PID pathogenesis and also help in identifying disease causing candidate PID genes.

ii) PID phenotype resource - Standardize PID phenotype terms and mapping to genes, mutation and disease as well as the construction of PID phenotype ontology resource for easy access and query of any observed symptoms against PID using semantic web technology

iii) PID functional domain analysis - Identify functionally significant domains that disrupt the interactions of PID genes associated with disease mutation through an integrated *in silico* approach

iv) PID structural elucidation tool - Accomplish sequence-structural analysis of PID mutations through modeling and docking studies to assess their functional impact on PID pathogenesis

A brief overview of the RAPID road map to this KnowledgeBase is shown in Fig. 2.

Inception of an integrated Arthritis KnowledgeBase for high-throughput data analysis

Integrated Arthritis database (IntARTdB) is a web based compendium of high-throughput data on arthritis. It serves as an integrated platform for arthritis-specific data collection, storage, annotation and analysis of genomics, transcriptomics, proteomics, metabolomics and other omics studies. The IntARTdB framework is based on the constructed model-view-controller architecture. We intend to provide the data annotation tool to registered users, with controlled access privilege, to share and contribute analyzed high-throughput data to IntARTdB.

This project has been recently initiated as an International collaboration with the Gobezie lab at Musculoskeletal Proteomics, The Case Center for Proteomics and Systems Biology, Case Western Reserve University - School of Medicine, USA.

Collaboration and funding

The PID project has been initiated in collaboration with the Institute of Bioinformatics (IOB, Bangalore, India) and the Immunogenomics research group at RIKEN RCAI, Japan. This laboratory was supported by The Asia S&T Strategic Cooperation Promotion Program, Special Coordination Funds for Promoting Science and Technology by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) between July, 2007 and March 2010. Now, it is being funded by RCAI, RIKEN internal funding source.

4. Atsushi Hijikata, Rajesh Raju, Shivakumar Keerthikumar *et al.* Mutation@A Glance: an integrative web application for analyzing mutations from human genetic diseases. ***DNA Res***, 17(3):197-208 (2010).

5. Shivakumar Keerthikumar, Sahely Bhadra, Kumaran Kandasamy, *et al.* Prediction of candidate primary immunodeficiency disease genes using a support vector machine learning approach - ***DNA Res***,16(6):345-51 (2009).

6. Shivakumar Keerthikumar, Rajesh Raju, Kumaran Kandaswamy, *et al.* RAPID: Primary Immunodeficiency disease database. ***Nucleic Acids Res***, 37 (Database issue):D863-7 (2009).

Laboratory for
Cellular Systems Modeling

Team Leader :
Mariko Okada (Hatakeyama)

Senior Scientist : **Shinji Kondo**

Research Scientists : **Masaki Nomura**
Hisaaki Shinohara

Technical Staff : **Noriko Yumoto**
Kaoru Takahashi
Ki Sewon

Student Trainee : **Hanna Iribe**

Recent sequencing projects have begun to reveal a coordinated effect of genomic mutation, epigenetic modification, micro and noncoding RNAs for determining cellular outcome. Yet, it is still unclear how all these components are dynamically connected and function to produce the desired biological outputs. Systems Biology thus meets the strong desire of biologists to understand mechanistic aspects of the molecular and cellular networks. As a result, one day, we may be able to define simple logic that drives proliferation, differentiation or death of the cells. One of the expected outcomes of Systems Biology research is to identify network designs and functions in the organism and to engineer them for industrial and medical application. Recent forefront studies of Systems Biology have begun to give us an idea about universal cellular logic designs and motifs distributed in living organisms regardless of the cell type.

The Laboratory for Cellular Systems Modeling has been carrying out research to identify biological networks and regulatory principles using computational, theoretical and experimental approaches. Our research interest is to identify and reconstruct biological regulatory circuits from experimentally observable data using computational and theoretical approaches to reveal the complexity of signal transduction networks, which determine cell fate in mammalian cells. By doing so, we are able to identify rules specified in immunological systems.

For this purpose, we take both bottom-up kinetic analysis and top-down omics approaches. These approaches complement each other in order to understand objects at a system level. For kinetic analysis, we constructed ordinary differential equation (ODE)-based mathematical models to describe digital induction of an early responsive gene, the c-Fos transcription factor, in a cell differentiation process (*Cell*, 2010). The model explains that AND-gate and negative feedback loops might shift the cell into the irreversible differentiated state and that a sustained ERK signal is a driving force of the system (Figure 1). This model is valid for production of transcription factors in B-cell development and neurite elongation of PC12 cells, therefore it is most likely that the model captures general network principles of this signal network during a cellular differentiation process. Using this logic, we were able to convert a transient signal to a sustained signal by membrane receptor mutation and, as predicted, this network conversion was accompanied by a differentiated cellular phenotype.

Recent publications

1. Morita M, Oike Y, Nagashima T, Kadomatsu T, Tabata M, Suzuki T, Nakamura T, Yoshida N, Okada M, Yamamoto T. Obesity resistance and increased hepatic expression of catabolism-related mRNAs in Cnot3(+/-) mice. ***EMBO J***. 30, 4678-4691 (2011).
2. Oyama M, Nagashima T, Suzuki T, Kozuka-Hata H, Yumoto N, Shiraishi Y, Ikeda K, Kuroki Y, Gotoh N, Ishida T, Inoue S, Kitano H, Okada-Hatakeyama M. Integrated Quantitative Analysis of the Phosphoproteome and Transcriptome in Tamoxifen-Resistant Breast Cancer. ***J. Biol. Chem.***, 286, 818-829 (2011).
3. Shiraishi Y, Kimura S, Okada M. Inferring Cluster-based Networks from Differently Stimulated Multiple Time-course Gene Expression Data. ***Bioinformatics*** 6, 1073-1081 (2010).

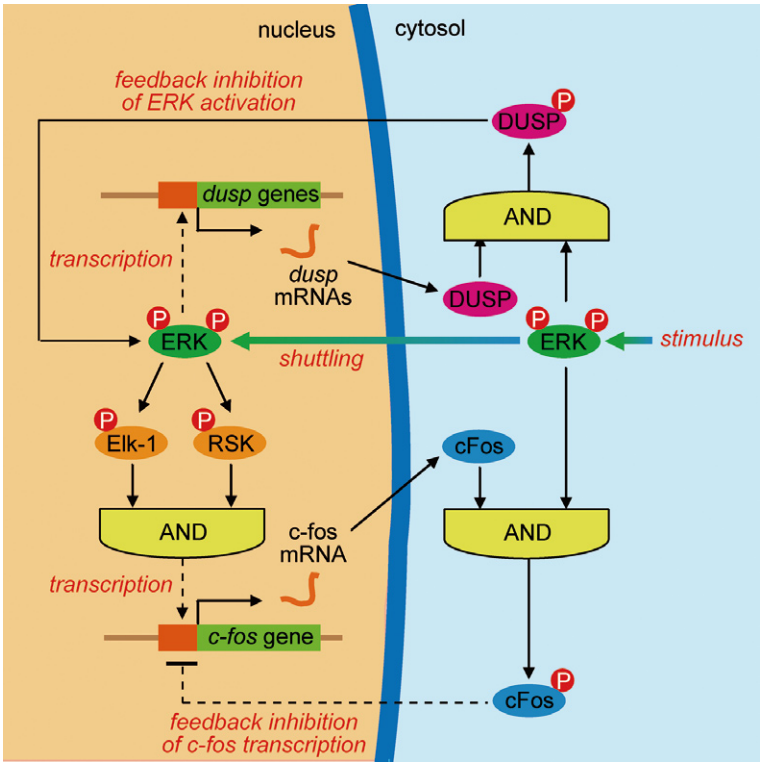


Figure: Network structure of ERK signal-induced c-Fos induction necessary for the early stage of cellular differentiation. ERK serves as a driving force to produce c-Fos using AND-gate loops (noise filtering). Thus, the c-Fos network is very robust to noise.

In addition, inspired by a relationship between the signal dynamics and cell fate decisions, we attempted to obtain binding kinetics of ligand-membrane receptor interaction in living cells (in collaboration with Dr. Yasushi Sako, RIKEN ASI). This analysis indicated that ligand-receptor binding kinetics are very similar in the cells whether they are undergoing proliferation or differentiation, therefore, intracellular kinetics rather than ligand-receptor kinetics seem to play an important role in cell fate decision.

As for the top-down approach, we perform several transcriptome studies to unveil transcription networks. For an example, we integrated phospho-proteome data and transcriptome data to understand drug-resistance mechanisms in breast cancer cells (*J. Biol. Chem.*, 2010). This integrative analysis indicated that AP-1 and CREB transcription factor activation was dysregulated in the drug-resistant cells. We proposed and clinically verified that GSK3beta is a drug-resistance marker. These studies showed that integrated analysis of signaling and transcription allows us to understand drug-resistant properties at a network level and to identify a therapeutic target. For this kind of large-scale data analysis, we also developed several computer algorithms and methods. Particularly, we developed a method to identify synergic effects of two transcription factors / or histone modifications responsible for gene expression (*Bioinformatics*, 2011). The method further allows us to evaluate combinatorial drug therapy for certain diseases using transcriptome data.

4. Nakakuki T, Birtwistle MR, Saeki Y, Yumoto N, Ide K, Nagashima T, Brusch L, Ogunnaike BA, Okada-Hatakeyama M*, Kholodenko BN.* Ligand-Specific c-Fos Expression Emerges from the Spatiotemporal Control of ErbB Network Dynamics. ***Cell*** 141, 884-896 (2010). * corresponding authors.

5. Suenaga A, Hatakeyama M, Kiyatkin AB, Radhakrishnan R, Taiji M, Kholodenko BN. Molecular Dynamics Simulations Reveal that Tyr-317 Phosphorylation Reduces Shc Binding Affinity for Phosphotyrosyl Residues of Epidermal Growth Factor Receptor. ***Biophys. J***. 96, 2278-2288 (2009).

Research Unit for
Thymic Environment

Unit leader :
Willem van Ewijk

Research scientist : **Kathryn Ischi-Schrade**

STAT 3 influences the development of thymic microenvironments

The development of thymic microenvironments formed by thymic epithelial cells (TECs) is a two-step phenomenon. The embryonic initiation of TECs occurs as a cell-autonomous phenomenon, regulated among others by the tissue specific transcription factor Foxn1 (Nehls et al., *Science* 272, 886-889, 1996; Balciunaite et al., *Nat. Immunol* 3, 1102-1108, 2002), while further expansion of TECs is controlled by developing thymocytes, a phenomenon earlier designated as "thymic crosstalk" (van Ewijk et al., *Immunol. Today* 15, 214-217, 1994). Analysis of the molecular signature underlying the lymphoid dependent phase of TEC development is at present a 'hot' topic in thymic research. This signature is now becoming increasingly clear in the development of medullary TECs (mTECs), where the NF-κB signaling pathways controls the proliferation and differentiation of mTECs, and involves cell surface receptors such as LTβR, RANK and CD40 on mTECs (Boehm et al., *J.Exp.Med*. 198, 757-769, 2003; Hikosaka et al., *Immunity* 29, 438-450, 2008; White et al., *J. Immunol*. 185, 4769-4776, 2010).

However, in cortical TECs (cTECs) this signature has yet to be unraveled, although *in vitro* experiments imply Notch signaling as an important regulator in functional TEC development (1).

STAT3

We have set out to analyze the role of signaling cascades other than NFκB in TEC development. Around 10 years ago, it was suggested that STAT3 signaling influenced TEC development (Sano et al., *Immunity* 15, 261-273, 2001). In this report, cre-mediated deletion of STAT3 under control of the Keratin-5 (K5) promotor was found to strongly affect TEC development mainly in the cortex, however, the influence of STAT3 deletion in the medulla remained less clear.

To study the STAT3 deletion in more detail, we created mice where STAT3 was deleted under control of the Foxn1 promotor. We employed Foxn1-cre mice (Hauri-Hohl et al., *Blood*, 112, 626-634, 2008) and GFP reporter mice (Kawamoto et al., *FEBS Lett*. 470, 263–268, 2000) to construct Foxn1-STAT3CKO mice. The thymus of these mice was normal sized, and T cell development (as determined by expression of CD4 and CD8) appeared normal (Fig 1A, B). Immunohistological analysis of cortical TECs (cTECs) revealed a normal architecture but mTECs, as identified by the mTEC specific marker ER-TR5, formed long cytoplasmic extensions. Moreover, mTECs in STAT3 deleted mice were less frequent and they formed smaller medullary domains (Fig 1C).

We next investigated whether the functional maturation of mTECs in Foxn1-STAT3CKO mice was impaired. For this purpose, we stained cryosections of the thymus of these mice with antibodies detecting UEA-1 and AIRE, molecules expressed in mature mTEC subsets involved in negative selection of autoreactive thymocytes. Fig. 2 shows that UEA-1- and AIRE-expressing mTECs are present in medullary domains in Foxn1-STAT3CKO thymi. These data there-

Recent publications

1. Masuda, K, Germeraad WTV , Satoh R, Itoi M, Katsura Y, van Ewijk W, Kawamoto H. Activation of Notch signaling in thymic epithelial cells induces development of thymic microenvironments. ***Mol. Immunol.*** 2009, 46: 1756-1767.

2. Vroegindeweij, E, Itoi M, Satoh R, Zuklys S, Crobach S, Germeraad WTV. Cornelissen JJ, Cupedo T, Holländer G, Kawamoto H, van Ewijk W. Thymic cysts originate from Foxn1 positive thymic medullary epithelium. ***Mol. Immunol.*** 2010 47(5):1106-1113.

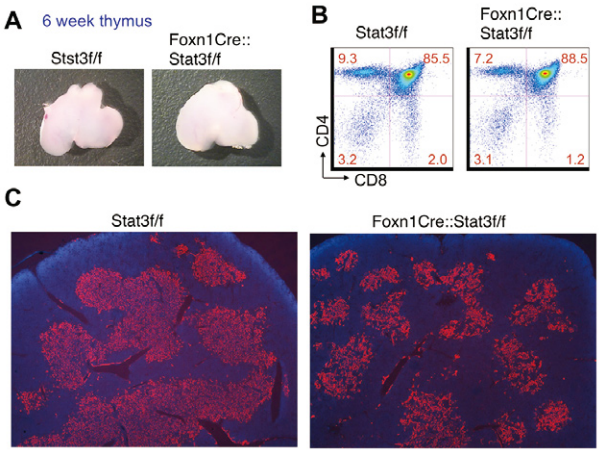


Figure 1: **A.** Thymi of Foxn1-STAT3CKO thymi and control thymi are equal in size.
B. Similar CD4/CD8 profiles of thymocytes in Foxn1-STAT3CKO mice.
C. Cryostat sections of thymi derived from Foxn1-STAT3CKO show a size reduction in the medullary domains, stained by ER-TR5 (*red*).

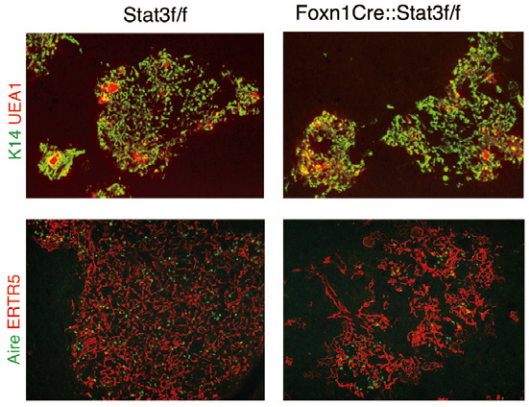


Figure 2: Cryostat sections of thymi derived from Foxn1-STAT3CKO show mature mTECs as identified by UEA-1 (*red; top panels*) and AIRE (*green; bottom panels*) staining.

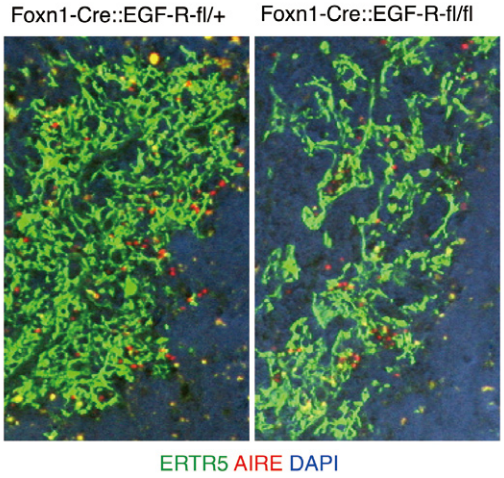


Figure 3: Cryostat sections of thymi derived from Foxn1-Cre::EGF-R-fl/+ (control) and Foxn1-Cre::EGF-R-fl/fl mice (deletion) stained with ER-TR5 (mTECs; *green*) and AIRE (*red*).

fore indicate that TEC development in itself is not impaired in absence of STAT3, also explaining the normal phenotypical T cell development observed in the Foxn1-STAT3CKO mice (Fig.1B). Thus, it seems likely that STAT3 controls the expansion rather than the final differentiation of mTECs.

In contrast to data reported by Sano et al (*Immunity* 15, 261-273, 2001), we did not find a cTEC phenotype in Foxn1-STAT3CKO mice. Overall, the three dimensional organization of cTECs in Foxn1-STAT3CKO thymi was found similar to that in control mice, and functional molecules like MHC I and II, and β5T were normally expressed by Foxn1-STAT3CKO cTECs (data not shown).

To exclude the possibility that genetic variations underlie the difference in TEC phenotype between Sano's paper and our results, we generated our own K5-STAT3 CKO mice. Surprisingly, and in contrast to the earlier report by Sano et al., the stromal phenotype of these mice was quite similar to the phenotype observed in Foxn1-STAT3CKO mice (data not shown).

We have so far no clear explanation for the phenotypic difference in the cTECs in the two studies. Since K5 is also expressed in the skin, which is a reason why these mice are also used as a model for atopic dermatitis (Sano et al., *Embo J.* 18,4657-4668, 1999), one could speculate that induction of dermatitis induced some stress that indirectly affected TEC development. However, stress related glucocorticoid levels in the K5 STAT3 were reported to be normal (Sano et al., *Immunity* 15, 261-273, 2001).

EGFR

EGFR and HGFR are known molecules upstream of STAT3 (Guo et al., *J Clin Invest.*;117:3846-3856, 2007). We generated EGFR and HGFR floxed mice and mated these mice to FoxN1-Cre mice in order to target the deletion to TECs. While HGFR-Foxn1CKO mice had no apparent epithelial phenotype, EGFR-Foxn1CKO mice had a TEC phenotype in the thymic medulla, similar (but slightly milder) to that in STAT3-Foxn1CKO mice (Fig.3).

In conclusion, our study indicates that (1) TEC development in the medulla, but not in the cortex, is under the control of STAT3, (2) STAT3 regulates expansion of mTECs rather than their differentiation. (3) The EGF receptor acts upstream of STAT-3 signaling in this process.

Research Unit for

Immunoepigenetics

Unit Leader :
Miguel Vidal

Research Scientist : **Kaori Hisada**

Polycomb group (PcG) complexes function as transcriptional repressors of genes controlling cell identity states and transitions between them. In stem cells, PcG complexes function in maintaining their developmental potential and its orderly deployment during their differentiation through selective silencing of their targets. PcG complexes act both at higher order chromatin structure and at cis-acting control region levels. Their function depends, at least in part, on their activities as histone modifiers (trimethylation of histone H3 and monoubiquitylation of histone H2A). Recruiting of PcG complexes to their targets and dissection of functional diversity of PcG subunits are subjects of active research. One of these subunits, the RYBP protein that we identified as a direct interactor with the PcG histone H2A monoubiquitin ligases Ring1A and Ring1B, appeared to be a good candidate for PcG recruiting. RYBP function, however, is poorly characterized, in part due to the embryonic lethal phenotype of constitutive RYBP mutant alleles.

RYBP function studies in an embryonic stem (ES) cell model

Embryonic stem (ES) cells cannot be derived from early embryonic structures constitutively deficient in RYBP and RYBP is found among interactors with Oct-4, the regulatory master of the pluripotency network of transcriptional control in ES cells. We have generated ES cells carrying both a floxed RYBP allele and a gene encoding a ubiquitously expressed Cre-ER recombinase inducible by hydroxytamoxifen. Inactivation of RYBP results in self-renewing ES cells that express markers of pluripotency (Fig. 1). ChIP on Chip studies showed that RYBP colocalizes with class I Polycomb products (for example Ring1B) on chromatin of wild type ES

Recent publications

1. Hisada, K., Sánchez, C., Endo, T., Endoh, M., Román-Trufero, M., Sharif, J., Koseki, H. and Vidal, M. RYBP represses endogenous retroviruses, preimplantation- and germline-specificgenes in mouse embryonic stem cells. ***Mol. Cell. Biol.*** 32:1139-1149 (2012)

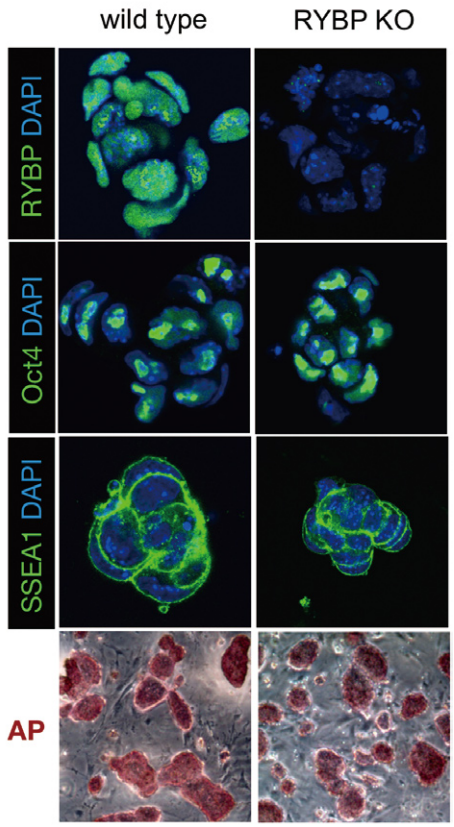


Figure 1: Pluripotency markers in RYBP-deficient ES cells. Oct4, SSEA1 and alkaline phosphatase, in control and tamoxifen-treated RYBPf/f ES cells

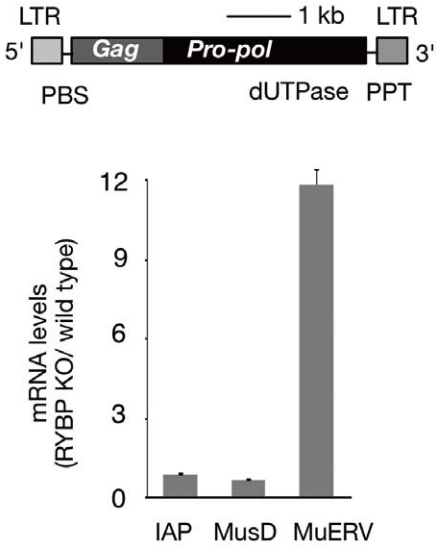


Figure 2: RYBP represses retrotransposons in ES cells. *Top*: schematic representation of MuERVs. *Bottom*: specific derepression of MuERVs but not other retrotransposons in RYBP-deficient ES cells.

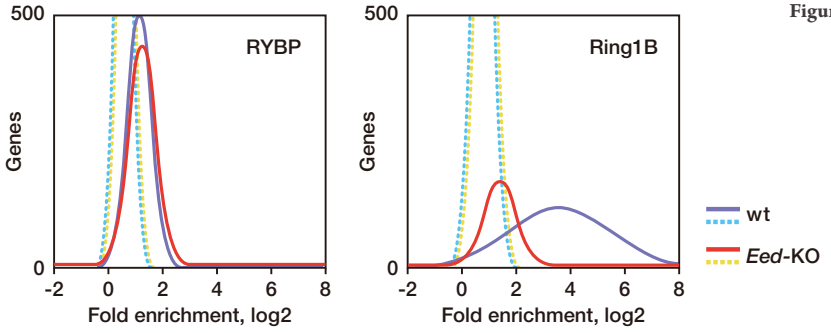


Figure 3: Contrasting sensitivity to H3K27me3 of RYBP and Ring1B association to chromatin. RYBP binding to targets in *Eed*-KO cells (without H3k27me3) was not altered, whereas Ring1B was acutely depleted from chromatin in mutant cells.

cells. However, RYBP-deficient ES cells showed little or no derepression of genes repressed by Ring1B (core Polycomb targets such as developmental regulators). Instead, transcripts originating in class III retrotransposons (MuERVs) are, together with preimplantation-specific genes, among the highest upregulated mRNAs in RYBP-deficient cells (Fig. 2). In some cases, at least, transcripts from preimplantation genes originate from nearby truncated transposon elements. This observation, together with the property of RYBP to associate with chromatin in ES cells lacking H3K27me3 (Fig. 3), the mark that recruits Ring1B complexes, and the expression pattern of RYBP (starting at the two-cell stage) suggest a role for RYBP in early developmental stages. Future work will address this hypothesis and also the function of ES cells conditionally deficient for Yaf2, the RYBP paralog.

Research Unit for

Immune Crosstalk

Unit Leader:

Hilde Cheroutre

Mucosal T cells March to the Beat of a Different Drummer

Our research aims at understanding and elucidating the differentiation, function and immune regulation of T cells that reside at the mucosal interface of the intestinal epithelium.

Unlike lymphoid T cells, intestinal intraepithelial lymphocytes (IEL) reside at epithelial surfaces and form the first line of defense against invading pathogens. Although indispensable for preventing the initial infection, IEL need to balance their protective function with safeguarding the integrity of the barrier and failure to do so compromises homeostasis of the organism.

These unique challenges drive specialized differentiation and regulation of IEL and the main goals of our research are 1) to elucidate regulatory mechanisms that preserve the mucosal barrier in the face of potentially destructive immune protection and 2) characterizing unique processes that drive the generation of mucosal effector memory T cells that warrant optimal pre-existing protective immunity.

We have made milestone discoveries in both areas and showed that, for example, retinoic acid (RA) is a key factor in controlling mucosal immune responses and under tolerogenic conditions. RA promotes the induction of TGF-β-dependent Foxp3 iTreg whereas it suppresses the differentiation of inflammatory TGF-β-driven Th17 cells.

Our research continues to elucidate mechanisms of mucosal immune regulation and in a recent study we uncovered an unexpected degree of plasticity for CD4 T helper (Th) cells which, upon antigenic stimulation, are able to terminate the expression of the Th transcription factor, ThPOK, and differentiate into CD8-CTL-like effector cells.

We recently also showed that an affinity-based selective process operates at the mucosal interface of the intestine and preserves the optimal effector cells to become long-lived effector memory T cells (T_{EM}) that establish pre-existing and heightened protective immunity (Huang et al., *Nature Immunology* 2011).

Unlike central memory T cells (T_{CM}) that reside in lymphoid tissues, T_{EM} gain the capacity to reside long-term in non-lymphoid tissues, such as the intestine. T_{CM} cells, which respond to antigen with a robust clonal expansion, are effective at protecting against infections by pathogens that replicate systemically, but they are inadequate to prevent transmission of viruses, including the human immunodeficiency virus (HIV), or intracellular bacteria, which penetrate across mucosal epithelia. Effective resistance against trans-

Recent publications

1. Huang Y, Park Y, Wang-Zhu Y, Larange A, Arens R, Bernardo I, Olivares-Villagómez D, Herndler-Brandstetter D, Abraham N, Grubeck-Loebenstein B, Schoenberger SP, Van Kaer L, Kronenberg M, Teitell MA, Cheroutre H**.** Mucosal memory CD8$^+$ T cells are selected in the periphery by an MHC class I molecule. ***Nat Immunol***. 2011 Oct 2;12 (11):1086-95.
2. Ichikawa S, Mucida D, Tyznik AJ, Kronenberg M, Cheroutre H. Hepatic stellate cells function as regulatory bystanders. ***J Immunol***. 2011 May 5;186(10):5549-55.
3. Cheroutre H. HIV vaccination: turning the spotlight on effector memory T cells as mucosal gatekeepers. ***F1000 Biol Rep***. 2009 Nov 26;1:89.

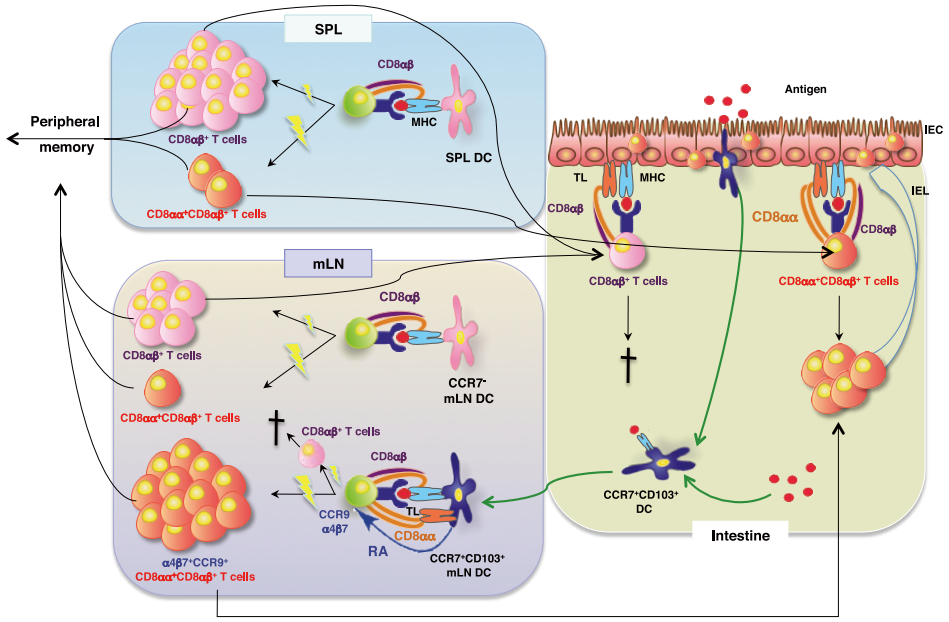


Figure : **Proposed model for the roles of TL and CD8αα in selection of high affinity CD8αβ T_{EM}.** CD8αα expression is selectively induced on high affinity/avidity primary effector CD8αβ$^+$ T cells and further enhanced by RA released by mucosal migratory (CCR7$^+$) DC, which also promote gut-tropism (α4β7$^+$CCR9$^+$) of the effector T cells. Activated migratory DC express the CD8αα high affinity ligand, TL, which when interacting with CD8αβ on activated T cells leads to TL-induced cell death (†,TICD). High affinity primary effector cells that induce CD8αα use it to escape TICD by sequestering TL away from CD8αβ, leading to affinity-based selective survival. TL constitutively expressed on the intestinal epithelial cells (IEC) mediates affinity maturation of the mucosal T_{EM} and eliminates low affinity/avidity primary and secondary effector cells that home to the gut and fail to induce CD8αα.

mission of such pathogens requires the presence of local, highly efficient antigen-specific T_{EM} prior to re-challenge. Nevertheless, because most of the current knowledge of immune memory has been gained from model systems that use systemic immunization routes for the generation of lymphoid T_{CM}, the generation of T_{EM} was very poorly understood.

We showed previously that the TCR repressor, CD8αα, induced on activated CD8αβ T cells, marks those primary effector cells that preferentially differentiate into memory cells (Madakamutil et al., *Science* 2004). The expression level of CD8αα is controlled by signal strength and it is most highly induced on those effector cells with the highest antigen-affinity. We found that mucosal T_{EM} are highly enriched for CD8αα-expressing memory cells, indicating that a selective mechanism might drive the preferential accumulation of high affinity effector cells at the mucosal interface of the intestine.

In an earlier study we had shown that the high affinity ligand for CD8αα, the non-classical MHC class I molecule thymus leukemia antigen (TL), is constitutively expressed on mucosal epithelial cells. Using oral infection with the food borne pathogen, *Listeria monocytogenes (Lm)*, we demonstrated that an affinity-based selection mechanism exists, controlled by TL expressed on APCs, which leads to the selective survival of high affinity, CD8αα$^+$CD8αβ memory precursor cells. Furthermore, TL on the intestinal epithelial cells (IEC) continues to impose selection pressure and drives the affinity maturation of the resident mucosal CD8αβ T_{EM} and warrants the optimal protective capacity of these memory cells.

Our findings represent a fundamentally new concept for immune memory differentiation and indicate that an affinity-based selective process operates *in vivo* that preserves the optimal effector cells to become long-lived T_{EM} that establish pre-existing and heightened protective immunity at mucosal borders.

Although unable to provide sterilizing protection, T_{EM} control the pathogen load and delay or prevent the initial infection and spreading of the pathogen, as well as reduce the potential for secondary transmission. Therefore, our finding that an endogenous TCR quality-based mechanism selects for the most avid effector cells to form mucosal T_{EM} has significant implications for the design of new and improved strategies to induce effective pre-existing protective immunity at the most vulnerable entry sites for pathogens.

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Central Facilities

Central Facilities in RCAI provide all researchers in the Center with access to the most advanced equipment and technologies. Central Facilities consist of five sections; the FACS, Confocal, and Monoclonal Antibody Laboratories managed by Dr. Takashi Saito, the Genomics Laboratory managed by Dr. Osamu Ohara, and the Animal Facility managed by Dr. Haruhiko Koseki.

FACS Lab.

Technical Scientist : **Hanae Fujimoto**
Technical Staff : **Yukiko Hachiman**
Visiting Scientist : **Ikuo Ishige** (BM Equipment Co. Ltd.)

The FACS Lab provides a range of support for flow cytometry and cell sorting, procedures that are essential for nearly all immunological experiments. In 2011, the FACS Lab newly installed a CyTOF (Fig. 1), which combines the functions of a flow cytometer and a mass spectrometer by using atomic mass spectrometry to allow immunophenotypic detection of up to 25 metal labeled intracellular/extracellular antibodies. Because the mass spectrometer provides at least 3 orders of magnitude resolution between adjacent detection channels, compensation is not required. This is the first CyTOF installed in Japan and, even throughout the world, only 4-5 institutions have installed the machine. The FACS Lab invited Dr. Sean C. Bendall from Stanford University and organized a seminar to introduce the application of this machine for single-cell analysis of immunological research.

Table 1: Instruments in the FACS Lab

Machine types	Machines	Number of machines
FACS cell analyzer	Calibur	5
	Canto Ⅱ	1
Imaging flow cytometer	ImageStreamX	1
Mass Cytometer	CyTOF	1
FACS cell sorter	Aria Ⅰ	2
	Aria Ⅱ	1
	Vantage	2
	Diva (digital vantage)	1

Figure 1 : CyTOF

For the users of FACS machines (cell analyzers and cell sorters), Hanae Fujimoto and Yukiko Hachiman provide various services, mainly in the following three areas.

1. Technical support and training
   In 2011, the facility offered 12 technical courses (5 for cell sorting, 4 for cell analysis and 3 for CyTOF) both in English and Japanese. Courses were held for 4 different machine types, Calibur, Canto II, CyTOF and Aria I/II. A total of 82 RCAI researchers took the courses in 2011.

2. Cell sorting operation service
   The FACS Lab provides a cell sorting operation service, in which researchers can ask an experienced operator to conduct the sorting experiment. In 2011, there were 283 such requests.

3. Management/maintenance of FACS machines
   FACS machines are available for registered users 24 hours a day and reservations are accepted up to one month in advance through an internal website. All the necessary information including instructions, reservations and user fees can be accessed via the intranet. In addition to the in-house FACS Lab staffs, engineers from Becton Dickinson visit once a week to provide maintenance and technical support.

Monoclonal Antibody Lab.

Technical Staff : **Tomomi Aoyama, Kazuyo Uchida**

The Monoclonal Antibody (mAb) Lab aims to produce mAbs that meet the needs of RCAI researchers, and also focuses on more targeted development of mAb with a strategic purpose, such as those for allergy-related molecules. This activity is now being done in cooperation with the program for Drug Discovery and Medical Technology Platforms for development of monoclonal antibody as diagnosis and drugs. Two technical staff members, Tomomi Aoyama and Kazuyo Uchida, are engaged in producing, purifying and analyzing mAbs. In 2011, the Lab produced mAbs against 13 different antigens, which were requested by 8 laboratories. The lab also prepared human chimeric antibodies, such as anti-Cry j 1 human IgE antibody.

Confocal Lab.

Visiting Scientist : **Yasutaka Wakabayashi** (Leica Microsystems K.K.)

The Confocal Lab provides imaging equipment and technical support. The Confocal Lab is managed in collaboration with Leica Microsystems and there are 6 confocal microscope systems:

1. Inverted system with a visual and multi-photon MP laser suitable for time-lapse imaging of living cells and organs.
2. Inverted system with a 405 nm laser suitable for a time-lapse imaging of living cells in a controlled environment (CO_2, temperature, and humidity).
3. Leica SP5 confocal system, the successor of the SP2. Brighter and more striking images can be produced because the optical system has been improved.
4. Upright system with visual and UV lasers suitable for standard fixed specimen observation.
5. Newly installed inverted intravital system with a visual, MP, and OPO laser and a high speed scanner which can be used for *in vivo* imaging of various tissues. In particular, the OPO laser can excite red fluorophores and excite them in a deeper region of the tissue because the tunable wavelength is longer than usual MP.
6. Intravital upright system with a single visual laser, double MP lasers and a high speed scanner that can be used for *in vivo* imaging and for some applications such as light stimulation.

During 2011, Dr. Wakabayashi from Leica Microsystems K.K. provided training for 14 researchers. The total running time of the microscopes was over 1980 hours.

Genomics Lab.

Research Scientist : **Takashi Watanabe, Yoko Kuroki**
Research Associate : **Mai Yamagishi, Atsushi Hijikata**
Technical Staff : **Tomoko Hasegawa, Nobutake Suzuki, Akio Kobayashi, Fumie Yokoyama, Emi Abe**

The Genomics Lab provides a wide variety of services to the members of the Center: proteomics analysis, *in vitro* translation, multiplex suspension array, DNA microarray, DNA sequencing, cDNA/Genomic clone distribution, and Primer/labeled probe distribution for qRT-PCR analysis of immune cells (Table 2).
Because genome technology has been progressing very quickly, the Genomics lab is keen to provide the Center with the most up-to-date technology on demand. We have thus decided to introduce two next generation sequencers, a GS Junior [Roche] and a HiSeq 1000 [Illumina] (Fig. 2). Both sequencers enable us to obtain high massively parallel DNA sequencing data for genome DNA, transcripts, and so forth. The GS Junior is already available while the members of the Center will be able to access the HiSeq 1000 in early 2012.

Table 2: Services provided by the Genomics Lab in 2011

Proteimics	# of samples	# of teams
Two-dimentional electrophoresis	10	2
Mass Spectrometry Analysis	78	2
In vitro translation (recombinant protein synthesis)	# of samples	# of teams
	1	1
Multiplex suspension array	# of samples	# of teams
	4,012	11
Affymetrix Genechip (Exon array, Gene array, miRNA array)	# of samples	# of teams
Human	118	5
Mouse	518	17
Total	636	22
DNA sequencing	# of samples	# of teams
36cm capillary	13,384	19
50cm capillary	13,152	17
Total	26,536	36
cDNA clone delivery	# of clones	# of teams
	62	7
Primer/labeled probe delivery	# of sets	# of teams
	145	3

Figure 2: GS junior (*left*) and HiSeq 1000 (*right*)

Animal Facility

Senior Technical Scientist	: **Takanori Hasegawa**
Technical Scientist	: **Shinobu Mochizuki, Masashi Matsuda**
Technical Staff	: **Tomoyuki Ishikura, Isamu Hisanaga, Yusuke Iizuka, Hiromi Tatsumi, Yurie Kawamoto, Chie Yoshida**

Animal caretakers (outsourced) : 21 people

Over 10,000 cages of SPF mice are maintained in the Animal Facility. The facility provides the following services for the users in the RIKEN Yokohama Institute.

1. Maintenance, generation and cryostocks of genetic resources
   The Animal Facility has been maintaining over 47,000 mice and 5 rats in the SPF area, 1,700 mice in an isolated area and several germfree mice. They have newly introduced 553 mouse lines into the SPF area by a combination of *in vitro* fertilization (IVF) and embryo transfer and generated cryostocks of genetic resources for 448 lines. They also maintain relatively large colonies for several commonly used strains such as NOD/SCID/CγKO mice, Rag1KO and cre deleters and provided them to users on demand. In addition, the animal facility provided technical assistance to generate chimeras (249 lines), transgenic and BAC-transgenic mice (12 lines), as well as to establish and maintain ES cells. Furthermore, they generated an internally available database for genetic resources.

2. Introduction of human BAC clones into NOD/SCID/CγKO mice
   This work is undertaken in collaboration with Dr. Osamu Ohara (RCAI, Immunogenomics Group) and Dr. Fumihiko Ishikawa (RCAI Human Disease Model Group).
   The Animal Facility has launched a new activity to improve the efficacy of transplantation of human hematopoietic stem cells into NOD/SCID/CγKO mice by "humanizing" the host strain. For this purpose, they have introduced large genomic fragments containing human genes encoding MHC, cytokines, adhesion molecules, virus receptors and others into NOD/SCID/CγKO mice. Up to now, they have established 19 BAC transgenics and confirmed the expression of human genes on a C57BL6 background and begun back-crossing onto the NOD/SCID/CγKO mice using the speed-congenic method.

3. Creation of germfree mice, maintenance and management
   This work is undertaken in collaboration with Dr. Hiroshi Ohno (RCAI, Epithelial Immunobiology Team).
   The Animal Facility attempted sanitization of knockout mice, and built a system that creates a germ-free mouse at the pace of 1 strain per month. They have created 4 germ-free mice in this year. They are bred in the germ-free state even now.

Figure 3: Bio-bubble for the maintenance of immunodeficient mice

Administrative Coorddination Office

Since the Administrative Coordination Office (AdmCO) was organized in April 2010, the office has been working to support the researchers of RCAI in a variety of ways. This year they extended their support to the scientists outside RIKEN. After the Great East Japan Earthquake, RCAI, as a member of RIKEN Yokohama Institute decided to provide support to the researchers in the Tohoku area. AdmCO took a lead in arranging to send them research materials, such as cell lines and mice, as well as laboratory equipment. AdmCO believes that these donations contributed greatly to the recovery of universities and research institutions there.

At the same time, to cope with the power shortages in summer 2011, Mr. Ogata, facilities staff, and others actively cooperated with the power saving committee in reducing the number of power-consuming machines, especially freezers and refrigerators.

The devastating earthquake greatly affected the center's events too. RCAI had to cancel most of the Center's international programs, such as RCAI-JSI International Symposium, RCAI International Summer Program, and the Harvard intern program after months of preparation. However, a new program called the RCAI 10th anniversary seminar series was launched instead. AdmCO invited 35 promising young scientists both from Japan and abroad. Each AdmCO staff member prepared for a seminar or two and the IT team assisted the speakers, providing for a better presentation environment.

To support the RCAI researchers AdmCO organized three working groups in the office. Each group works on MTAs, mouse import and export support, and the ordering system, respectively. The MTA group processed more than 150 MTAs and the mouse group supported 17 exports. The order system staff dealt with many daily inquiries, requests and problems, the numbers reaching almost 600 in a half year. The working groups did their tasks quite efficiently.

Next year AdmCO plans to expand the MTA group further and to build the RCAI intellectual property data by consolidating these agreements, which up until now have been scattered in discontinued teams. Also, AdmCO plans to improve the internal website just revamped by the IT team. AdmCO is always considering better ways of supporting RCAI and its members.

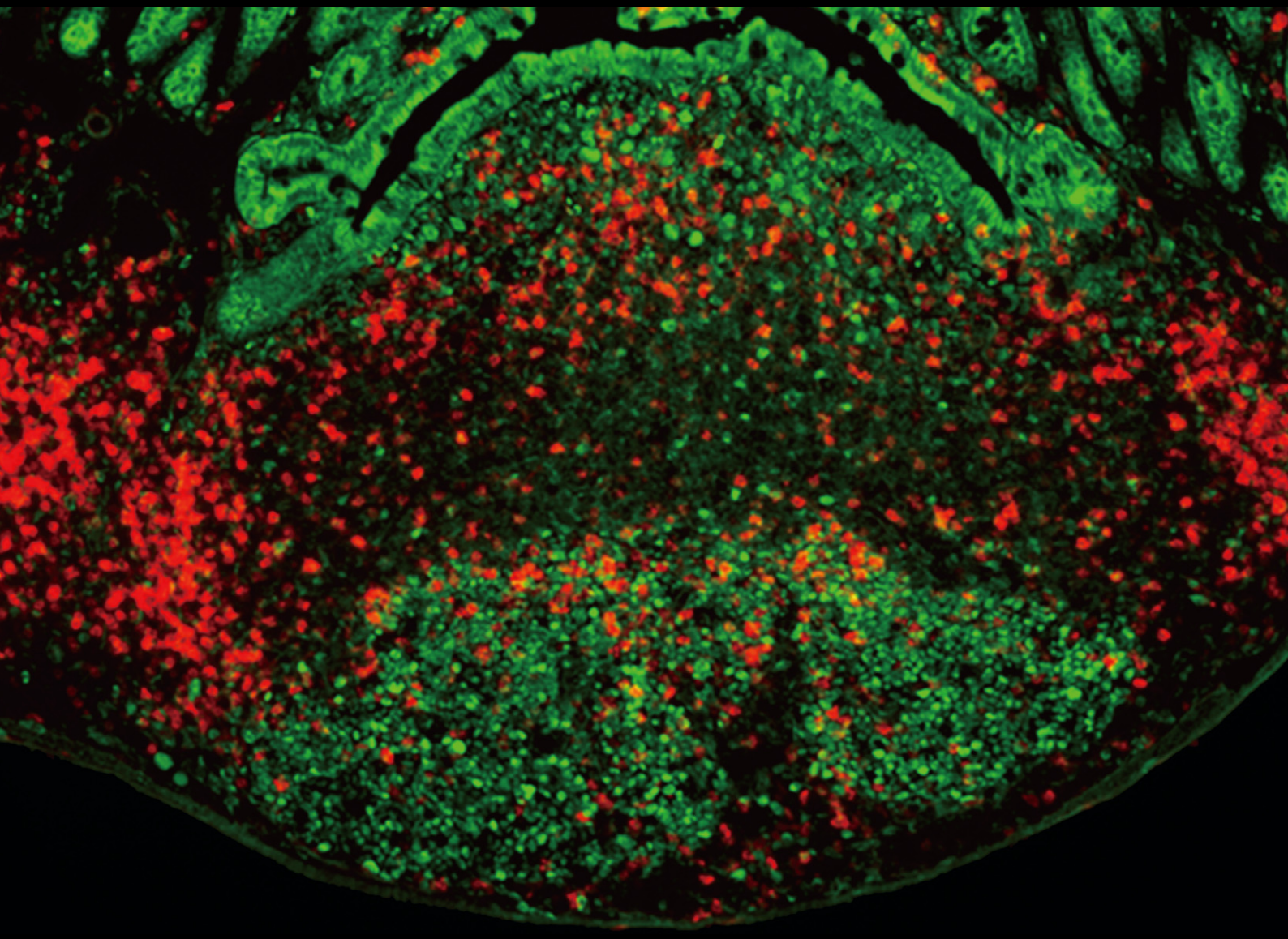
Table : Members of the Administrative Coordination Office

Position	Name
Office Manager	Ichiro Taniuchi
Administration	
Chief Assistants	Hiroko Tanabe
	Hiroko Yamaguchi
	Mari Kurosaki
Assistants	Sachiko Dohi
	Chiaki Fukushima
	Sachiko Haraguchi
	Akiko Imai
	Akiko Imaizumi
	Aiko Iyama
	Shihoko Kato
	Reiko Kimura
	Satomi Law
	Rie Morita
	Rieko Okoshi
	Toshiko Nakamura
	Kazuyo Nomura
	Yuko Ochi
	Norie Takeuchi
	Yuuki Yamada
	Mio Yoshioka
	Motoko Yoshioka
IT-team	
Technical Scientists	Yasuaki Murahashi
	Miho Izawa
Technical Staff	Aoi Ozawa
Facilities	
Technical Scientist	Toshihiko Ogata

2011

Part 7

Data and Statistics



Publications (Apr. 2011 - Mar. 2012)

Journals	IF(2010)	FY2011
Nature	36.1	1
Cell	32.4	1
Cancer Cell	26.9	1
Nat Immunol	25.7	4
Nat Med	25.4	1
Immunity	24.2	6
J Exp Med	14.8	6
Genome Res	13.6	1
PLoS Biol	12.5	1
Gastroenterology	12.0	1
Blood	10.6	5
EMBO Journal	10.1	2
Proc Natl Acad Sci USA	9.8	6
Trends Immunol	9.5	1
Cell Res	9.4	1
J Allergy Clin Immun	9.3	2
Arthritis Rheum	8.4	1
Stem Cells	7.9	1
Adv Immunol	7.2	2
Development	6.9	2
Genome Biol	6.9	1
Sci Signal	6.4	2
Mol Cell Biol	6.2	1
Mol Biol Cell	5.9	1
J Immunol	5.7	8
J Biol Chem	5.3	2
J Virol	5.2	1
Eur J Immunol	4.9	1
Br J Haematol	4.9	1
Bioinformatics	4.9	1
DNA Res	4.8	2
Am J Respir Cell Mol Biol	4.4	1
PLoS One	4.4	2
Intl Immunol	3.3	5
Other Journals		28
Total		**103**

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Invited Presentations

Date	Speaker	Title	Meeting	Place
Apr - 11	**Cheroutre, H.**	Stretching CD4 Effector T Cells Beyond Plasticity	Keystone Symposium on Immunoregulatory Networks	Breckenridge, USA
Apr - 11	**Wang, J.-Y.**	The lysosomal protein LAPTM5 negatively regulates cell surface TCR and BCR expression and T and B cell activation	Peking University Special Seminar	Beijing, China
Apr - 11	**Fagarasan, S.**	PD-1 deficiency skews the gut microbiota by altering the IgA selection in gut	The 2012 Spring Conference of the Korean Association of Immunologists	Seoul, Korea
Apr - 11	**Kurosaki, T.**	Function of Calcium in Lymphocytes	Advances in Immunology and Cancer Biology	Istanbul, Turkey
Apr - 11	**Kurosaki, T.**	Function of Store-Operated Calcium Entry in B Lymphocytes	Keystone Symposia, B Cells: New Insights into Normal versus Dysregulated Function	Whistler, Canada
Apr - 11	**Fukuda, S.**	Elucidation of the mechanisms of host-bacterial crosstalk in the gut by multiple omics approach	Global COE program, The University of Tokyo	Kashiwa, Japan
Apr - 11	**Taniuchi, I.**	Transcriptional control of thymocytes development	Seminar at SIgN	Singapore, Singapore
Apr - 11	**Ohara, O.**	Integration of New Technologies for Accurate and Rapid Molecular Diagnosis of Primary Immunodeficiencies	The 7th Congress of Asian Society for Pediatric Research	Denver, USA
May - 11	**Tokunaga, M. & Sakata-Sogawa, K.**	Strategies adapted by cells examined by single molecule quantification using fluorescence imaging	"The next technical breakthrough" Bio-finance Guild Seminar	Tokyo, Japan
May - 11	**Ohara, O.**	Integration of Microfluidics Technology with Advanced Genomics for Prognosis/Diagnosis of Allergic and Immunological Diseases	150 Jahre Wirtschaftsbeziehungen Deutschland-Japan	Dusseldorf, Germany
May - 11	**Ohno, H.**	Mechanism of protection of mouse O157-infectious death by bificobateria using multi-omics approach	1st JAXA Space Medicine and Biology Research Workshop in 2011 "How to prevent immune suppressor - from astronautes to the elderly -"	Tokyo, Japan
May - 11	**Kaisho, T.**	Dendritic cell sensing and responses for nucleic acid adjuvants	KSBMB 2011 Annual Meeting	Seoul, Korea
May - 11	**Fagarasan, S.**	Bacteria and retinoic acid impact on follicular dendritic cells function in Peyer's patches	The 76th Japanese Society of Interferon and Cytokine Resesarch The 19th International Symposium on Molecular Cell Biology of Macrophages	Osaka, Japan
May - 11	**Saito, T.**	Visualizing the dynamics of immune synapse by TIRF	XXVI Congress of the International Society for Advancement of Cytometry (CYTO2011)	Baltimore, USA
May - 11	**Ishikawa, F.**	Targeting chemotherapy-resistant human AML stem cells	XXVI Congress of the International Society for Advancement of Cytometry (CYTO2011)	Baltimore, USA
May - 11	**Koseki, H.**	Polycomb-dependent regulation for differentiation programs of stem cells and progenitors	5th Annual Meeting of Japanese Society for Epigenetics	Kumamoto, Japan
May - 11	**Kawamoto, H.**	Lineage restriction process from hermatopoietic stem cells to T cell progenitors: An essential developmental checkpoint for the production of T cell lineage	Hematology Lecture at Erasmus University	Rotterdam , The Netherland
May - 11	**Taniuchi, I.**	Transcriptional control of thymocytes development	Seminar at Max Planck Institute, Freiburg	Freiburg, Germany
May - 11	**Taniuchi, I.**	Transcriptional control of thymocytes development	Seminar at University of Tubingen	Tubingen, Germany
Jun - 11	**Fagarasan, S.**	T cell-independent and T cell-dependent IgA synthesis in the gut	Master Class Istituto di Ricerca in Biomedicina.	Bellinzona, Switzerland
Jun - 11	**Hirano, T.**	IL-6 amplifier and autoimmune	The 2th Symposium on Joint Research Core Network Institute for Enzyme Research, Institute for Enzyme Research,The University of Tokushima	Tokushima, Japan
Jun - 11	**Ishii, Y.**	Uncovering adjuvant potential of natural killer T (NKT) cell ligand, alpha-galactosylceramide	5th World Vaccine Congress Asia 2011	Singapore
Jun - 11	**Saito, T.**	Spatiotemporal regulation of T cell co-stimulation	FASEB Summer Conferences-Signal Transduction in the Immune System	Snowmass Village, USA
Jun - 11	**Kurosaki, T.**	Calcium signaling in immune cells	FASEB Summer Conferences-Signal Transduction in the Immune System	Snowmass Village, USA
Jun - 11	**Hase, K.**	Biological significance of M cells in gut mucosal immunity	The 15th Annual Meeting of Intestinal Microbiology	Tokyo, Japan
Jun - 11	**Ohno, H.**	Multi-omics analysis for elucidating the host-commensal bacteria interaction	The 15th Annual Meeting of Intestinal Microbiology	Tokyo, Japan
Jun - 11	**Ohno, H.**	Multi-omics analysis for elucidating the host-commensal bacteria interaction - Mechanism of protection of mouse O157-infectious death by bificobateria	The 16th Tokyo Liver Symposium "Next generation sequence and genomics"	Tokyo, Japan
Jun - 11	**Fukuda, S.**	Carbohydrate transporters confer a probiotic effect to bifidobacteria	The International Scientific Conference on Probiotics and Prebiotics	Kosice, Slovakia
Jun - 11	**Taniuchi, I.**	Epigenetic regulation of T cell developemnt	Environment and Hormone Meeting	Tokyo, Jaoan
Jun - 11	**Ishikawa, F.**	Leukemia stem cell research using humanized mouse	21st Japan Soceity of Cytometry Meeting	Kyoto, Japan
Jun - 11	**Kawamoto, H.**	Lineage commitment process during hematopoiesis: Visualization of developmental checkpoints	21st Japan Soceity of Cytometry Meeting	Kyoto, Japan
Jun - 11	**Okada, T.**	Imaging of lymphocyte migration and interactions during immune responses	The 63rd Annual Meeting of The Japan Society for Cell Biology (Satellite Symposium 1)	Sapporo, Japan
Jul - 11	**Ishikawa, F.**	Investigating human immunity and diseases using humanized mouse system	15th Japan Society of Cancer Immunology meeting	Osaka, Japan
Jul - 11	**Fagarasan, S.**	Dynamic interactions between bacteria and immune cells in the gut	Seminar Series at the Max Planck Institute of Immunobiology and Epigenetics	Freiburg, Germany
Jul - 11	**Fukada, T.**	Slc39a14/Zip14: its involvement in regulation of systemic growth and GPCR signal transduction	The 22nd Annual Meeting of the Japan Society for Biomedical Research on Trace Elements	Kyoto, Japan
Jul - 11	**Ishii, Y.**	Therapeutic potential of immunoregulatory liposomal alpha-GalCer	38th BMS Conference	Hakone, Japan
Jul - 11	**Tanaka, M.**	Regulation of anti-tumor immunity by clearance of dead tumor cells	Gordon Research Conferences Apoptotic Cell Recognition & Clearance	Lewiston, USA
Jul - 11	**Ohno, H.**	Multi-omics approach reveals that bifidobacteria protect from enteropathogenic Escherichia coli infection through production of acetate	The 9th International Workshop on Advanced Genomics	Tokyo, Japan
Jul - 11	**Ishikawa, F.**	Targeting AML stem cells	9th Japan Soceity of Medicao Oncology meeting	Yokohama, Japan
Jul - 11	**Taniuchi, I.**	Transcriptional control of thymocytes development	Seminar at RIMD Osak Univ	Osaka, Jaoan
Jul - 11	**Koseki, H.**	SAM domain-mediated polymerization of mammalian polyhomeotic homologues mediates recruitment of PRC1 to Polycomb target genes and their condensation	3rd X-Inactivation Meeting	Oxford, UK
Jul - 11	**Hori, S.**	Control of autoimmunity by regulatory T cells	The 35th Aso Symposium	Kumamoto, Japan
Aug - 11	**Kaisho, T.**	Molecular mechanisms for dendritic cell functions	JSI Immunology Summer School	Sendai, Japan

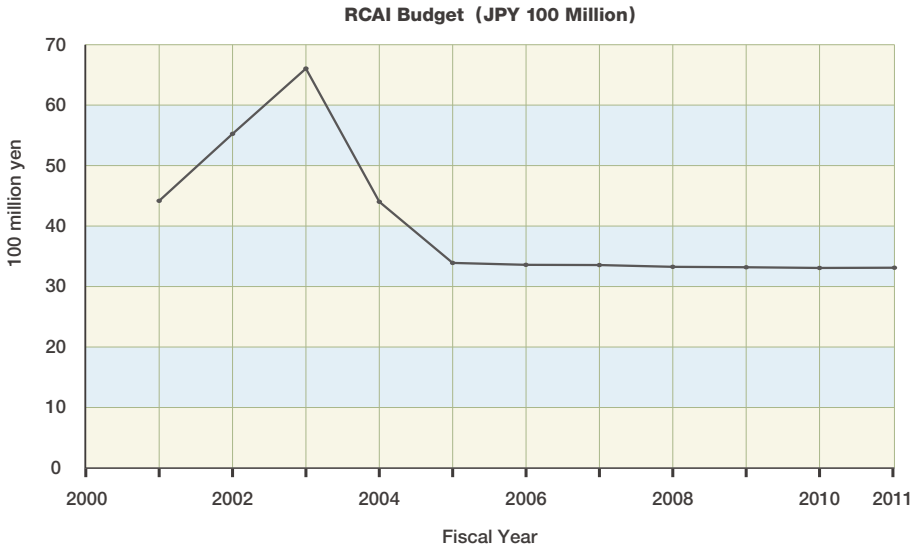
Aug - 11	**Saito, T.**	**Dynamic regulatory mechanism of T cell activation**	JSI Immunology Summer School	Zao, Japan
Aug - 11	**Taniuchi, I.**	**Transcriptional and epigenetic control of helper vs cytotoxic lineage decision in thymus**	Seminar at Stanford University	Stanford, USA
Aug - 11	**Taniuchi, I.**	**Genetic and molecular analyses of Runx-dependent silencers during T cell development**	18th International Runx Workshop	San Diego, USA
Aug - 11	**Ohno, H.**	**Analysis of Host-intestinal microbiota interaction by multi-omics approach**	The 23rd Takato Symposium " The cell revisited"	Ina, Japan
Aug - 11	**Fukuda, S.**	**Understanding the gut ecosystem by multiple omics approach**	The 6th Seminar of the Creative Research Program, University of Tsukuba	Tsukuba, Japan
Sep - 11	**Saito, T.**	**Spatiotemporal regulation of T cell activation and costimulation**	1st POSTECH International Symposium of Bio-Imaging	Pohang, Korea
Sep - 11	**Ohno, H.**	**Function and differentiation of M cells, a unique subset of intestinal epithelial cells important for mucosal immunity**	ComBio2011, Plenary Lecture	Cairns, Australia
Sep - 11	**Ohno, H.**	**Multi-omics approach reveals that bifidobacteria protect from enteropathogenic Escherichia coli infection through production of acetate**	ComBio2011, symposium	Cairns, Australia
Sep - 11	**Ohno, H.**	**The role of M-Sec in tunneling nanotube formation**	The EMBO Meeting 2011	Vienna, Austria
Sep - 11	**Ishikawa, F.**	**Phenotype, Distribution, and Heterogeneity of Leukemic Stem Cells**	2011Working Conference on Cancer Stem Cells	Vienna, Austria
Sep - 11	**Fukada, T.**	**How does the zinc homeostasis system control mammalian growth? Lessons from molecular and genetic studies of zinc transporters Zip13 and Zip14**	The 5th International Conference on Metals and Genetics (ICMG 2011)	Kobe, Japan
Sep - 11	**Taniuchi, I.**	**Transcriptional and Epigenetic Regulation of CD4/CD8 Lineage Choice**	Cold Spring Harbor Asia Conference	Suzhou, China
Sep - 11	**Cheroutre, H.**	**Protecting the Border with a Fake ID: CD4 T Cells Stretch Beyond Plasticity**	4th International Conference on Crossroads between Innate and Adaptive Immunity	Mykonos, Greece
Sep - 11	**Ishii, Y.**	**Generation of vaccines for polliniosis**	59th allergy meeting for kanto otolaryngologist	Tokyo, Japan
Sep - 11	**Taniguchi, M.**	**Early NKT Precursor**	6th International Symposium on CD1 and NKT Cells	Chicago, USA
Sep - 11	**Ohno, H.**	**Food Allergy, Intestinal Immune System, and Oral Tolerance**	The 18th Annual Meeting of The Japanese Society of Immunotoxicology	Chiba, Japan
Sep - 11	**Taniguchi, M.**	**iPS-derived NKT cells as a clinical application for cellular immunotherapy**	The 39th Annual Meeting of the Japan Society for Clinical Immunology	Tokyo, JAPAN
Sep - 11	**Taniuchi, I.**	**Transcriptional Control of Helper versus Cytotoxic Lineage Choice**	Austrian Society for Allergology and Immunology Annual Meeting 2011	Graz, Austria
Sep - 11	**Ishido, S.**	**Novel immune regulation by ubiquitination**	The 84th Annual Meeting of the Japanese Biochemical Society	Kyoto, Japan
Sep - 11	**Fukada, T.**	**The zinc transporter Zip14 controls systemic growth and GPCR signal transduction**	The 84th Annual Meeting of the Japanese Biochemical Society	Kyoto, Japan
Sep - 11	**Nishida, K.**	**Role of adaptor molecule Gab2 in mast cell-mediated anaphylaxis response**	The 84th Annual Meeting of the Japanese Biochemical Society	Kyoto, Japan
Sep - 11	**Yamasaki, S**	**The role of intracellular Zn signaling on mast cell activation**	The 84th Annual Meeting of the Japanese Biochemical Society	Kyoto, Japan
Sep - 11	**Okada, M**	**Kinetic analysis of transcriptional signal network for activation of c-Fos transcription factor**	The 84th Annual Meeting of the Japanese Biochemical Society	Kyoto, Japan
Sep - 11	**Fukuda, S.**	**Multi-omics analysis elucidates gut environmental ecosystems**	Research Workshop for Lactic Acid Bacteria and Intestinal Bacteria	Tokyo, Japan
Oct - 11	**Okada, M.**	**Quantitative and predictive analysis of cancer signaling and transcription network**	The 70th Annual Meeting of the Japanese Cancer Association	Nagoya, Japan
Oct - 11	**Fujii, S.**	**New type of immunotherapy using artificial adjuvant vector cells (aAVCs) linking innate and adaptive immunity.**	The 70th Annual Meeting of the Japanese Cancer Association	Nagoya, Japan.
Oct - 11	**Watarai, H.**	**Function of Pathogenic Invariant Natural Killer T Cells Contribute to the Induction of Airway Hyperreactivity**	Joint Congress of Asia Pacific Association of Pediatric Allergy, Respirology and Immunology (APAPARI 2011) & 48th Annual Meeting of Japanese Society of Pediatric Allergy and Clinical Immunology (48th JSPACI)	Fukuoka, JAPAN
Oct - 11	**Ohno, H.**	**Mechanism of protection of mouse from enterohemorrhagic E. Coli O157-infectious death by bifidobacteria using multi-omics approach**	The 94th Meeting of the Japanese Society for Bacteriology Kanto Branch	Tokyo, Japan
Oct - 11	**Fujii, S.**	**New type of immunotherapy using NKT cell-induced DC activation in situ**	The 73rd Annual Meeting of Japanese Society of Hematology	Nagoya, Japan.
Oct - 11	**Ishikawa, F.**	**Creating therapeutic strategies targeting AML stem cells**	The 73rd Annual Meeting of Japanese Society of Hematology	Nagoya, Japan.
Oct - 11	**Fukuda, S.**	**Multi-omics analysis uncovers an ecosystem of gut environment**	The 6th Metabolome Symposium, Japan	Osaka, Japan
Nov - 11	**Okada, M.**	**Identifying logics of signal-transcription network for cellular differentiation**	IFReC / IPR Joint Seminar	Osaka, Japan
Nov - 11	**Okada, M.**	**Cell fate decisions determined by switch-like responses of genetic networks**	Theory of Biomathematics and Its Applications VIII	Kyoto, Japan
Nov - 11	**Kurosaki, T.**	**Function of Calcium Signaling in B Lymphocytes**	The 1st International Meeting on Ion Channel Signaling Mechanisms: from Basic Science to Clinical Application	Marrakesh, Morocco
Nov - 11	**Hirano, T.**	**The role of Interleukin 6 amplifier in inflammatory diseases.**	Bio-Rheumatology International Congress (BRIC) Tokyo, The 8th GARN Meeting	Tokyo, Japan
Nov - 11	**Sato, K.**	**Molecular mechanism for the establishment of oral tolerance**	Japan Bioindustry Association Seminar	Tokyo, Japan
Nov - 11	**Taniuchi, I.**	**Transcriptional and epigenetic regulation of thymocytes fate decision**	Seminar at Albany University	Albany, USA
Nov - 11	**Taniuchi, I.**	**Transcriptional and epigenetic control of helper versus cytotoxic lineage choice**	Seminar at Washington University	St. Louis, USA
Nov - 11	**Saito, T.**	**Regulatory mechanism of T cell activation by imaging analysis**	The 61st Annual Meeting of Japanese Society of Allergology	Tokyo, Japan
Nov - 11	**Nishida, K.**	**Role of Zinc in immmune/inflammatory response**	The 61st Annual Meeting of Japanese Society of Allergology	Tokyo, Japan
Nov - 11	**Okada, T.**	**Imaging of lymphocyte dynamics during the germinal center formation**	The 61st Annual Meeting of Japanese Society of Allergology	Tokyo, Japan
Nov - 11	**Taniguchi, M.**	**A novel NKT cell subtype which contributes to the induction of allergic reactions**	The 61st Annual Meeting of Japanese Society of Allergology	Tokyo, JAPAN
Nov - 11	**Tanaka T.**	**PDLIM2 inhibits T helper 17 cell development and granulomatous inflammation through degradation of STAT3**	The 40th Annual Meeting of the Japanese Society for Immunology	Chiba, Japan
Nov - 11	**Tanaka, M.**	**The role of CD169 macrophages in anti-tumor immunity**	The 40th Annual Meeting of the Japanese Society for Immunology	Chiba, Japan

Nov - 11	**Yokosuka, T.**	The PD-1 microclusters: negative costimulatory signalosomes directly targeting TCR downstream	The 40th Annual Meeting of the Japanese Society for Immunology	Chiba, Japan
Nov - 11	**Kurosaki, T.**	Contribution of transcription factors to rapid responsiveness of IgG type memory B cells	The 40th Annual Meeting of the Japanese Society for Immunology	Chiba, Japan
Nov - 11	**Cheroutre, H.**	Activation-induced silencing of Thpok defines a new type of CD4 effector T lymphocytes	The 40th Annual Meeting of the Japanese Society for Immunology	Chiba, Japan
Nov - 11	**Wang, J.-Y.**	The Fc receptor for IgM (FcμR) positively regulates B cell survival and activation	The 40th Annual Meeting of the Japanese Society for Immunology	Chiba, Japan
Nov - 11	**Okada, T.**	Imaging of lymphocyte dynamics during the germinal center formation	The 40th Annual Meeting of the Japanese Society for Immunology	Chiba, Japan
Nov - 11	**Fukuda, S.**	Acetate-producing bifidobacteria equipped with 'probiotic transporters' protects the host against enteropathogenic infection	The 40th Annual Meeting of the Japanese Society for Immunology	Chiba, Japan
Nov - 11	**Hori, S.**	Analysis of IPEX mutations reveals a critical role of the KLRG1$^+$ subset of regulatory T cells in the regulation of autoimmunity	The 40th Annual Meeting of the Japanese Society for Immunology	Chiba, Japan
Nov - 11	**Kawamoto, H.**	Molecular mechanisms for the production and maintenance of the T cell lineage	The 40th Annual Meeting of the Japanese Society for Immunology	Chiba, Japan
Nov - 11	**Cheroutre, H.**	Mucosal Immunity: At the Borders, It Matters Who You Are	The 6th Chiba University Global COE Symposium	Chiba, Japan
Dec - 11	**Yokosuka, T.**	Dynamic regulation of T cell activation by TCR microclusters	Japan-China-Korea Immunology Symposium 2011	Osaka, Japan
Dec - 11	**Hase, K.**	Epithelial immune functions at the interface between self and non-self	Japan-China-Korea Immunology Symposium 2011	Osaka, Japan
Dec - 11	**Cheroutre, H.**	Activation-induced silencing of Thpok defines a new type of CD4 effector T lymphocytes	University of Michigan-RIKEN RCAI Joint Workshop	Yokohama, Japan
Dec - 11	**Fujii, S.**	DC targeting immunotherapy by antigen mRNA-transfected, allogeneic cells loaded with NKT cell ligand as artificial adjuvant vector cells (aAVC)	University of Michigan-RIKEN RCAI Joint Workshop	Yokohama, Japan
Dec - 11	**Ohno, H.**	The epithelia-specific membrane trafficking factor AP-1B secures gut immune homeostasis in mice	University of Michigan-RIKEN RCAI Joint Workshop	Yokohama, Japan
Dec - 11	**Tanaka T.**	HSP70 is essential for PDLIM2-mediated termination of NF-κB signaling	University of Michigan-RIKEN RCAI Joint Workshop	Yokohama, Japan
Dec - 11	**Taniuchi, I.**	Mechanisms of Helper versus Cytotoxic Lineage Choice	University of Michigan-RIKEN RCAI Joint Workshop	Yokohama, Japan
Dec - 11	**Udono, H.**	Dendritic cell requires HSP90 as a cytosolic translocator of extracellular antigen for cross-presentation	University of Michigan-RIKEN RCAI Joint Workshop	Yokohama, Japan
Dec - 11	**Yokosuka, T.**	Imaging of T cell activation regulation: - Negative regulation of TCR signaling by PD-1 microcluster –	University of Michigan-RIKEN RCAI Joint Workshop	Yokohama, Japan
Dec - 11	**Nomura, M.**	Practical application of the cmgraph method in using real data	Workshop on Applied Dynamical Systems	Kyoto, Japan
Dec - 11	**Hase, K.**	Functional roles of GP2 and M-Sec in intestinal M cells	Matrix of Infection Phenomena Syposium on "The future of Infectious Disease Study"	Tokyo, Japan
Dec - 11	**Sato, K.**	Crucial role of mesenteric lymph node dendritic cells in the establishment of oral tolerance	The 32nd Special Seminar on Institute of Natural Medicine, University of Toyama	Toyama, Japan
Jan - 12	**Fagarasan, S.**	PD-1 deficiency skews the gut microbiota by altering the IgA selection in gut	IFReC-SIgN Winter School on Advanced Immunology	Awaji, Japan
Jan - 12	**Fagarasan, S.**	Regulation of IgA synthesis in Peyer's patches: new aspects	The 4th Symposium for the MEXT Priority Research on Immunological Self	Kyoto Japan
Jan - 12	**Hori, S.**	Plasticity of Foxp3$^+$ T cells: its implications in Treg cell lineage commitment	The 4th Symposium for the MEXT Priority Research on Immunological Self	Kyoto, Japan
Jan - 12	**Hirano, T.**	Zinc as a signaling molecule	International Society for Zinc Biology 2012 Conference	Melbourne, Australia
Feb - 12	**Fagarasan, S.**	New aspects of T cell-dependent IgA synthesis in gut	New Horizons in the Immune System	Tokushima, Japan
Feb - 12	**Kaisho, T.**	Molecular mechanisms for regulating dendritic cell subset functions	The 29th Annual Meeting of Japanese Society of Immunology & Allergology in Otolaryngology	Ooita, Japan
Feb - 12	**Hori, S.**	Resolving the controversy over regulatory T cell plasticity	The 14th International Conference on Lymphocyte Activation and Immune Regulation: T Cell Differentiation and Plasticity	Newport Beach, USA
Feb - 12	**Saito, T.**	Negative regulation of TCR signaling by PD-1 microcluster	RCAI-LIAI Joint Workshop	La Jolla, USA
Feb - 12	**Hori, S.**	Genetic and environmental control of regulatoy T cell fitness in peripheral tissues	RCAI-LIAI Joint Workshop	La Jolla, USA
Feb - 12	**Ohno, H.**	M Cells and Antigen Delivery to DCs in the Gut	Keystone Symposium on Dendritic Cells and the Initiation of Adaptive Immunity	Santa Fe, USA
Feb - 12	**Sato, K.**	Control of immune response by dendritic cells	Seminar on Nippon Institute for Biological Science	Tokyo, Japan
Feb - 12	**Cheroutre, H.**	Protecting the Border with a Fake ID: CD4 T Cells Stretch Beyond Plasticity	Keystone Symposium on Mucosal Biology: A Fine Balance between Tolerance and Immunity	Vancouver, Canada
Feb - 12	**Sato, K.**	Plasmacytoid dendritic cells in innate and adaptive immunity	Seminar on WPI Immunology Frontier Research Center, Osaka University	Osaka, Japan
Feb - 12	**Koseki, H.**	Application of iPS Cell Technology for NKT Cell-targeted Adjuvant Therapy for Tumors in Mice	14th US-Japan Cellular and Gene Therapy Conference	Bethesda, USA
Feb - 12	**Kawamoto, H.**	A revised model of hematopoiess	2011 Japan Research Association For Immunotherapeutics	Tokyo, Japan
Feb - 12	**Kawamoto, H.**	Myeloid-based model of hematopiesis: revison of the classical myeloid-lymphoid dichotomy concept	The 34th Annual Meeting of the Japan Society for Hematopoietic Cell Transplantation	Osaka, Japan
Mar - 12	**Fagarasan, S.**	Dynamic interactions between bacteria and immune cells in gut	ESF-JSPS Frontier Science Conference for Young Researchers Cutting Edge Immunology and its Clinical Application	Hulshorst, Holland
Mar - 12	**Saito, T.**	Negative regulation of TCR signaling by PD-1 microcluster	Keystone Symposium	Keystone, USA
Mar - 12	**Tokunaga, M.**	Dynamic feature of life examined by single molecule imaging	Public lecture on frontier researches at Tokyo Institute of Technology	Tokyo, Japan
Mar - 12	**Ohno, H.**	Mechanism of protection of mouse O157-infectious death by bificobateria using multi-omics approach	Bridge Seminar Series (Biken/Buinkai-Ridge Seminar Series)	Osaka, Japan
Mar - 12	**Cheroutre, H.**	Stretching the CD4 T helper (Th)-Lineage Beyond Plasticity	2011 Cancer and Immunology Colloquium Seminar Series at Cedars-Sinai Medical Center	Los Angeles, USA
Mar - 12	**Nishida, K.**	Role of adaptor molecule Gab2 in mast cell-mediated allergy response	The 132th Annual Meeting of the Pharmaceutical Society of Japan	Hokkaido, Japan

Date	Title	Speaker	Affiliation	Country	
05-Jun-11	**Regulation of fate determination and plasticity of germ cells**	**Yasuhisa Matsui**	Institute of Development, Aging and Cancer, Tohoku University	Japan	
29-Jun-11	**Coupling of metabolism and epigenome by nuclear protein complex SAMIT**	**Kazuhiko Igarashi**	Tohoku University School of Medicine	Japan	
14-Jul-11	**The role of the immune system in prion neuroinvasion from the intestine**	**Neil A Mabbott**	The Roslin Institute & Royal(Dick) School of Veterinary Sciences University of Edinburgh	UK	
28-Jul-11	**Exploration of Host-Parasite Interface Possibly Reveals a New Aspect of Immunology**	**Masahiro Yamamoto**	Graduate School of Medicine, Osaka University	Japan	The RCAI 10th Anniversary Seminar Series/Seminar 1
01-Aug-11	**Donor mesenchymal stem cells trigger chronic graft-versus-host disease following minor antigen mismatched bone marrow transplantation**	**Yumi Matsuzaki**	Center for Integrated Medical Research, Keio University	Japan	The RCAI 10th Anniversary Seminar Series/Seminar 2
03-Aug-11	**Proteinomic analysis of cell signaling networks with micro-western arrays**	**Richard B. Jones**	Institute for Genomics and Systems Biology, The University of Chicago	USA	The RCAI 10th Anniversary Seminar Series/Seminar 3
08-Aug-11	**Systems Approach to Hematopoietic Stem/Progenitor Cell Biology**	**Jun Seita**	Institute for Stem Cell Biology & Regenerative Medicine, and Department of Pathology, Stanford University School of Medicine	USA	The RCAI 10th Anniversary Seminar Series/Seminar 4
29-Aug-11	**Signaling pathways activated by innate receptors and their roles in host defense**	**Taro Kawai**	Laboratory of Host Defense, Immunology Frontier Research Center, Osaka University	Japan	The RCAI 10th Anniversary Seminar Series/Seminar 5
05-Sep-11	**Role of Crosstalk between Adipocytes and Endothelium in Sepsis**	**Kiichiro Yano**	Cardiovascular-Metabolic Laboratories, Daiichi-Sankyo Co., Ltd.	Japan	The RCAI 10th Anniversary Seminar Series/Seminar 6
05-Sep-11	**Current status and Prospect of iPS Cell Research**	**Takashi Aoi**	Center for iPS Cell Research and Application , Kyoto University	Japan	
12-Sep-11	**Natural helper cells play a critical role in IL-33-dependent eosinophilia in the lung**	**Kazuyo Moro**	Department of Microbiology and Immunology, Keio University School of Medicine	Japan	The RCAI 10th Anniversary Seminar Series/Seminar 7
28-Sep-11	**High-speed interstitial T cell migration in lymph node involves LFA-1-dependent and -independent mechanisms controlled by stromal cell network**	**Tomoya Katakai**	Kansai Medical University	Japan	The RCAI 10th Anniversary Seminar Series/Seminar 8
06-Oct-11	**MicroRNA Regulation of Energy Metabolism in Mammalian Immune System**	**Taro Fukao**	Max-Planck Institute of Immunobiology and Epigenetics	Germany	The RCAI 10th Anniversary Seminar Series/Seminar 9
10-Oct-11	**Retinoic acid and gut-homing T cells in intestinal immune homeostasis**	**J. Rodrigo Mora**	Massachusetts General Hospital & Harvard Medical School	USA	The RCAI 10th Anniversary Seminar Series/Seminar 10
12-Oct-11	**LRF Transcription Factor Maintains HSC Homeostasis by Preventing Lymphoid-Primed LT-HSCs from Excessive Differentiation**	**Takahiro Maeda**	Hematology Division, Brigham and Women's Hospital	USA	The RCAI 10th Anniversary Seminar Series/Seminar 11
17-Oct-11	**Forced expression of reprogramming factors *in vivo***	**Yasuhiro Yamada**	Center for iPS Cell Research and Application, Kyoto University	Japan	
18-Oct-11	**C-type lectins: their roles in the host defense against fungal infection**	**Shinobu Saijo**	Medical Mycology Research Center, Chiba Univerisity	Japan	The RCAI 10th Anniversary Seminar Series/Seminar 12
24-Oct-11	**Regulatory B cells, lessons from the model of IBD**	**Atsushi Mizoguchi**	Harvard Medical School, Massachusetts General Hospital	USA	The RCAI 10th Anniversary Seminar Series/Seminar 13
01-Nov-11	**Molecular mechanisms required for thymic self-tolerance**	**Taishin Akiyama**	Institute of Medical Science, University of Tokyo	Japan	The RCAI 10th Anniversary Seminar Series/Seminar 14
15-Nov-11	**Mediator Lipidomics in Inflammation Research**	**Makoto Arita**	Graduate School of Pharmaceutical Sciences, The University of Tokyo	Japan	The RCAI 10th Anniversary Seminar Series/Seminar 15
20-Nov-11	**Transcription factors in cytotoxic T cell development**	**Takeshi Egawa**	Washington University School of Medicine	USA	The RCAI 10th Anniversary Seminar Series/Seminar 16
20-Nov-11	**Towards an Experimental and Systems Biology Framework for Cancer Cell Therapeutics**	**Petar M. Mitrasinovic**	Indian Institute of Technology Roorkee, India & Belgrade Institute of Science and Technology, Serbia	Serbia	
24-Nov-11	**The nature of B and T cell memory generated in germinal centers**	**David Tarlinton**	Walter and Eliza Hall Institute	USA	
07-Dec-11	**Drosophila models to study chronic inflammation**	**Hidehiro Fukuyama**	Institute of Molecular and Cellular Biology	France	The RCAI 10th Anniversary Seminar Series/Seminar 17

07-Dec-11	**Targeted gene correction of a1-antitrypsin deficiency in induced pluripotent stem cells**	**Kosuke Yusa**	Wellcome Trust Sanger Institute	UK	The RCAI 10th Anniversary Seminar Series/Seminar 18
11-Dec-11	**Revisiting 'Thymic Nurse Cell': Unique lympho-epithelial complexes in the thymic cortex**	**Takeshi Nitta**	National Center for Global Health and Medicine	Japan	The RCAI 10th Anniversary Seminar Series/Seminar 19
15-Dec-11	**Primary immunodeficiency in Japan**	**Kosuke Imai**	Department of Community Pediatrics, Perinatal and Maternal Medicine Tokyo Medical and Dental University	Japan	The RCAI 10th Anniversary Seminar Series/Seminar 20
20-Dec-11	**Bone marrow niches for hematopoietic stem and progenitor cells**	**Takeshi Nagasawa**	Institute for Frontier Medical Sciences, Kyoto University	Japan	
21-Dec-11	**Intestinal microbiota shapes the immune system**	**Kenya Honda**	Department of Immunology,Graduate School of Medicine, The University of Tokyo	Japan	The RCAI 10th Anniversary Seminar Series/Seminar 21
25-Dec-11	**Central nervous system autoimmunity and gut flora**	**Takashi Yamamura**	Department of Immunology, National Institute of Neuroscience, NCNP	Japan	The RCAI 10th Anniversary Seminar Series/Seminar 22
18-Jan-12	**Deciphering pancreas development and diseases**	**Matthias Hebrok**	UCSF Diabetes Center	USA	The RCAI 10th Anniversary Seminar Series/Seminar 23
23-Jan-12	**HLA variation and disease susceptibility**	**Katsushi Tokunaga**	Department of Human Genetics, Graduate School of Medicine, University of Tokyo	Japan	RCAI-CGM Joint Seminar
23-Jan-12	**HLA-DRB1polymorphism and anti-citrullinated peptide antibody in rheumatoid arthritis**	**Yuta Kochi**	RIKEN Center for Genomic Medicine	Japan	RCAI-CGM Joint Seminar
24-Jan-12	**Germinal Center Selection and the Development of Memory B and Plasma Cells**	**Mark Shlomchik**	Laboratory Medicine and Immunolobiology, Yale University	USA	The RCAI 10th Anniversary Seminar Series/Seminar 24
30-Jan-12	**The role of the ERM protein moesin in lymphocyte homeostasis**	**Takako Hirata**	Kyoto University Graduate School of Medicine	Japan	The RCAI 10th Anniversary Seminar Series/Seminar 25
31-Jan-12	**The role of the thymus in populating the T cell pool and some thoughts on the commercialisation of basic research**	**Roland Scollay**	Nursing & Health Science, Monash University, Melbourne, Australia	Australia	
06-Feb-12	**Microbial and cytokine detection controls antigen degradation by autophagy and subsequent endogenous MHC II-restricted presentation in dendritic cells**	**Philippe Pierre**	Centre d'Immunologie de Marseille Luminy	France	
12-Feb-12	**Interpreting the CpG island signal**	**Rob Klose**	Department of Biochemistry, Oxford University	UK	The RCAI 10th Anniversary Seminar Series/Seminar 26
13-Feb-12	**Signaling functions of reactive oxygen species and electrophiles: regulatory mechanisms and implications in chronic inflammation-associated diseases**	**Tomohiro Sawa**	Department of Microbiology, Graduate School of Medical Sciences, Kumamoto University	Japan	The RCAI 10th Anniversary Seminar Series/Seminar 27
19-Feb-12	**Paradox of Immunodeficiency and Inflammation & Autoimmunity in Human Aging**	**Sudhir Gupta**	University of California	USA	The RCAI 10th Anniversary Seminar Series/Seminar 28
19-Feb-12	**Lipid, vitamin, and nucleotide in the regulation of gut immunity**	**Jun Kunisawa**	Division of Mucosal Immunology, Institute of Medical Science, The University of Tokyo	Japan	The RCAI 10th Anniversary Seminar Series/Seminar 29
22-Feb-12	**A systems biology approach to Parkinson's disease: Research at the new Luxembourg Centre of Systems Biomedicine**	**Rudi Balling**	Luxembourg Centre for Systems Biomedicine (LCSB)	Luxembourg	The RCAI 10th Anniversary Seminar Series/Seminar 30
22-Feb-12	**Identification of claudin-expressing medullary thymic epithelial stem cells that maintain the functional medulla during life**	**Yoko Hamazaki**	Graduate School of Medicine, Kyoto University	Japan	The RCAI 10th Anniversary Seminar Series/Seminar 31
27-Feb-12	**Unraveling governing principles from immune cell dynamics**	**Kumar Selvarajoo**	Institute for Advanced Biosciences, Keio University	Japan	The RCAI 10th Anniversary Seminar Series/Seminar 32
27-Feb-12	**Immune regulatory functions of DOCK family proteins in health and disease**	**Yoshinori Fukui**	Medical Institute of Bioregulation, Kyushu University	Japan	The RCAI 10th Anniversary Seminar Series/Seminar 33
07-Mar-12	**Allele-specific profiling of genome-wide epigenetic marks and sequence polymorphisms**	**Yoshiko Mito**	Massachusetts General Hospital/ Harvard Medical School	USA	The RCAI 10th Anniversary Seminar Series/Seminar 34
22-Mar-12	**Regulatory effects of IgG on B cell functions**	**Toshiyuki Takai**	Institute of Development, Aging and Cancer, Tohoku University	Japan	The RCAI 10th Anniversary Seminar Series/Seminar 35

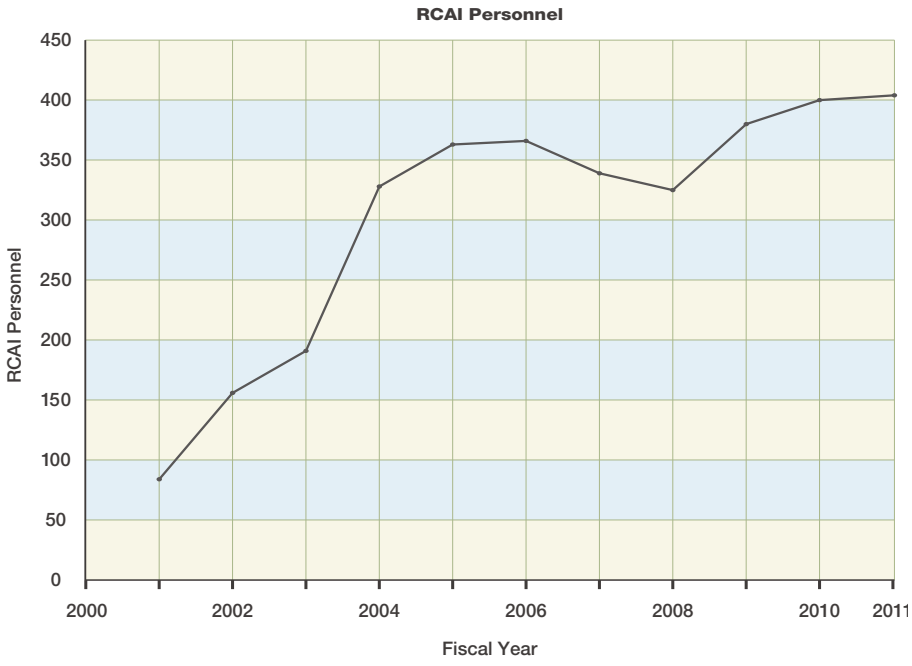
Budget, Personnel and Patents



RCAI Budget FY2001-2011 (JPY 100 Million)

Note: Budgets for FY2001-2003 include construction expenses for RCAI facility

RCAI Budget (JPY 100 Million)	
2001	41.74
2002	54.23
2003	60.48
2004	40.10
2005	39.02
2006	35.90
2007	34.56
2008	32.61
2009	31.86
2010	30.83
2011	31.06



RCAI Personnel	
2001	84
2002	156
2003	191
2004	328
2005	363
2006	366
2007	339
2008	326
2009	380
2010	400
2011	404

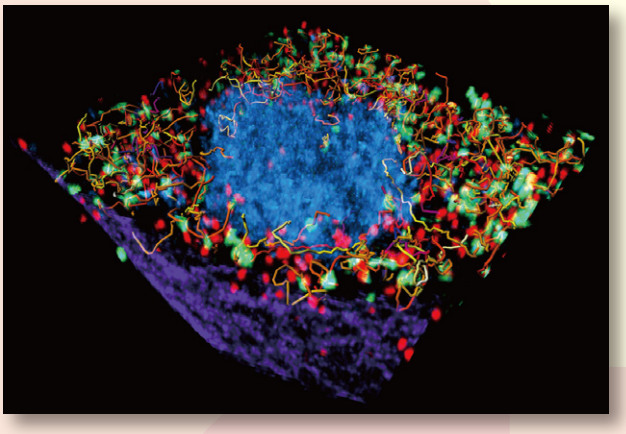
RCAI staff composition (as of Mar. 2012)

Category	Number
Director	1
Senior Advisor	2
Group Director	8
Team Leader	12
Unit Leader	11
Coordinator	1
Senior Scientist	12
Research Scientist	49
Special Postdoctoral Researcher	9
Foreign Postdoctoral Researcher	1
Research Associate	6
Junior Research Associate	11
International Program Associate	8
Student Trainee	63
Research Fellow	1
Research Consultant	3
Visiting Scientist	68
Senior Technical Scientist	1
Technical Scientist	6
Technical Staff I	40
Technical Staff II	63
Assistant	24
Temporary Employment	4
Total	404

RCAI Patents FY2011 (as of Mar. 2012)

There were 17 patents filed from April 2011-March 2012.

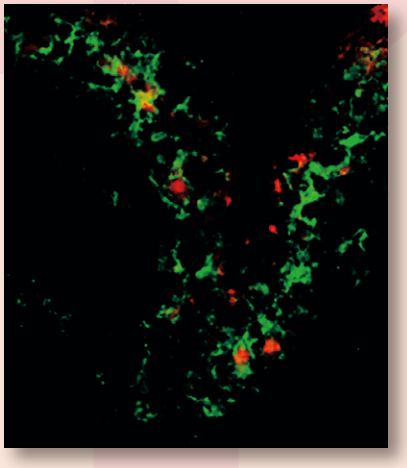
Cover and Section Heading Photo Legends



Cover: Two-photon live imaging analysis reveals that Bcl6 is essential for entry of antigen-engaged B cells into the germinal center

Using two-photon microscopy, The Research Unit for Immunodynamics visualized a 3D-reconstructed slice (360 µm x 360 µm x 30 µm) of a B cell follicle in a mouse lymph node and viewed from an angle. Bcl6-sufficient antigen-engaged B cells (labeled with cyan fluorescent protein, *light blue*) are forming a nascent germinal center in the B cell follicle. Migration of Bcl6-deficient, antigen-engaged B cells (labeled with green fluorescent protein, *green*) was analyzed to show that they were unable to enter the nascent germinal center just like the non-antigen-specific polyclonal, mostly naïve B cells (labeled with rhodamine, *red*). This study highlights the critical role of Bcl6 in the entry of antigen-engaged B cells into the germinal center. Changes in the colors of the migration tracks of Bcl6-deficient, antigen-engaged B cells indicate the time after the beginning of the 60-min time-lapse recording (from dark blue to purple to red to orange to yellow). The lymph node capsule, which was visualized by detecting second harmonic generation signals, is also shown (*dark purple*).

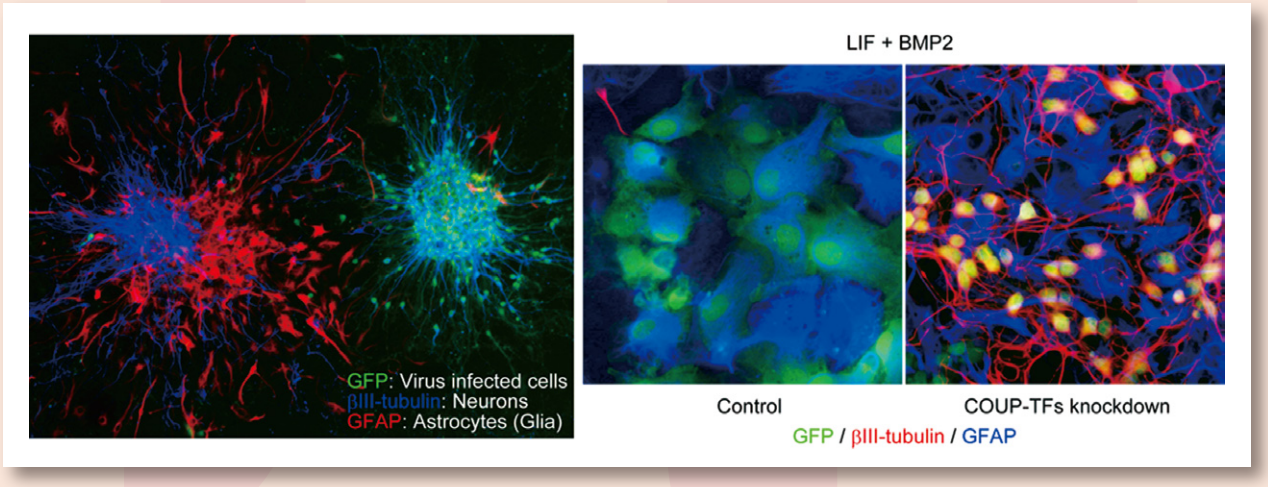
Image courtesy of the Research Unit for Immunodynamics



Front page of Part 1: CD169-positive macrophages in the lymph node sinus engulf subcutaneously injected dead tumor cells

The lymph sinus is a highway that links afferent and efferent lymph and is believed to be a filtering zone for the lymphatic system. The Laboratory for Innate Immunity discovered that CD169-positive macrophages (*green*) residing within the lymph sinuses rapidly consume the dead tumor cells (*red*) and play a critical role in cross-presenting tumor antigens to cytotoxic T cells.

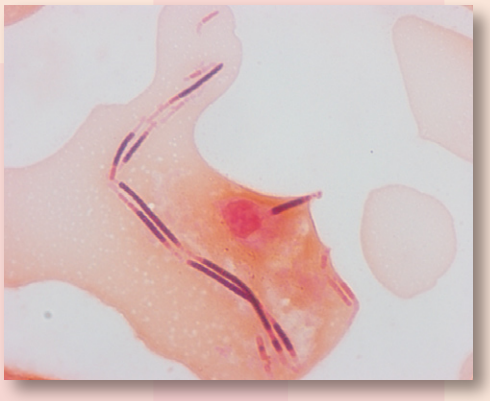
Image courtesy of the Laboratory for Innate Immunity

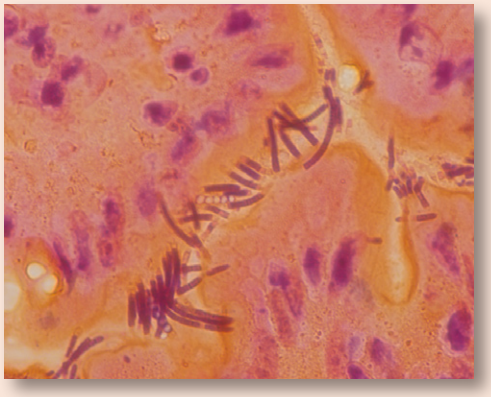


Front page of Part 2: Alteration of responsiveness to gliogenic cytokines

Young Chief Investigator Laboratory for Stem Cell Competency has been investigating the molecular mechanisms governing the differentiation of neural stem cells (NSCs). They identified *Coup-tfI* and *II* (*Coup-tfs*) as critical molecular switches for the neurogenic-to-gliogenic transition of NSCs. *Coup-tfs* knockdown neurospheres continued to produce neurons even in the late developmental stage and resisted the induction of gliogenesis by gliogenic cytokines, LIF and BMP2.

Image Courtesy of the Young Chief Investigator Laboratory for Stem Cell Competency





Front page of Part 3 and 6: Crosstalk between prokaryotic and eukaryotic kingdoms

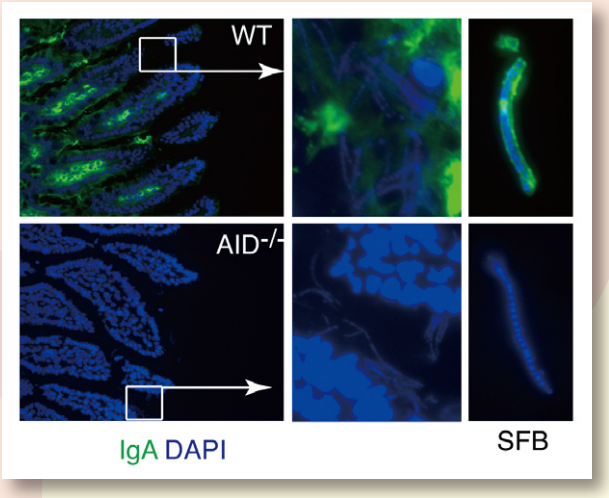
A Gram stain of a section from the small intestine showing Gram-positive segmented bacteria (*violet*) trapped in the mucus (*yellow layer*) or interacting with immune cells.

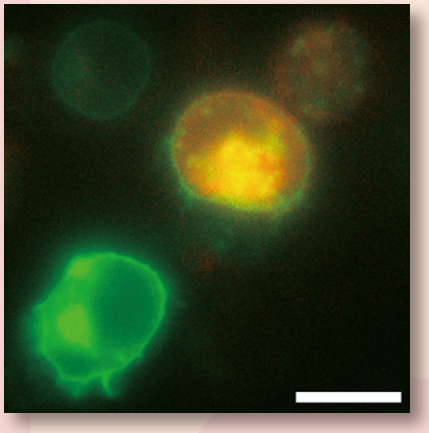
Image courtesy of the Laboratory for Mucosal Immunity

Front page of Part 4: Bacteria shielding by IgA in the gut

The production of IgA by plasma cells in the gut is critical for the containment of the gut commensal microbiota. The Laboratory for Mucosal Immunity demonstrated expansion of segmented filamentous bacteria (SFB) in the absence of IgA coating, such as in $AID^{-/-}$ mice. Pictures show IgA plasma cells (*green*) in lamina propria and secretory IgAs coating bacteria in the intestinal lumen. Blue stains the DNA of eukaryotic and prokaryotic cells. Note the absence of IgA and the attachment of SFB directly to the epithelium in $AID^{-/-}$ mice.

Image courtesy of the Laboratory for Mucosal Immunity





Front page of Part 5: Introduction of Cy3-labeled calmodulin in LAT-GFP expressing Jurkat cells

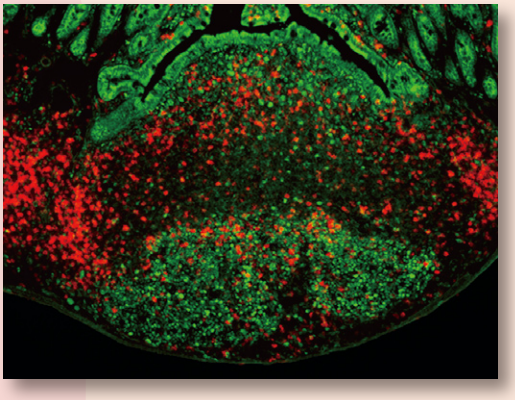
The Research Unit for Single Molecule Imaging, in collaboration with the Research Unit for Molecular Systems Immunology, established a method to visualize cell signaling using single molecule fluorescence microscopy. Calmodulin was labeled with a single molecule of Cy3 *in vitro* and introduced into Linker of Activated T cells (LAT)-GFP Jurkat cells by electroporation. The yellow fluorescence represents successful Cy3-labeling of calmodulin and its colocalization with LAT, a raft-resident protein. Bar: 10 µm.

Image courtesy of the Research Unit for Single Molecule Imaging, Research Unit for Molecular Systems Immunology and Dr. Nobuhiro Hayashi at the Tokyo Institute of Technology

Front page of Part 7: Enlarged germinal centers and expanded TFH cells in Peyer's patches of PD-$1^{-/-}$ mice

The Laboratory for Mucosal Immunity discovered that PD-1 regulates repertoire selection of IgA in the germinal centers of Peyer's patches. PD-1 deficiency leads to an expansion of follicular helper T (T_{FH}) cells, resulting in a large number of IgA B cells receiving T_{FH} help and differentiating into IgA plasma cells.

Image courtesy of the Laboratory for Mucosal Immunity



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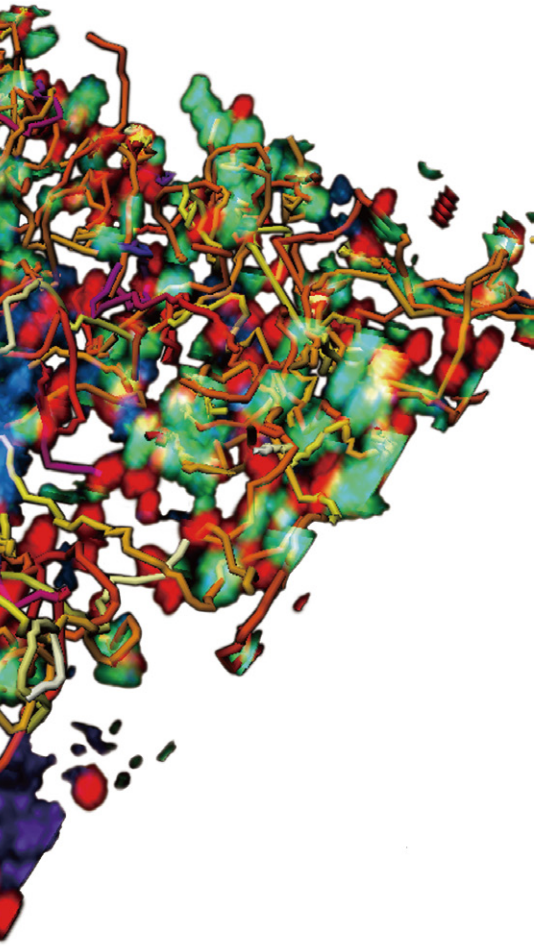

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