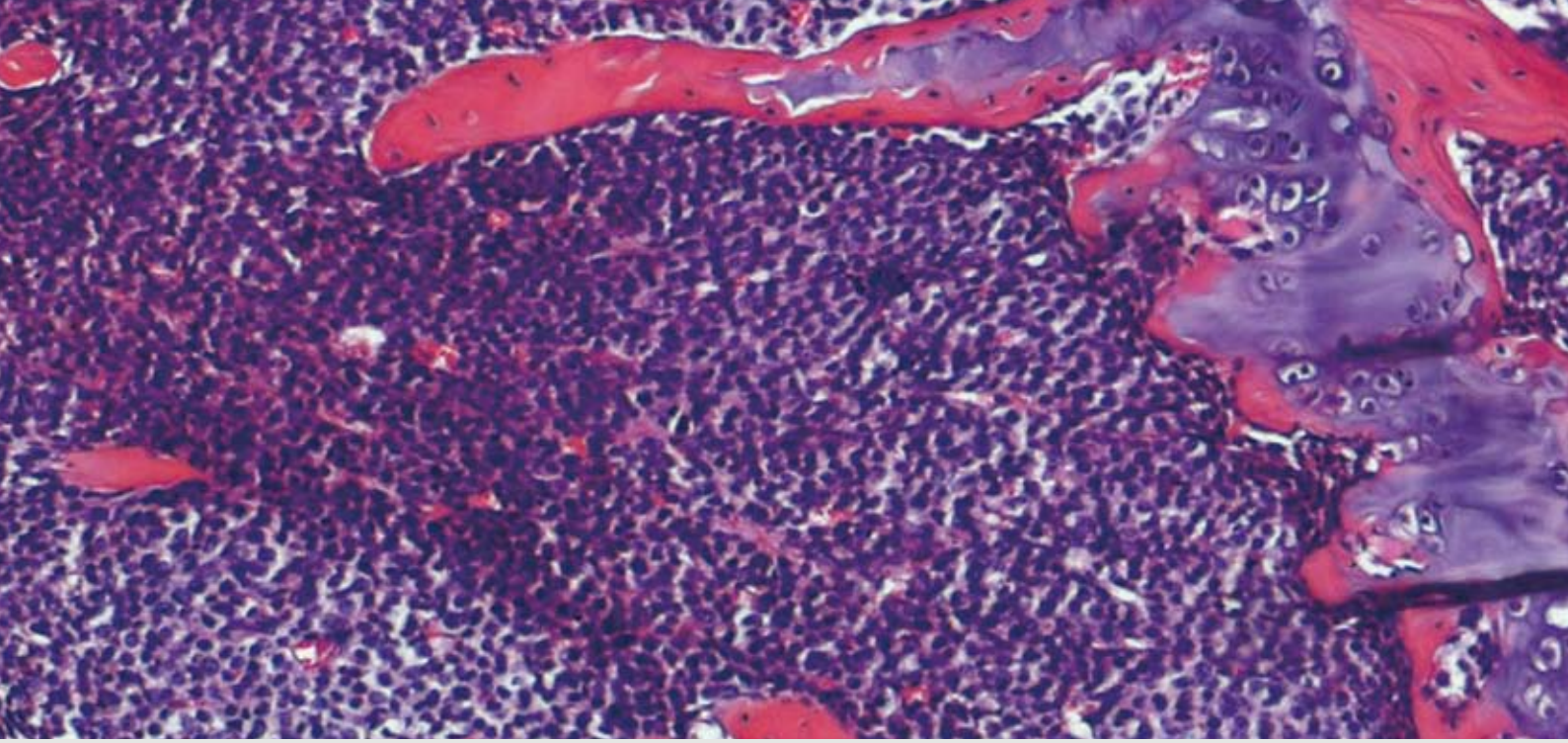
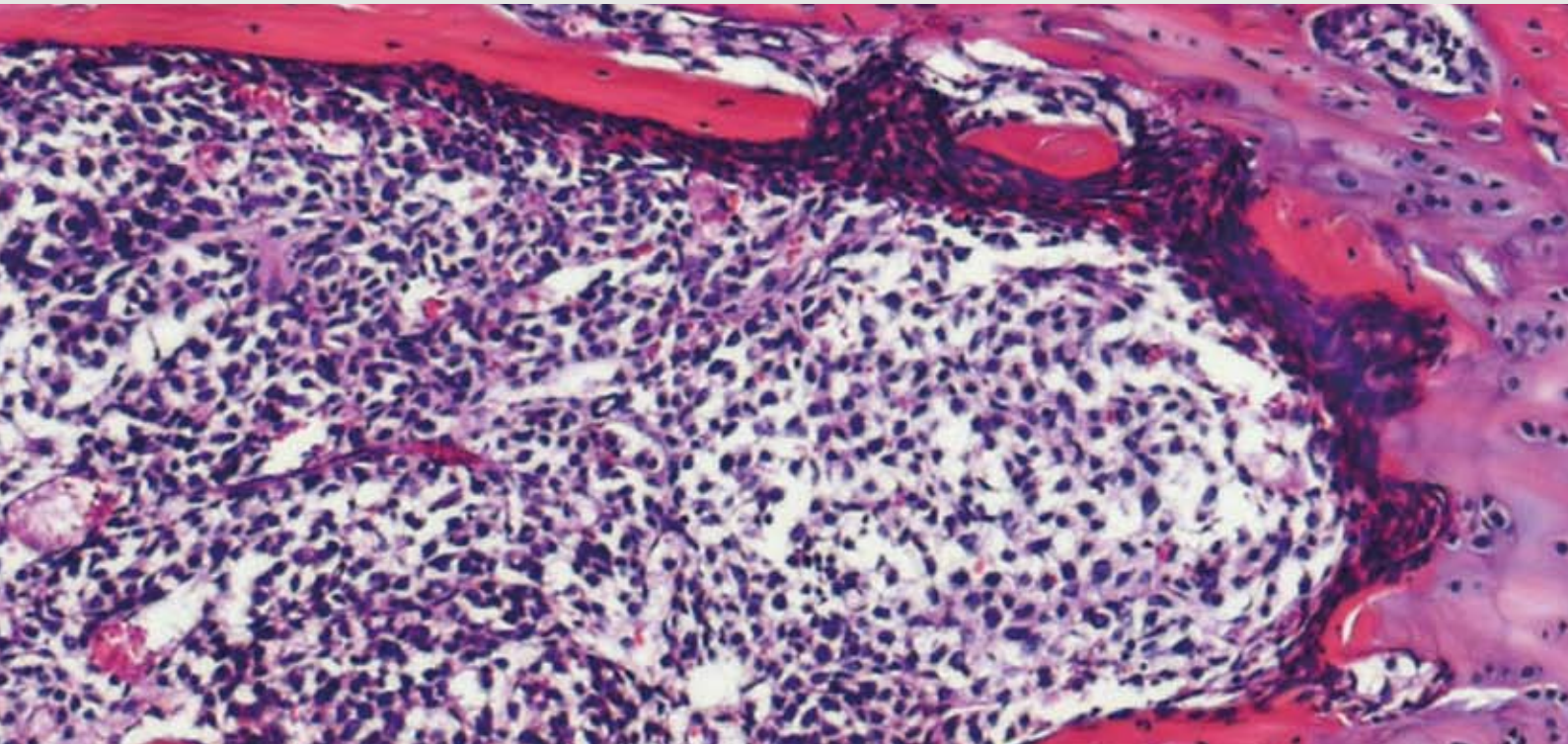


RIKEN RCAI Annual Report 2007

RIKEN Research Center for Allergy and Immunology



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Kiyoshi Takatsu
Dale Umetsu
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Unit for Human Disease Model	**Fumihiko Ishikawa M.D., Ph.D.**
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Unit for Cellular Immunotherapy	**Shin-ichiro Fujii M.D., Ph.D.**
Unit for Immunoinformatics	**S. Sujatha Mohan Ph.D.**
Unit for Lymphocyte Cloning	**Andrei Rybouchkin Ph.D.**
Unit for Single Molecule Immunoimaging	**Kumiko Sakata-Sogawa Ph.D.**
Unit for Therapeutic Model	**Kanako Shimimzu M.D., Ph.D.**

International Research Units

Unit for Thymic Environment	**Willem van Ewijk Ph.D.**
Unit for Immunogenetics	**Miguel Vidal Ph.D.**

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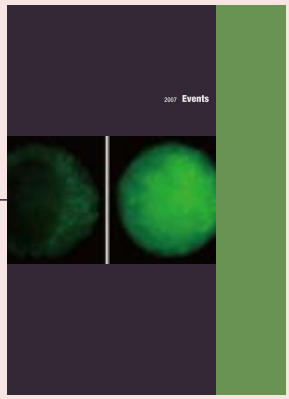

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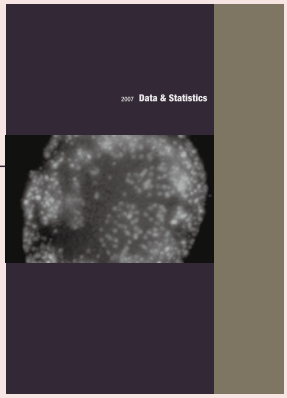


Data and Statistics

Director's Report

This is the fourth annual report of the RIKEN Research Center for Allergy and Immunology (RIKEN RCAI). Four years have now passed since all research groups moved from universities into the new building in RIKEN Yokohama campus in 2004.

RCAI – Current status 2007

The RCAI now houses 32 independent laboratories and the demographics have changed somewhat since the last report. Eighteen percent of the principal investigators (PIs) are non-Japanese and 15% are women. Nearly half of the PIs are less than 45 years old and 20% are under 40. Since RCAI has no internal graduate program, students must be recruited from universities. Half of the PIs have appointments as adjunct professors at universities including Osaka, Tokyo Medical and Dental, Chiba, and Yokohama City. As a result of these interactions, 45 graduate students joined RCAI last year. RCAI continues to be a vibrant evolving institution and during the last three years ~ 20 percent of researchers moved in or out.

The Research Coordination Office is now in its second year of operation and supports many important Center activities including assisting researchers in applications for external funding, international programs (see below), maintenance of the English RCAI webpage, and production of the Annual Report. Application for external funding is becoming especially critical since the research support from the Japanese government has been decreasing by about >5% per year. Drs. Toshitada Takekmori and Haruka Iwano are in charge of this important effort.

The World Premier International Research Center (WPI) Initiative was established by Japan's Ministry of Education, Culture, Sports, Science and Technology (MEXT) in FY2007. Only five of these institutes were selected amongst all scientific fields and the Osaka University Immunology Frontier Research Center (IFReC), Shizuo Akira Director, was one of them. In an exciting new development, we are initiating a collaborative research agreement with IFReC that will establish RCAI as a satellite center. Drs. Kurosaki, Saito, and Hirano will be appointed to the IFReC and will be able to have laboratories in Osaka or RIKEN or both.

Several recommendations were made by RCAI advisory council (AC) during its last full meeting, and we continue to implement them. Our efforts to develop an effective vaccine for cedar pollen allergy had reached an advanced stage and the AC felt that future development to the point of clinical trials would be best managed by the private sector. Two RIKEN Venture Company spinoff companies were established in 2006: REGiMMUNE will move forward with the human vaccine development and Animal Allergy Clinical Laboratories is responsible for immunological diagnosis and immune therapies for animals. At RCAI we have been continuing our fundamental research in this area and are developing a new allergy vaccine that does not react with human anti-cedar pollen IgE, thus eliminating the risk of anaphylaxis, but can suppress IgE responses in mice. The AC also recommended

that we further develop our clinical research activities through several mechanisms: 1) collaborating with spinoff companies, translational research centers at universities, and pharmaceutical companies. We are collaborating with the RIKEN Venture Companies to move some of our discoveries into clinical trials and will continue to expand our efforts in the other two areas. 2) Creation of a Clinical Advisory Council. We have established a Strategic Research Advisory Council that meets once or twice a year to evaluate and provide advice on RCAI translational research projects. The Council is composed of experts on Research and Development and clinical professors and is chaired by Dr. Nobuyuki Miyasaka. 3) In particular, the AC recommended that we establish a network with clinicians to study clinical allergy and primary immunodeficiency diseases, and that endeavor has been so successful that it is discussed separately below (section 4). Finally, the AC was very impressed with our progress in the humanized mouse model and recommended that we move forward to create the third generation model that would contain human MHC and cytokine genes, thus improving T cell function and stem cell survival. These efforts are currently underway.

RCAI - Mid-term plan

RCAI instituted its second mid-term (6 years) plan in 2007, allowing us to establish our research priorities one year earlier than RIKEN. During the next six years, RCAI will focus on four key areas:

- Spatiotemporal Dynamics of Molecules in Immune Cells
- Regulatory Mechanisms in the Immune System
- Creation of Application Platforms
- Translational Research

For basic research, imaging will be the key word. In the Spatiotemporal Dynamics of Molecules in Immune Cells area, we will target the development of methods to visualize the molecular dynamics in immune cells. For that purpose, we will expand our existing single molecule imaging capabilities and develop new technologies to measure and simulate the molecular dynamics in viable cells using single-molecule microscopy (Tokunaga Nature Methods, 2008). This area also includes basic research on immune cells, including elucidation of the molecular basis for regulation of lymphocyte functions, activation and survival, and antigen presentation. We also have the ENU mutagenesis project for screening of recessive genes that cause immunologic diseases. In the area of "Regulatory Mechanisms in the Immune System" we focus on the elucidation of basic mechanisms that regulate the immune system. We will integrate information concerning the dynamics and kinetics of immune cells and build upon this information to attain an understanding of the overall regulation of the immune system. We focus on systems such as mucosal immunity, immune responses including allergic, autoimmune, and anti-tumor responses, immune system development, and technology development for in vivo imaging.

RCAI is different from universities especially because of its mission oriented research projects. During the next six years, we plan to strengthen the platform for clinical applications and so have added a new research area "Creation of application platforms" to our focus. Research projects on artificial lymph nodes (Watanabe Nature Biotechnology, 2007), humanized mice (Ishikawa Nature. Biotechnology 2007), adjuvant vaccines, and allergy vaccines will take place in this arena. These projects will provide a platform for "translational research". Among translational research projects, we finished a phase I/IIa clinical study on NKT cell therapy for lung cancer patients. Median survival time was 18.3 months, which was much better than the 4.5 months in the control group treated with conventional chemotherapy. We will start second stage trials in which we will target postoperative patients. The tumor recurrence rate for these patients is more than 75% and we hope to be able to improve on this dismal outcome with NKT cell therapy. In the area of allergy vaccines, we developed a fusion protein of cedar pollen allergens that is unable to cause anaphylaxis but induces specific suppression of IgE responses. The important absence of an anaphylactic reaction seems to be due to the fact that the fusion protein does not react with cedar pollen-specific human IgE, as tested so far with sera obtained from 100 cedar pollinosis patients, yet can induce IgG antibody and T cell responses. In animal models, we have confirmed that this vaccine induces antigen-specific suppression of IgE responses and has both prophylactic and therapeutic activities. We hope to proceed to the stage of generating GMP grade materials for human trials, but it is a pity that we have so far not found a pharmaceutical company willing to collaborate with us on this project.

International Programs

A major goal of RCAI is to be an international center of world class immunologic research. In keeping with this globalization strategy, we have created new positions, the RIKEN RCAI Distinguished International Research Units. The aim of this new program is to establish non-Japanese investigators in fully independent research positions at RCAI. Under this program, Dr. Sujatha Mohan from the Institute of Bioinformatics, India, joined RCAI as the leader of new Immunoinformatics Research Unit. Dr. Andrei Rybouchkin has been promoted from a Research Scientist to the leader of the Lymphocyte Cloning Research Unit. In addition, I am happy to report that our International Research Collaboration Award expanded the international exchanges of scientists in various forms. Dr. Willen van Ewijk established a laboratory which aims to develop an artificial thymus. Two of his technicians from the Netherlands stayed in his RCAI laboratory for an entire year, enhancing both the scientific and the cultural exchanges. Dr. Miguel Vidal started a laboratory for stem cells and immunoepigenetics. Dr. Peter Burrows, a professor at the University of Alabama at Birmingham, started to support the center as a Science Advisor. His presence and availability for consultation has been a great benefit for RCAI researchers.

RCAI carried out joint symposia with three institutes this year. In May, we had a first joint seminar with the Max Planck Institute of Immunobiology, Max Planck Institute for Infection Biology, and the German Arthritis Research Center. In June, there was a joint symposium with Singapore A*STAR, and in March, 2007, we had a joint symposium with INSERM. Dr. Saito has expended great effort to make these events successful. This summer we will begin the Harvard Summer School, an internship program in which Harvard undergraduate students will come to RCAI to do research while earning credits at Harvard.

RCAI has also collaborated with the Japanese Society for Immunology to promote immunological research in Japan. The RCAI-JSI International Symposium on Immunology, organized by Dr. Hirano, took place in June, 2007. The theme of the symposium was "Transcriptional Factors and T Lymphocyte Development." Attendance from foreign countries exceeded 23%, a positive reflection on the international recognition of this symposium. We also held the second RCAI-JSI Workshop at RCAI. The topic this time was primary immunodeficiencies and clinicians and basic researchers gathered at RCAI to discuss and exchange their data.

RCAI's second International Summer Program (RISP) was a great success. Under the guidance of the organizer, Dr. Kurosaki, many people cooperated, leading to a highly successful program. Applications to RISP have increased steadily so that last year, there were about 130, an indication of the growing reputation of this program in the international immunology community. Forty three students from 16 countries were ultimately accepted.

This year I also made a significant change in our September

RCAI research retreat program. Previously, outside participants were excluded from the retreat because much of the data presented there was preliminary and unpublished. However, this year we invited senior editors from Nature Immunology, Nature Medicine and Immunity to participate. I appreciate their involvement in discussions and I also appreciate the efforts of the organizers, Dr. Udono and Dr. Kaisho.

Primary immunodeficiency project

Primary immunodeficiency (PID) describes a collection of diseases caused by mutations in genes required for the normal development and function of the human immune system. The PIDs are clinically heterogeneous, but typically are characterized by increased susceptibility to infections and, in their most severe form, are fatal if untreated. In the 2006 Annual Report I described the establishment of the Human Immunodeficiency Network. At that time, RCAI, the Clinical Study Group for PID, and the Kazusa DNA Research Institute established a platform to combine genetic diagnosis and basic and clinical research. The goal of this Network is to encourage synergistic collaboration between clinical and basic researchers, to identify new PID genes, and to develop better approaches for the early diagnosis and treatment of PID. The basic goals remain the same, however the Network has expanded considerably during the past year and is now a collaboration between RCAI and 13 universities in Japan, Kazusa, the Institute of Bioinformatics (IOB) in Bangalore, India, and the Jeffrey Modell Foundation in the United States. Dr. Sujatha Mohan of the IOB, an institute renowned for its expertise in bioinformatics, has become an RCAI unit leader to spearhead the expansion of the PID database for predicting the onset of PID pathology and defining optimal treatments. In addition to serving as a repository for PID patient samples and the analysis of their genetic defects, the Network has a strong research component with a focus on recapitulating human PID in the humanized mouse model, where immunophenotypic analyses can be readily performed and therapeutic strategies can be evaluated. The activity of RCAI in the area of PID came to the attention of the Jeffrey Modell Foundation (JMF), an American non-profit organization founded by Fred and Vicki Modell in memory of their son Jeffrey, who died at the age of 15 from pneumonia resulting from a PID. Established in 1987, the foundation is dedicated to early and precise diagnosis, meaningful treatments, and ultimately cures of PID throughout the world. RCAI and JMF have agreed to a joint project, the RIKEN Jeffrey Modell Diagnostic and Research Center for Primary Immunodeficiencies. The Center will contribute to the study of basic human immunology and will also play a major role in speeding up the diagnosis of PID and the selection of optimal treatments. To open the new center, RCAI held an inaugural dedication ceremony that was attended by JMF founders Mr. Fred and Mrs. Vicki Modell, Peter Turner the President of CSL Behring, a major financial backer of the JMF, Jan Bult the President of the Plasma Protein Therapeutic Association (PPTA), and representatives of Tsubasa no Kai, a Japanese advocacy group for PID patients and their families.

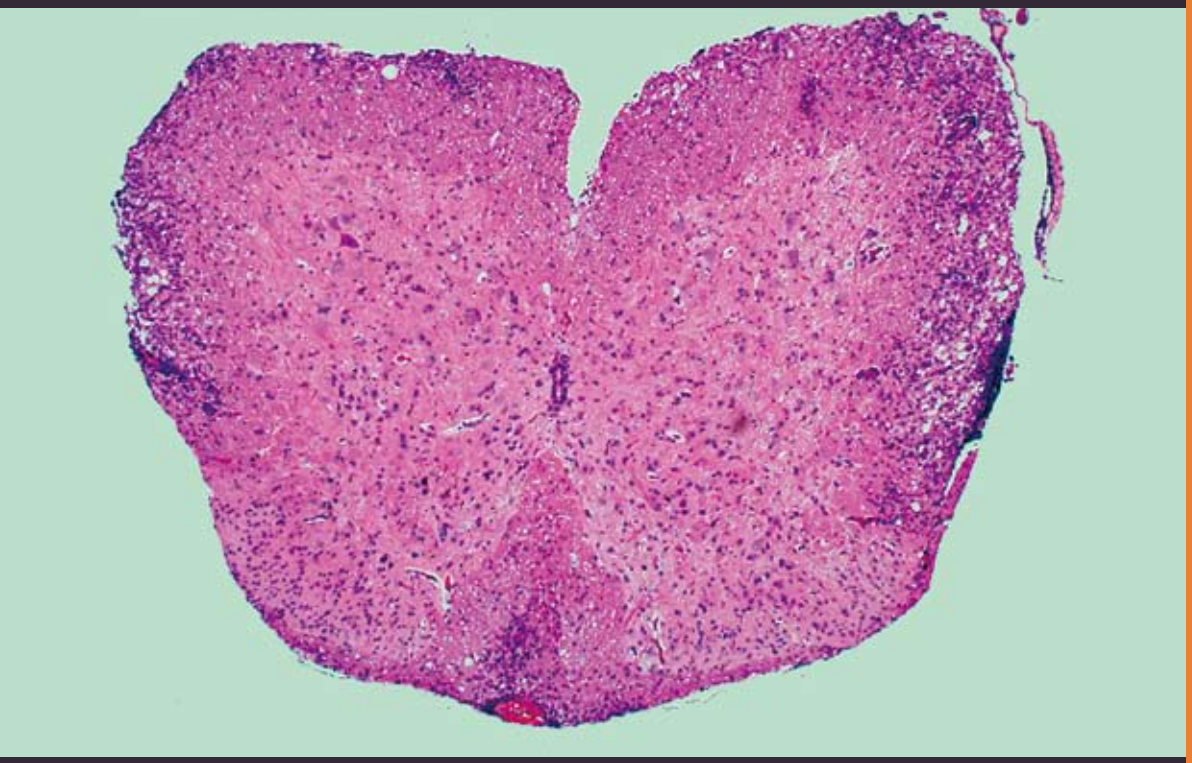
Publications by young investigators

Overall, RCAI investigators had an excellent year in that 25% of 126 publications from our institute were in high impact journals, i.e. those with an impact factor greater than ten. As one of my goals is to develop the potential of young RCAI investigators, it was rewarding to see significant progress this year as measured by the number of young investigators who published highly significant papers. Dr. Taniuchi studies cell fate decisions using CD4/CD8 lineage commitment in the thymus as a model. He has found that the Runx transcription factor complex actively suppresses the expression of another transcription factor required for CD4 T cell development, thus allowing for differentiation of cytotoxic T cells (Science 2008). The transcription factor NF-κB is a central regulator of host immune and inflammatory responses, and its activity must be tightly regulated in order to prevent inflammatory diseases. Dr. Kaisho identified a novel nuclear ubiquitin ligase that terminates NF-κB activation through intranuclear sequestration and subsequent degradation (Nature Immunology 2007). Dr. Sato has identified a new type of dendritic cell, DCreg, and found that these regulatory dendritic cells protect against cutaneous chronic graft-versus-host disease by generating regulatory T cells (Blood 2007). Dr. Ishikawa has used his humanized mouse model to identify and characterize the leukemic stem cells in acute myelogenous leukemia. He found that quiescence of human LS cells may be a mechanism underlying resistance to cell cycle-dependent cytotoxic therapy, and validated the humanized mouse as potentially useful model for development of novel therapeutic strategies (Nature Biotechnology. 2007). Dr. Kawamoto has previously proposed a model for fetal heamtopoiesis in which developing cells maintain myeloid potential even after segregation toward B and T cell lineages. He now has convincing data demonstrating that the "myeloid based" model holds true for adult heamtopoiesis as well (Nature 2008). Dr. Watarai discovered a novel TREM family receptor expressed only on TLR activated plasmacytoid dendritic cells, which plays a crucial role in Type I interferon production after TLR activation. His studies provide new insights into the early events after pathogen infection (PNAS 2008). Injection of apoptotic cells can induce suppression of immune responses to cell-associated antigens. Dr. Tanaka has shown that intravenous injection of apoptotic cells expressing a fragment of myelin oligodendrocyte glycoprotein prevented the development of EAE, a mouse model for human multiple sclerosis. Marginal zone macrophages are a critical cell type for this immunosuppressive effect (J. Clin. Invest. 2007). Dr. Tokunga has developed a highly inclined thin illumination system that enables clear single-molecule imaging in cells. This method will permit researchers to visualize and quantify molecular dynamics, interactions and kinetics in cells for molecular systems biology (Nature Methods 2008). Dr. Fujii found that cross-presentation of glycolipid from tumor cells loaded with alpha-galactosylceramide could induce T cell mediated immunity via dendritic cells. This novel experimental therapeutic approach led to highly protective and long-lived adaptive immunity directed toward tumor antigens (J. Exp. Med. 2007).

Senior investigators have also published milestone papers. Dr. Koseki studies epigenetic inheritance and has indentified a protein, Np95, that is essential in vivo to maintain global and local DNA methylation and to repress transcription of retrotransposons and imprinted genes (Nature 2007). Calcium is a well know intracellular second messenger in cell signaling but Dr. Hirano has found that zinc is as well. He was able to visualize a zinc wave following cross-linking of the FcεR1 on mast cells (J. Cell Biology 2007). Dr. Saito found that the Bcl-10 adaptor–binding partner CARD9 is a critical common adaptor protein in myeloid cells for signaling through both ITAM-associated receptors and Toll-like receptors (Nature Immunology 2007). Dr. Kurosaki discovered that the calcium-binding endoplasmic reticulum protein STIM1 is critical to mast cell function. STIM1 critically regulates FceRI Ca influx, activation of the NF-κB and NFAT transcription factors, degranulation, and in vivo anaphylaxis responses (Nature Immunology 2007).

Masaru Taniguchi
March, 2008

2007 Research Highlights



Haruhiko Koseki

Good housekeeping

A protein with an important role in regulating gene expression may have other duties relating to chromosome maintenance

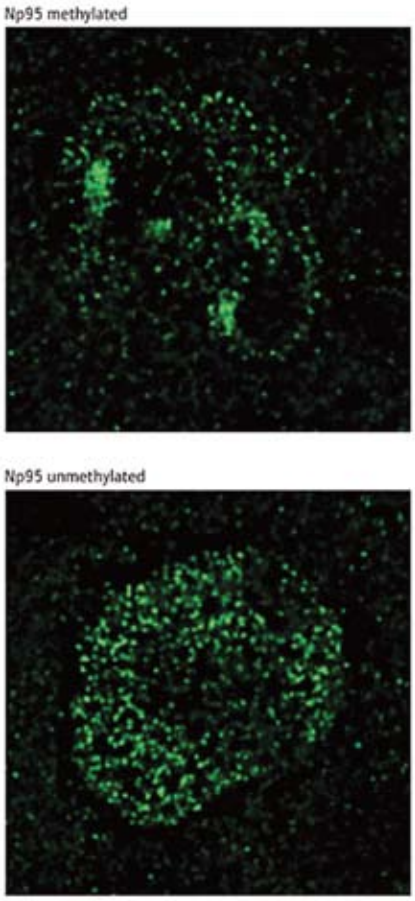


Figure In cells undergoing DNA synthesis in the presence of methylated nucleotides (*top*), Np95 (*green*) specifically localizes to the chromosomes, revealing preferential association with hemimethylated DNA. In cells treated with unmethylated nucleotides (bottom), no such localization is seen and the Np95 is diffused throughout the nucleus.

Most genetic information is directly encoded by the sequence of nucleotides in a chain of DNA, but further instructions may also be provided by adding chemical modifications to those nucleotides, just like inserting footnotes can alter the meaning of a text. One important modification is DNA methylation, which generally has the effect of 'silencing' transcriptional activity of marked genes—a secondary but essential level of regulation.

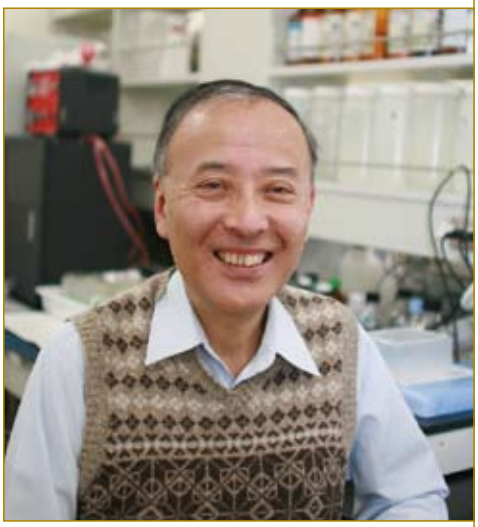
DNA methylation patterns can be transmitted from parent to child, an important process known as genomic 'imprinting.' Maintaining these patterns is an active process, as each cycle of DNA replication results in the production of chains that are only hemimethylated, and full methylation is subsequently restored by the enzyme DNA methyltransferase 1 (Dnmt1).

Other proteins are known to assist this process. Recent work from a group led by Haruhiko Koseki of the RIKEN Research Center for Allergy and Immunology in Yokohama, and Masaki Okano of the RIKEN Center for Developmental Biology in Kobe, has highlighted the important role of one particular protein, Np95, in directing Dnmt1 to hemimethylated DNA targets.

Koseki's group initially found that Np95 associates with imprinting-related proteins, and subsequent experiments in mouse embryonic stem cells (ESCs) extended these findings, demonstrating that Np95 co-localizes with Dnmt1 and other associated proteins at hemimethylated chromosomal regions after DNA replication (Figure). Eliminating the expression of Np95 altogether resulted in a marked reduction of DNA methylation in cultured ESCs, and led to full developmental arrest in mouse embryos.

Methylation does more than silence genes; it also helps stabilize DNA elements known as retrotransposons, which are otherwise capable of physically 'jumping' into other chromosomal regions—and potentially disrupting other genes. Koseki's group found that Np95 helps to lock down these retrotransposons. "At the least, Np95 is essential to maintain genomic imprinting, which in turn permits normal development of embryonic and extraembryonic tissues," he says. "But it could be possible that this process may also be important to ensure genomic stability."

Intriguingly, Koseki's and his colleagues also found evidence suggesting that Np95's function may not be limited to restoring DNA methylation, but could encompass other chromosomal maintenance tasks as well—a possibility he is keen to investigate further. "Hemimethylated DNA is not simply a transient status that appears after DNA replication … it may potentially form a specific signal that could be sensed by Np95," he says. "We presume that Np95 may form a platform that helps to organize not only DNA methylation but also other types of modifications."



Masahiro Muto

ORIGINAL RESEARCH PAPER

Sharif, J., Muto, M., Takebayashi, S., Suetake, I., Iwamatsu, A., Endo, T.A., Shinga, J., Mizutani-Koseki, Y., Toyoda, T., Okamura, K. et al. The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. *Nature* 450, 908–912 (2007).

Poor transport cuts to the bone

Researchers link skeletal disorders with sugar chain production

Haruhiko Koseki

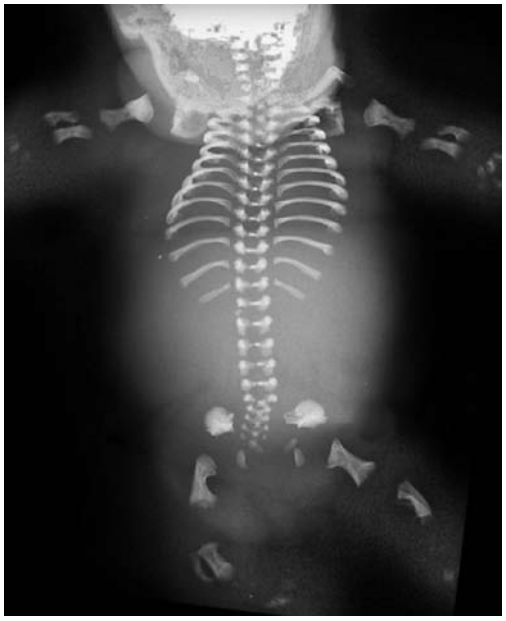


Figure A human skeleton affected by Schneckenbecken dysplasia.

An international research team with strong RIKEN representation has uncovered a molecular basis—and developed a mouse model—for some types of congenital human skeletal disorders, including the rare, lethal Schneckenbecken dysplasia (Figure). The team, led by Shiro Ikegawa of RIKEN's SNP Research Center and Haruhiko Koseki of RIKEN Research Center for Allergy and Immunology in Yokohamma, has accumulated persuasive evidence that the underlying problem is an inoperative form of the molecule responsible for transporting sugars—critical to the construction of the skeleton—across the membrane of the cellular organelle known as endoplasmic reticulum (ER).

The skeleton is formed from a matrix of compounds secreted by cartilage and bone cells. A family of large molecules called proteoglycan is intimately involved in the process. The proteoglycan molecules consist of a protein core to which long chains of sugars, such as chondroitin sulfate, are attached. Chondroitin sulfate is made in the ER and another cellular organelle, known as the Golgi apparatus, from two different sugar compounds. Its composition reflects, and may even control, the growth activity of the bone. The sugars are moved into the ER by molecules known as nucleotide-sugar transporters (NSTs). More than 10 genes for NSTs are known in humans.

In a recent *Nature Medicine* paper, researchers from Japan, the US, Germany and Holland, detailed how they developed a line of mice incorporating a recessive mutation in *SLC35D1*, the gene coding for the NST involved in chondroitin sulfate synthesis. They confirmed that mice in which this mutation is expressed died as newborns, and displayed skeletal abnormalities, in particular short, thick limbs. The researchers then used cell staining and radioactive labeling to confirm that, at a molecular level, these developmental problems were associated with a severe reduction in the length and number of sugar chains of the proteoglycans.

The mutant mice bore many features in common with humans suffering from Schneckenbecken ('snail pelvis') dysplasia. When the team investigated two human cases of this dysplasia, they were able to show that the NSTs involved in chondroitin sulfate synthesis were inactive. They were also able to trace the problem back to mutations in the human *SLC35D1* gene.

This is the first demonstration of the consequences in humans of such abnormal biosynthesis of proteoglycans sugar chain. Examples of other human disorders associated with proteoglycans have recently been discovered. "We expect that our results will open many avenues of research in the fields of connective tissue disorders, vertebrate development and glycobiology," Ikegawa says.

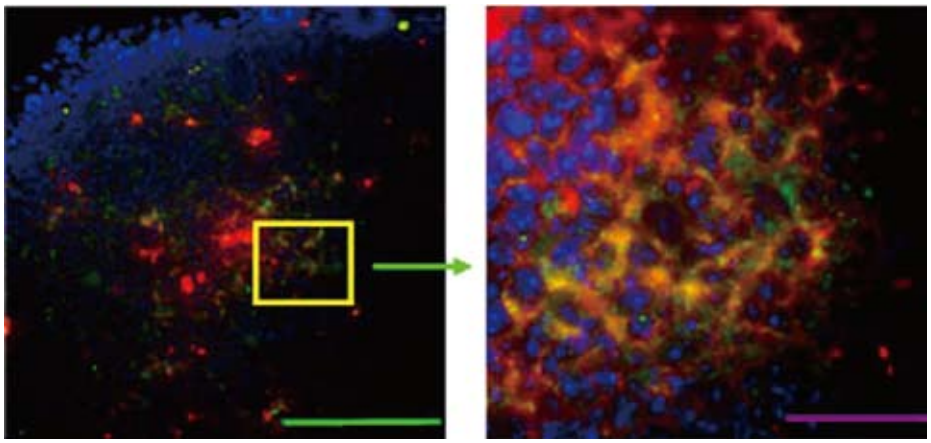
ORIGINAL RESEARCH PAPER

Hiraoka, S., Furuichi, T., Nishimura, G., Shibata, S., Yanagishita, M., Rimoin, D.L., Superti-Furga, A., Nikkels, P.G., Ogawa, M., Katsuyama, K., Toyoda, H., Kinoshita-Toyoda, A., Ishida, N., Isono, K., Sanai, Y., Cohn, D.H., Koseki, H. & Ikegawa, S. Nucleotide-sugar transporter SLC35D1 is critical to chondroitin sulfate synthesis in cartilage and skeletal development in mouse and human. *Nature Medicine* 13, 1363–1367 (2007).

Hiroshi Ohno

Cell fusion may create niche for immune cell education

Researchers identify possible precursors of lymphoid tissue cellular network



Figure

Lymphoid follicle-like structures contain FDC (*red*) with B220$^+$ characteristics (*green*).

Japanese researchers have identified a subset of cells they believe may induce the formation of a network of follicular dendritic cells (FDC) in the spleen and lymph nodes.

A recent paper published by Hiroshi Ohno and colleagues at the RIKEN Research Center for Allergy and Immunology, Yokohama, suggests that in the mouse, spleen cells expressing the cell surface marker proteins CD35, involved in processing and clearance of immune complexes, and B220, found on almost all immune system cells, can induce the formation of these networks and ultimately lymphoid follicles (Figure).

These so-called FDC form a reticular network of cells in the spleen and lymph nodes that trap immune complexes of antibodies, antigens and associated molecules. The network plays a critical role in the development and maturation of the antibody-producing B lymphocytes (B cells). If a B cell binds weakly to an antigen trapped on the surface of an FDC, it undergoes programmed cell death (apoptosis). On the other hand, a B cell that has a high affinity for the trapped antigen survives to become an antibody-producing plasmablast, and ultimately a memory B cell. This is the fundamental process that underpins the ability of the immune system to respond quickly to attack by pathogenic infection.

But it is not simply a matter of recognition; it appears that a complex interaction of cells and molecules and cellular architecture within the dynamic microenvironment of the lymphoid follicle is required for B cell maturation. The players include connective tissue cells called stromal cells.

"The intrigue of the lymphoid follicle stems from the complexity of its microarchitecture, comprising immune cells and stromal cells, adhesion molecules, cytokines and antigen-antibody complexes, and the relationships between these components, in the formation of B cell-follicular dendritic cell aggregates and the regulation of B cell differentiation," says Takaya Murakami, the first author of the paper.

Results from a series of experiments both *in vitro* and *in vivo* suggest that the splenic cells with the CD35 and B220 proteins on their surface (CD35$^+$B220$^+$ cells) interact with stromal cells to create a niche for migrating B cells, forming cell clusters. The researchers believe that this may play a critical role in FDC network development and the subsequent formation of lymphoid follicles. There is also some evidence that the stromal cells may fuse with the CD35$^+$B220$^+$ cells during this process.

Further investigation of the role of stromal cells in the development of the lymphoid follicles and B cell maturation is planned, says Murakami.

Takuya Murakami

ORIGINAL RESEARCH PAPER

Murakami, T., Chen, X., Hase, K., Sakamoto, A., Nishigaki, C. & Ohno, H. Splenic CD19$^-$CD35$^+$B220$^+$ cells function as an inducer of follicular dendritic cell network formation. *Blood* 110, 1215–1224 (2007).

Interferon production mechanisms revealed

Researchers are a step closer to understanding the mechanisms underlying a major immune defense against viruses

Masaru Taniguchi

RIKEN researchers based at the RIKEN Research Center for Allergy and Immunology in Yokohama have unraveled part of the mechanism for the production of Type I interferons by immune system cells known as plasmacytoid dendritic cells (PDCs).

PDCs are specialized producers of type I interferons: proteins, produced in response to viral and other infections, which play an important role in the so-called innate immune response, which is the body's first line of defense against infection (Figure). Type I interferons, including IFN-α and IFN-β, rapidly act to inhibit viral replication within infected cells, giving the infected host time to marshal a full immune response. But the mechanisms underlying interferon production are poorly understood.

Now, the research team, led by Masaru Taniguchi, has identified a protein on the surface of PDCs that is expressed after stimulation through the so-called toll-like receptor (TLR) pathway. TLR is known to be required to activate interferon production in PDCs, but previous studies have shown that signaling through TLR is not the only trigger for interferon production.

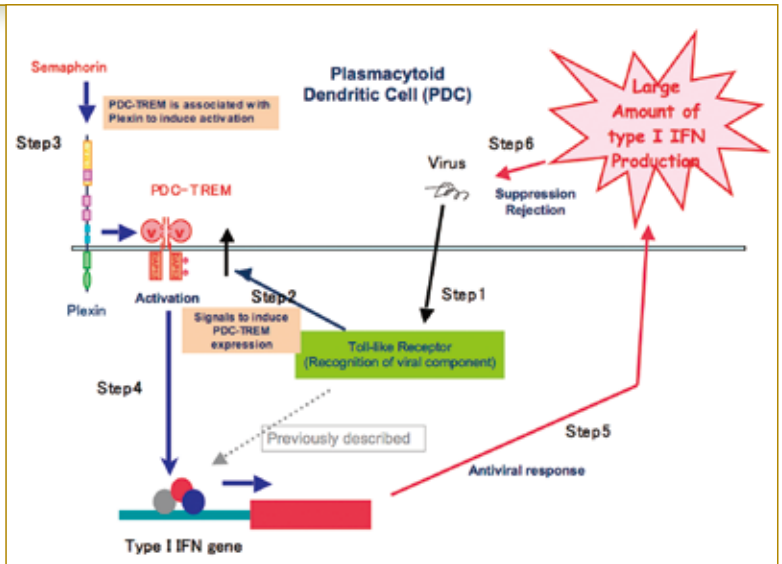


Figure The mechanisms underlying the immune defense against viruses

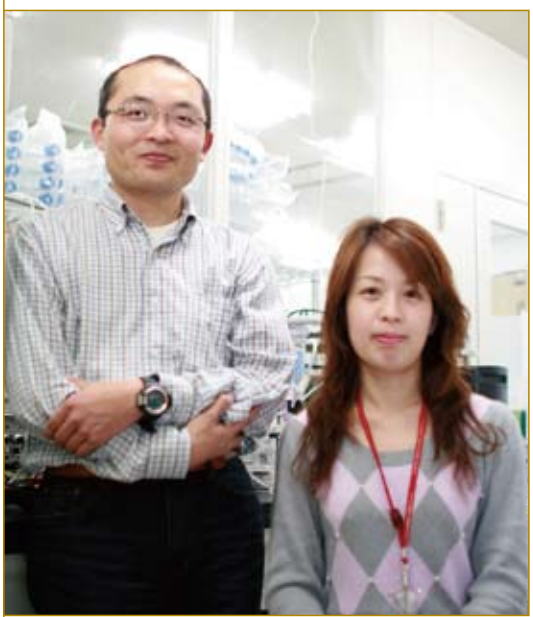
The researchers demonstrated that the protein, known as PDC-TREM, was associated on the PDC cell surface with at least two other proteins, Plexin A1 and DAP12. Under the condition of limited TLR stimulation, PDC-TREM was expressed on PDCs, but interferon production was not triggered.

However, when TLR stimulation took place in the presence of a protein that binds to Plexin A1, known as Sema6D, interferon was produced. Further investigation revealed that PDC-TREM mediated the activation of a cascade of signaling molecules that ultimately up-regulated the production of IFN-α.

Finally, the team demonstrated that inhibition of PDC-TREM expression, blocking of its binding to Plexin A1, and DAP12 deficiency all resulted in significantly reduced activation of these signaling molecules and subsequent production of IFN-α.

According to team-member Hiroshi Watarai, the results clearly demonstrate that PDC-TREM plays a central role in inducing interferon production in PDCs, which may provide possibilities for treatment of viral infections and other diseases in which interferon plays a role.

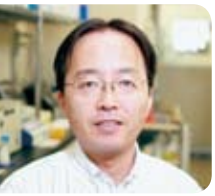
"Serum in patients with autoimmune diseases such as systemic lupus erythmatosus and Crohn's disease contains high level of type I interferons," notes Watarai "These results indicate that modulation of PDC-TREM signaling results in the regulation of type I interferon production from PDCs, so the PDC-TREM pathway may be a target for therapeutic use in anti-viral and autoimmune diseases."



Hiroshi Watarai *(left)* and Etsuko Sekine *(right)*

ORIGINAL RESEARCH PAPER

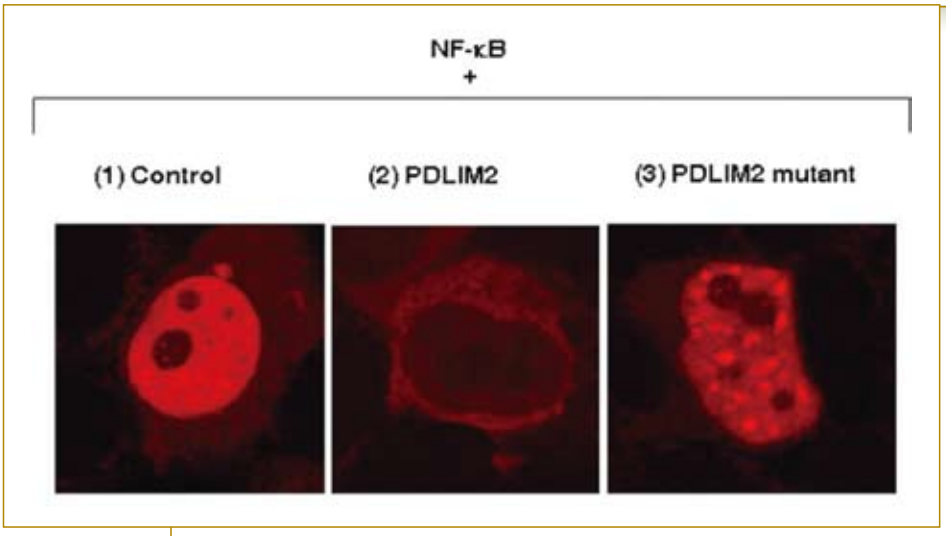
Watarai, H., Sekine, E., Inoue, S., Nakagawa, R., Kaisho, T. & Taniguchi, M. PDC-TREM, a plasmacytoid dendritic cell-specific receptor, is responsible for augmented production of type I interferon. *Proceedings of the National Academy of Sciences USA* 105, 2993–2998 (2008).



Tsuneyasu Kaisho

Halting the inflammation overload

Researchers discover a key molecule involved in regulating our immune response



Figure

PDLIM2 has two major activities, polyubiquitination of p65 for subsequent degradation and intranuclear targeting of p65 into nuclear bodies. The control (*left*) shows expression of p65 (stained in red) in the nucleus. The central image demonstrates the absence of nuclear p65, suggesting that p65 is degraded when PDLIM2 is present. The image on the right shows that PDLIM2 transports p65 into nuclear bodies. In this image, the red spotty staining represents the nuclear bodies where p65 has accumulated, since this PDLIM2 mutant has impaired ubiquitin ligase activity and intact intranuclear trafficking activity.

Our immune system protects us against microbial pathogens that invade our cells and cause illness. When receptors on the cell surface detect microbes, a cascade of signals and activities within the cell is triggered, resulting in inflammation, which is part of our early defense against pathogens.

Immunologists from the RIKEN Research Center for Allergy and Immunology, Yokohama, and the Harvard School of Public Health, US, recently published a study on the regulation of this system. Without regulation, an unstoppable immune reaction leads to excessive inflammation, which causes conditions such as asthma and arthritis.

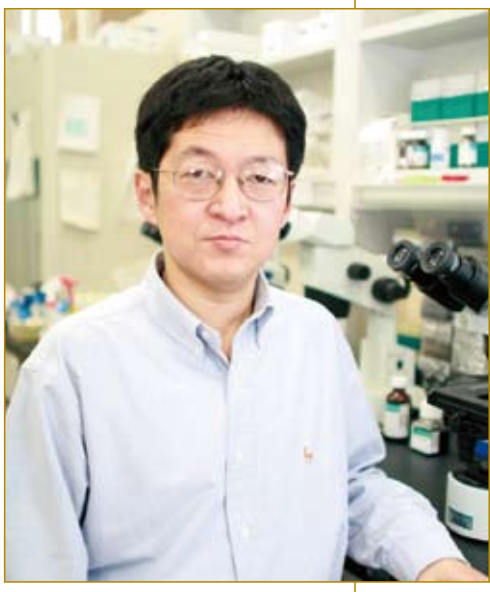
The researchers studied a molecule called NF-κB that contains two different subunits known as p65 and p50 and normally resides in the cytoplasm of cells. When this molecule receives the appropriate signal, it enters the cell nucleus and switches on immunoregulatory genes that encode pro-inflammatory molecules. If the process is not stopped, the immune reaction continues. To terminate this reaction promptly, it is important that the p65 molecule that starts this sequence of events is degraded once it has done its job.

RIKEN's Tsuneyasu Kaisho and his team have identified a pathway that leads to the degradation of p65. Their work shows that the process involves the specific attachment of the protein molecule ubiquitin to p65 followed by transportation of the 'ubiquitinated' p65 to distinct sub-nuclear domains, called nuclear bodies, where it is ultimately degraded by the proteins found there (Figure).

Critically, the researchers have described a factor called PDLIM2 that has two highly important roles in the regulation of the immune response. It helps ubiquitin to bind to p65 and then targets this complex to the appropriate nuclear bodies for degradation.

The team showed that PDLIM2-deficient mouse cells had uncontrolled immune responses due to the constant activity of NF-κB and augmented production of molecules that cause inflammation. *In vivo* studies showed mice lacking PDLIM2 were more sensitive to stimulation of the immune response than mice with normal levels of the molecule.

Developing treatments for inflammatory and autoimmune diseases by modifying the PDLIM2-mediated pathways to terminate NF-κB p65 activation is the future aim of the team. According to team member, Takashi Tanaka, the next step towards this goal is to clarify how PDLIM2 activity itself is regulated. This is very important for developing a way to modify its activity in living cells, he says.



Takashi Tanaka

ORIGINAL RESEARCH PAPER

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

How eating cell 'corpses' reduces inflammation

Specialized immune cells orchestrate proper elimination of dead cells to prevent inflammation

Masato Tanaka

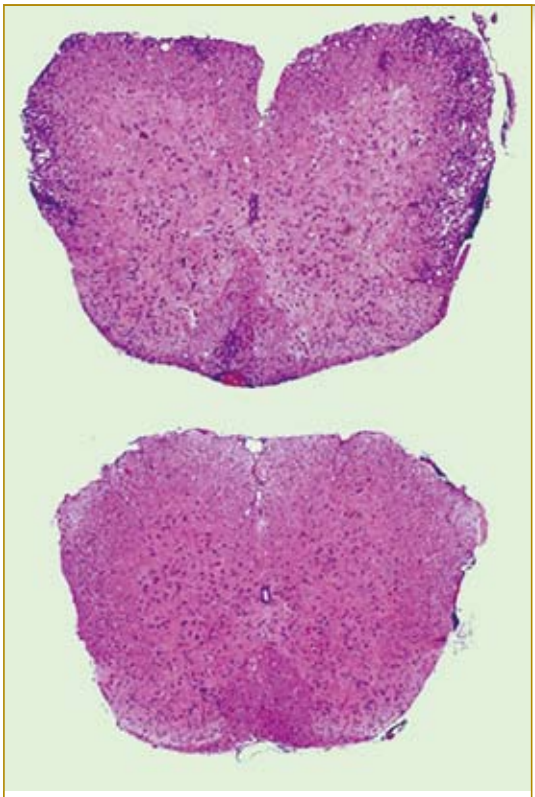


Figure Normal (*top*) and inflamed spinal cord (*bottom*). The multiple sclerosis-like disease occurs when specialized macrophages that prevent inflammation are depleted. © J. Clin. Invest./ American Society for Clinical Investigation /117/2273 (2007)

Reporting in the August issue of *The Journal of Clinical Investigation*, a team of Japanese researchers has found that immune cells called 'marginal zone macrophages' prevent inflammation by promoting the elimination of cells that have just died—so-called cell 'corpses'.

It has been long known that certain types of dead cells can suppress inflammation. A cell 'programmed' to die goes through a tranquil process called apoptosis, whereas traumatically killed cells die by a process called necrosis. Only apoptotic corpses can suppress inflammation.

Led by Masato Tanaka at the RIKEN Research Center for Allergy and Immunology, Yokohama, the team observed that apoptotic corpses injected into experimental mice migrate to specific locations in the spleen and lymph nodes and then disappear—phenomena associated with suppression of experimentally-induced inflammation. Intriguingly, marginal zone macrophages are found in the same locations.

Testing whether the macrophages were important for the disappearance of the corpses and reduced inflammation, the team depleted the macrophages from mice and then injected apoptotic cells. They found that the corpses were present much longer and experimentally-induced brain inflammation could no longer be suppressed (Figure).

Digging deeper to understand this, the team looked at other nearby immune cells and found differences in two types of cells called dendritic cells, one of which was known to suppress inflammation.

Studying how the two types of dendritic cells responded to apoptotic corpses when the macrophages were present or absent, Tanaka's team found that the dendritic cell type known to suppress inflammation could do so only when the macrophages were present. In the absence of the macrophages, the other dendritic cells caused inflammation.

Further observations indicated a difference in the way the dendritic cells responded to the apoptotic corpses—which are normally 'eaten' by the inflammation-suppressing dendritic cells. The team noticed that when the macrophages were absent, the second type of dendritic cells could ingest the apoptotic corpses, which caused inflammation.

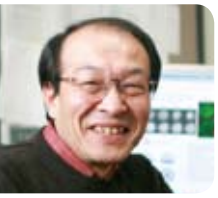
"We are now currently investigating the differences between the two [types of] dendritic cells," says Tanaka. One possibility is that the specialized macrophages transport apoptotic corpses selectively to the dendritic cells that suppress inflammation, thus physically preventing the other type of dendritic cells from promoting inflammation.

Exactly how marginal zone macrophages and two types of dendritic cells effect this complex processing of apoptotic corpses remains unknown, says Tanaka. Nevertheless, the observations are clear and represent a potential interesting avenue of research in causes of inflammation.

Yasunobu Miyake (*left*) and Hitomi Kaise (*right*)

ORIGINAL RESEARCH PAPER

Miyake, Y., Asano, K., Kaise, H., Uemura, M., Nakayama, M. & Tanaka, M. Critical role of macrophages in the marginal zone in the suppression of immune responses to apoptotic cell-associated antigens. *The Journal of Clinical Investigation* 117, 2268–2278 (2007).



Takashi Saito

Signaling simplified

Japanese immunologists find differential usage of essential components of signal pathways in innate and acquired immune responses

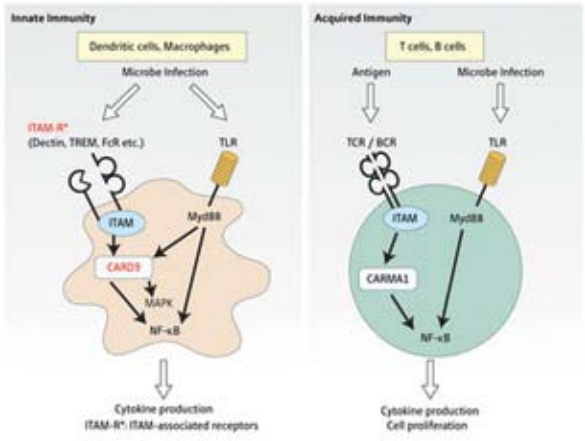


Figure Activation of immune cells through ITAM-receptors. Receptors on the surface of lymphocytes induce signal transduction pathways resulting in the production of cytokines

A team of researchers, led by Takashi Saito from the RIKEN Research Center for Allergy and Immunology in Yokohama, has given clear insights into the way constituent parts of the immune system communicate with each other. In their study recently published in *Nature Immunology*, the team describes a key factor through which urgent chemical messages are routed when the immune response is triggered.

Adaptor proteins CARD9 and CARMA1

Saito and colleagues have concentrated on the adaptor CARD9 by studying a population of mice lacking this protein (Figure). They found that CARD9 is active in myeloid cells and is intrinsic to the signal pathway that results in the innate immune reaction. Many receptors in myeloid cells mediate signals through protein molecules that contain a module called an ITAM (immunoreceptor tyrosine-based activation motif) and one of the myeloid cell receptors for fungus, Dectin-1, contains an ITAM-like motif as part of its structure.

Using the mouse population, the RIKEN team has provided genetic evidence that all myeloid receptors that initiate signals through an ITAM-containing protein need CARD9 and two other adaptor proteins, Bcl-10 and MALT1, to activate the innate immune response. Toll-like receptors do not involve the ITAM motif in their signal pathways. However, the team has demonstrated that toll-like receptor-dependent signaling pathways do converge on CARD9, but then are routed elsewhere.

CARD9 has disparate functions in the ITAM-related and TLR pathways; it links ITAM-related receptors to NF-κB activation and toll-like receptors to another gene-activating molecule MAPK (mitogen-activated protein kinase).

In addition to their study of myeloid cells, the researchers have provided evidence that another adaptor protein, known as CARMA1, acts like CARD9, but in the adaptive immune response in lymphocytes. They concluded this after studying a population of mice lacking CARMA1. The team found that it is essential in lymphocytes but dispensable in the myeloid receptor pathway and thus CARMA1 seems to be the CARD9 counterpart in lymphoid cells. Similarly to CARD9, CARMA1 functions in combination with the adaptors Bcl-10 and MALT1

Immune signal routes

The team has provided genetic evidence for the essential role played by CARD9 in innate immunity. This gives us a simplified picture of the way information is directed through myeloid cells. ITAM-associated receptors transfer their chemical messages through CARD9 to stimulate transcription factor NF-κB activation and TLRs route their signals through CARD9 to trigger MAPK. Both avenues lead ultimately to gene activation and the production of cytokine molecules which promote resistance to and security against infection.

"Therapeutic approaches targeting either the lymphoid CARMA1 complex (L-CBM) or the myeloid CARD9 complex (M-CBM) might provide a strategy for specifically modulating lymphoid versus myeloid cells for the activation or inhibition of their functional responses," Saito says. Such control would ultimately benefit the treatment of devastating, but unfortunately common, illnesses such as rheumatoid arthritis and harmful infectious diseases.

Hiromitsu Hara

ORIGINAL RESEARCH PAPER

Hara, H., Ishihara C., Takeuchi, A., Imanishi, T., Xue, L., Morris, S.W., Inui, M., Takai, T., Shibuya, A., Saijo, S., Iwakura, Y., Ohno, N., Koseki, H., Yoshida, H., Penninger, J., Saito, T. The adaptor protein CARD9 is essential for the activation of myeloid cells through ITAM-associated and Toll-like receptors. *Nature Immunology* 8, 619–629 (2007).

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Identification of a novel intracellular second messenger

Zinc acts as second messenger in the cell to propagate extracellular signals

Toshio Hirano

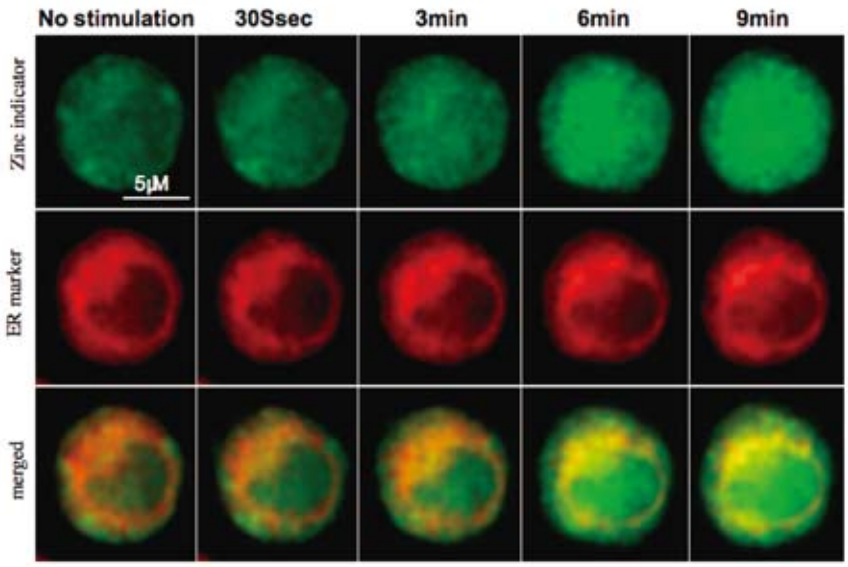


Figure
Bone-marrow derived mast cells were stimulated and intracellular fluorescence signals of zinc ion levels (*green*) and ER (*red*) were assessed.

Researchers from Japan have shown that zinc can be used by the cell to transduce extracellular signals into cellular responses by propagating intracellular signaling pathways.

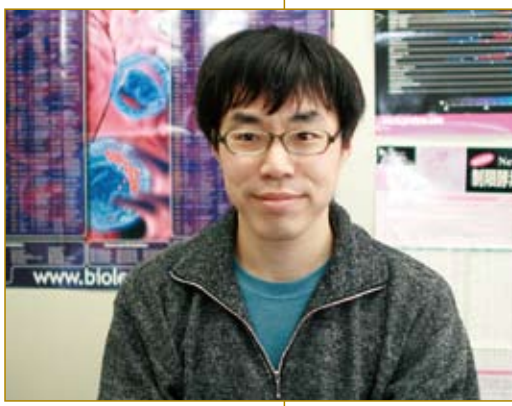
Cells that comprise the tissues of many different systems in the body must respond to extracellular molecular signals, such as hormones, toxins, cytokines, and metabolic by-products, in order to generate an appropriate and coordinated response. These external stimuli are often blocked by the plasma membrane that surrounds these cells, so they rely on so-called 'second messengers' within the cell to propagate the signaling cascade from the cell's exterior. Numerous reports indicate that certain small molecules play this role, the best studied being calcium.

Now Toshio Hirano at the RIKEN Research Center for Allergy and Immunology in Yokohama and his colleagues have added zinc to this list of second messengers. The group's findings elevate our understanding of the biological importance of zinc beyond its previously identified role as a neurotransmitter and a co-factor for protein folding and function.

Working with mast cells, which are immune cells involved in the allergic response, Hirano's group showed that when they mimicked the immune activation of these cells by stimulating a membrane-bound receptor on their surface, a wave of zinc was generated that washed across the cell a few minutes after the stimulation (Figure). They then showed that a rapid influx of calcium from outside the cell was required before this zinc-wave occurred. According to Hirano and his team, the zinc-wave seemed to originate from the endoplasmic reticulum, but they could not rule out other intracellular sources, such as the nucleus.

Investigating further, the researchers showed that one role of the zinc-wave, at least, is to inhibit an important class of enzymes that remove the activation signals from other signaling proteins. This inhibition extends the activation time of these signaling proteins and therefore allows the continuation of the cellular signaling cascade. In the case of the mast cells, this resulted in further expression of IL-6 and TNFα—two key immunoregulatory cytokines that, in turn, can influence the behavior of a number of other cell types.

Hirano hopes to perform future experiments that will determine the molecular mechanisms that coordinate calcium influx with intracellular zinc flow. Also in his sights is identifying other cell types that use zinc as a second messenger and the targets within those cells.



Satoru Yamasaki

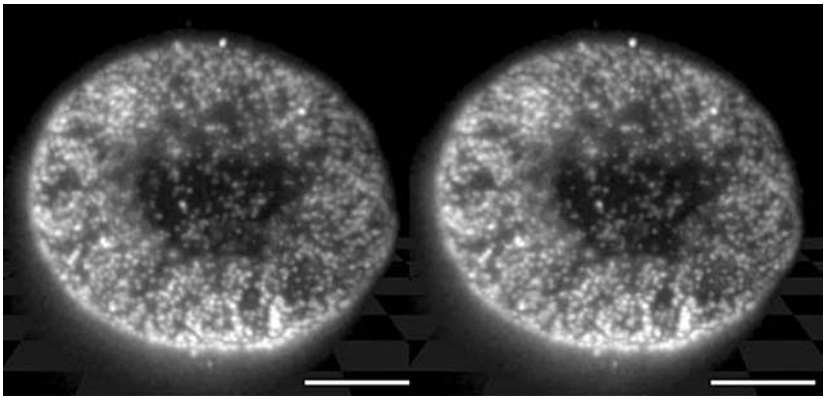
ORIGINAL RESEARCH PAPER

Yamasaki, S., Sakata-Sagawa, K., Hasegawa, A., Suzuki, T., Kabu, K., Sato, E., Kurosaki, T., Yamashita, S., Tokunaga, M., Nishida, K. & Hirano, T. Zinc is a novel intracellular second messenger. *Journal of Cell Biology* 177, 637–645 (2007).

Makio Tokunaga

Looking beneath the surface

A new imaging technique allows scientists to effectively visualize individual molecules within living cells in real-time



Figure

A stereo pair of images demonstrating the three-dimensional reconstruction of the distribution of nuclear pore complexes within the nuclear membrane from a serial set of HILO images. Scale bars = 5.0 μm.

Historically, analysis of the behavior of individual proteins has required the physical destruction of the cells in which they are found. More recently, however, a new generation of microscopy techniques has emerged that make it possible to directly visualize individual fluorescently tagged molecules within the living cell, giving scientists unprecedented capabilities to observe biological processes in their natural context.

"These techniques enable us to visualize molecular dynamics and interactions, to analyze molecular mechanisms quantitatively, and to detect biomolecules with great sensitivity in living cells," explains Makio Tokunaga, an imaging specialist at the RIKEN Research Center for Allergy and Immunology in Yokohama.

One popular technique is total internal reflection fluorescence (TIRF) microscopy, which takes advantage of the physics of refraction to specifically excite fluorescent molecules in the immediate proximity of the microscope objective. Unfortunately, although TIRF is useful for single-molecule visualization, its limited depth of visualization means that it can only observe targets located near the cell surface.

In order to overcome this limitation, Tokunaga's team developed a new variant of TIRF, which they term highly-inclined and laminated optical sheet (HILO) microscopy. HILO makes use of an alternative refraction strategy, in which the laser beam that illuminates the sample is converted into a thin sheet that passes through the center of the specimen. HILO is capable of imaging targets at depths of tens of microns — well beyond the cell membrane — and can be used to generate three-dimensional reconstructions via the computerized assembly of multiple scans into a single image.

In testing out HILO, the researchers started big — relatively — by imaging nuclear pore complexes (NPCs), the massive multiprotein assemblies that act as the gateway between the nucleus and cytoplasm. The researchers obtained remarkably clear images of these complexes, with virtually no background haze (Figure). They subsequently used HILO to visualize the movement of fluorescently labeled importin β, a protein that shuttles other molecules into the nucleus via the NPCs, generating videos that show the kinetics of nuclear import at high resolution and in real-time.

Tokunaga sees HILO as a promising tool for immunology research. "We intend to visualize signaling pathways from the cell membrane to the nucleus after stimulation using single-molecule microscopy," he says. By combining this real-time imaging data with sophisticated computational modeling strategies, it should be possible to gain unprecedented insight into complex cellular pathways. "We aim to open up new frontiers for understanding immune cells as molecular systems," concludes Tokunaga.

Kumiko Sakata-Sogawa

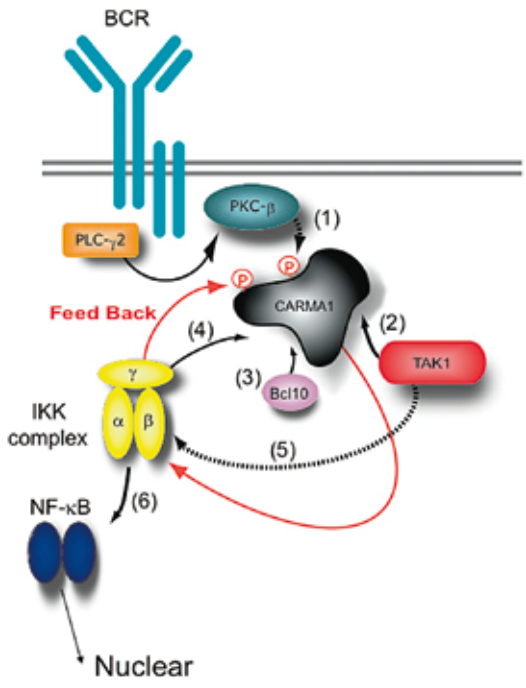
ORIGINAL RESEARCH PAPER

Tokunaga, M., Imamoto, N. & Sakata-Sogawa, K. Highly inclined thin illumination enables clear single-molecule imaging in cells. *Nature Methods* 5, 159–161 (2008).

Regulating antibody production

New insight into an important part of the immune response could lead to treatments for immune disorders

Tomohiro Kurosaki



Figure

A model of BCR-mediated NF-κB activation.
Stimulation of BCR leads to activation of proximal protein tyrosine kinase inducing Syk and Btk. Btk phosphorylates several tyrosine residues on PLC-γ2, and subsequently activates PKCβ. Activated PKCβ phosphorylates CARMA1 (1) directly or indirectly, which is able to recruit TAK1 to the phosphorylated CARMA1 (2). Meanwhile, the IKK complex, probably through the Bcl10 complex, is recruited to the phosphoryated CARMA1 (3 and 4). These interactions (CARMA1-IKK and CARMA1-TAK1) contribute to the access of two key protein kinases, TAK1 and IKK, leading to activation of the IKK complex (5). IKK accelerates nuclear translocation of NF-κB and activation of gene translations (6) and also induce positive feedback loop by phosphorylating upstream CARMA1.

A RIKEN research group has puzzled out the molecular details of a key part of the complex communication network that regulates the production of antibodies by the immune system. The findings are an important step towards understanding immune disorders and how some types of tumors are initiated, as well as developing treatments for these conditions.

The work concentrates on the molecular events that occur immediately after an immune system B-cell is stimulated by a foreign body or antigen. From previous studies it is known that antigens bind with a surface protein known as a B-cell receptor (BCR) and that this triggers a complicated cascade of biochemical reactions involved in the immune response and its regulation.

One important biochemical pathway in this response involves two families of proteins called nuclear factor-κB (NFκB) and inhibitor of nuclear factor-κB kinase (IKK). Generally NFκBs are bound in the body of the cell to IKKs. When a BCR is triggered by an antigen, however, one consequence is the phosphorylation or addition of phosphate groups to IKKs which activates them to break up. This liberates NFκBs which can then enter the nucleus and interact with specific genes to trigger the immune response.

In a paper published recently in the *Journal of Experimental Medicine*, researchers from RIKEN's Research Center for Allergy and Immunology in Yokohama led by Tomohiro Kurosaki describe work in which they were able to unravel the interactions of these molecules more precisely.

Hisaaki Shinohara

Two of the compounds known to be involved in the phosphorylation of specific amino acids in IKKs are protein kinase C β (PKCβ) and a complex called CBM built of three proteins: CARMA1, Bcl10 and MALT1. Using antibodies engineered to bind to and pinpoint amino acids which have phosphates added, the researchers found that PKCβ not only initiates the addition of phosphates to IKKs, but also adds phosphate directly to CARMA1 and this activates it to build the CBM complex. The researchers also found that one of the components of the IKK complex, IKKβ, phosphorylates CARMA1 as well. IKKβ therefore plays a positive feedback role when the IKK complex degenerates, amplifying the break down process.

"Both prolonged activation and inhibition of NFκBs are able at times to induce tumors," says Hisaaki Shinohara, first author on the paper. "So the molecules included in this feedback loop provide good candidates as cancer drug targets."

ORIGINAL RESEARCH PAPER

Shinohara, H., Maeda, S., Watarai, H. & Kurosaki, T. IκB kinase β-induced phosphorylation of CARMA1 contributes to CARMA1-Bcl10-MALT1 complex formation in B cells. *Journal of Experimental Medicine* 204, 3285–3293 (2007).

Tomohiro Kurosaki

Immune cells stimulated by calcium levels

Calcium sensors may play a pivotal role in the allergic response

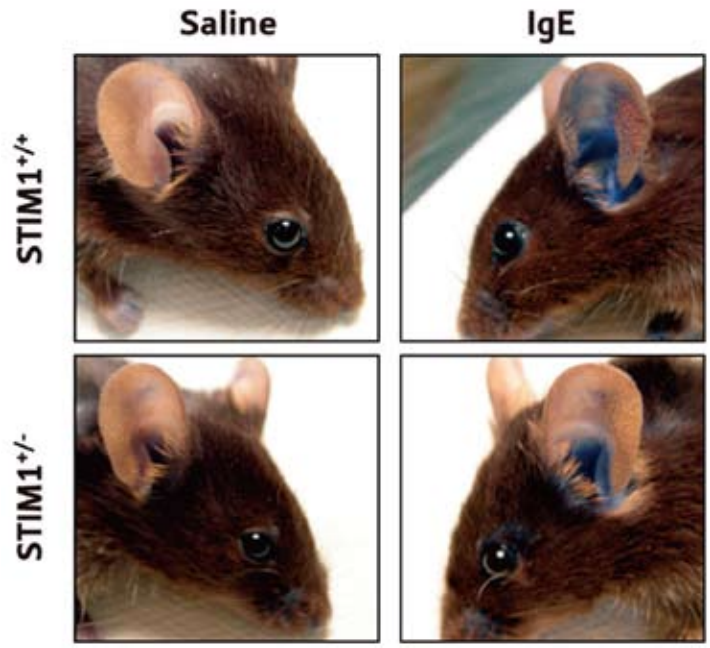


Figure Comparison of in vivo anaphylactic responses in mice with either two functional copies of the STIM1 gene ($Stim1^{+/+}$) or one functional copy ($Stim1^{+/-}$). One ear was injected with IgE antibodies against a specific antigen. The other ear was injected with saline as a control. After 16–18 h the antigen was administered intravenously together with a blue dye and the amount of dye leaking out into the ear tissue was measured.

The team, led by Tomohiro Kurosaki at the RIKEN Research Center for Allergy and Immunology in Yokohama, is unraveling the role played by calcium ion influx in mast cells and other immune system cells. They focused their work on STIM1: a protein located on the endoplasmic reticulum, a membranous structure within the cell.

Previous studies by Kurosaki and his colleagues have demonstrated that the STIM1 protein physically relocates from the endoplasmic reticulum to regions just under the cell membrane where it is involved in the activation of specific calcium channels known as calcium release-activated channels. This process is known as store-operated calcium influx and is an important mechanism in the activation of mast cells.

Now, using a genetically engineered mouse strain that does not express the STIM1 protein, the researchers have shown that the calcium influx mediated by stimulation of specific receptors, known as FcεR1, on mast cells was severely impaired. However, this genetic mutation was lethal with few fetuses surviving beyond birth.

To circumvent the lethality of the mutant mice, the researchers used fetal liver-derived mast cells FLMCs from mice lacking STIM1 to examine calcium influx. After depleting calcium stores of FLMCs, extracellular calcium was added back to the culture. Calcium influx in the FLMCs lacking STIM1 was significantly suppressed compared to levels seen in normal FLMCs. A similar result was seen in FLMCs following stimulation of the FcεR1 receptors, suggesting that STIM1-dependent calcium influx was the main mechanism used during FcεR1 signaling.

Kurosaki and team have also confirmed the function of STIM1 on the allergic response *in vivo*. In an experiment using mice with only one functional copy of the gene, the researchers were able to show that the sensitivity of immediate-type allergic responses was diminished, when compared to the response exhibited by mice with two functional copies.

In sensitized mice with only one functional copy of the STIM1 gene, the amount of dye entering the tissues was significantly lower than in mice with two copies (Figure), suggesting that the protein is required for antigen-induced, mast cell-mediated anaphylactic responses in vivo.

There is no doubt now that the protein plays an important role in regulating intracellular calcium levels in mast cells. “STIM1 may represent a new therapeutic target for allergic diseases,” team-member Yoshihiro Baba says.

Yoshihiro Baba (*left*) and Yoko Fujii (*right*)

ORIGINAL RESEARCH PAPER

Baba, Y., Nishida, K.,Fujii, Y., Hirano, T., Hikida, M. & Kurosaki, T. Essential function for the calcium sensor STIM1 in mast cell activation and anaphylactic responses. *Nature Immunology* 9, 81–88 (2008).

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Silencing allergic inflammation

Researchers reveal a new step in the regulation of allergic mediators

Ichiro Taniuchi

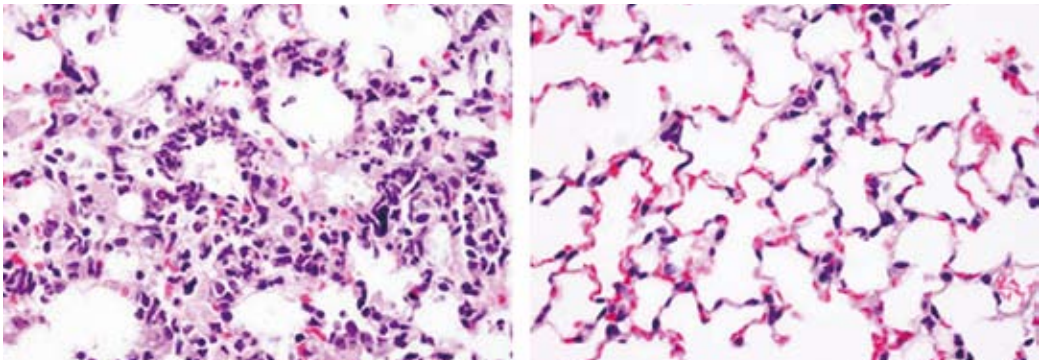


Figure Spontaneous airway infiltration was observed in mice lacking the CBFβ protein (*left*) but not in wild-type (*right*) mice.

New work by Japanese scientists shows that a family of DNA-binding complexes prevents secretion of factors that trigger allergic inflammation. These complexes, which consist of so-called Runx proteins that bind to specific DNA sequences and the Cbfβ protein, exert anti-allergic effects in immune cells called helper T lymphocytes.

During immune responses, T lymphocytes acquire the capacity to produce different types of soluble factors called cytokines. Type 1 (T_H1) cytokines help eradicate intracellular bacteria and viruses, but if not controlled can exacerbate autoimmune diseases. Type 2 (T_H2) cytokines help combat extracellular bacteria and parasites, but when produced in excess can worsen allergies and asthma.

T lymphocytes produce either T_H1 or T_H2 cytokines. These mutually exclusive cellular 'fates' are shaped and maintained by distinct sets of transcription factors that bind to DNA sequences that are dedicated to enhancing or silencing expression of individual cytokine genes.

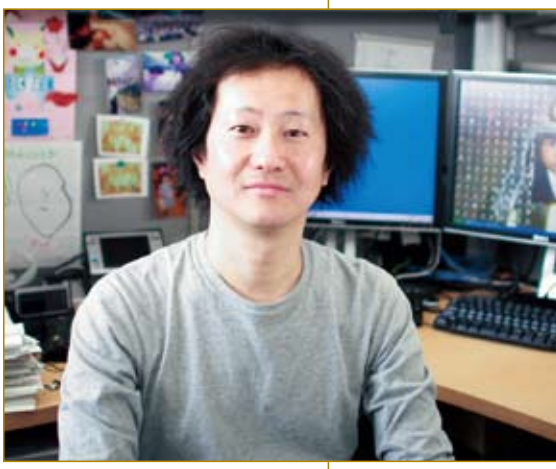
A team led by Ichiro Taniuchi, a scientist at the RIKEN Research Center for Allergy and Immunology in Yokohama, has determined that Runx complexes, which dampen expression of other genes in T lymphocytes, bind to and activate the silencer of *Il4*, a T_H2 cytokine gene. Their findings were published in a recent issue of *The Journal of Experimental Medicine*.

Using gene-targeting techniques, the researchers generated mice lacking either Runx3, or Cbfβ, specifically in T lymphocytes. Mice lacking CBFβ exhibited spontaneous lung infiltration (Figure). Mice lacking Runx3 displayed milder versions of these asthma-like symptoms.

Incubation with T_H1-promoting cytokines 'skews' naive T cells towards a T_H1 fate and silences T_H2 cytokine gene expression. CBFβ-mutant T cells, and, to a lesser extent their Runx3-deficient counterparts, were resistant to T_H1 polarization and failed to suppress T_H2 cytokine production.

Runx complexes bind directly to the *Il4* silencer in un-polarized T cells and T_H1 cells, but not in T_H2 cells. Forced expression of GATA3, a factor known to promote the T_H2 cell fate, in T_H1 cells prevented Runx3 binding to the *Il4* silencer. Precisely how GATA3 'expels' Runx complexes from the *Il4* silencer gene remains to be investigated.

Encouragingly, as human RUNX3 lies adjacent to a cluster of genes thought to influence asthma susceptibility in humans, these data may hold clinical significance. "The *Il4* gene might not be the only immunologically relevant target of Runx complexes. Further studies focusing on the roles of Runx complexes in mice may provide further insight into the molecular pathogenesis of allergic and autoimmune human diseases," says Taniuchi.



Yoshinori Naoe

ORIGINAL RESEARCH PAPER

Naoe, Y., Setoguchi, R., Akiyama, K., Muroi, S., Kuroda, M., Hatam, F., Littman, D.R. & Taniuchi, I. Repression of interleukin-4 in T helper type 1 cells by Runx/Cbfβ binding to the Il4 silencer. *The Journal of Experimental Medicine* 204, 1749–1755 (2007).

Ichiro Taniuchi

How cells switch from killers to helpers

Researchers illuminate a pivotal network of closely linked checkpoints controlling the destiny of key immune cells.

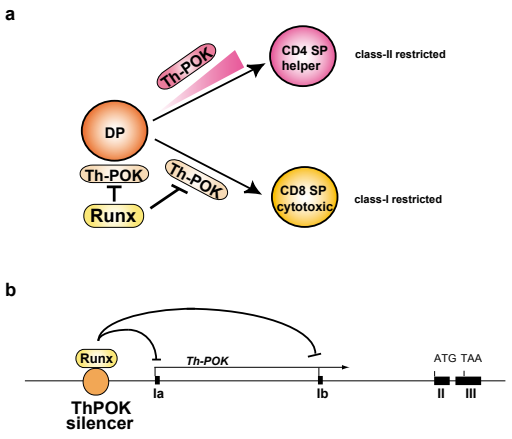


Figure Model of *Th-POK* gene regulation during thymocyte differentiation. (a) Expression of *Th-POK* gene is actively repressed by the Runx complexes in DP thymocytes and in developing thymocytes toward cytotoxic-lineage. (b) Runx complexes associate with a transcriptional silencer, the ThPOK silencer that is essential to repress *Th-POK* expression in DP thymocytes and in cytotoxic-linage cells.

A cell's fate - how it progresses from an immature state into one that is specialized for a particular purpose - is a vital but poorly understood area of biology. In a critical and exciting breakthrough in this field, a team of scientists from the RIKEN Research Center for Allergy and Immunology, the Japan Science and Technology Agency's Precursory Research for Embryonic Science and Technology (PRESTO), and the Kyoto Prefectural University of Medicine, have described that newly uncovered details of how special immune cells commit to a specific lineage.

In the mammalian thymus, precursor white blood cells mature and finally develop into 'T' (for thymus-derived) cells. As integral components of the vertebrate body's elaborate adaptive immune system, T cells must constantly probe their environment, using a variety of 'CD' proteins and the T cell receptor (TCR) expressed on the cell surface, for the presence of invading pathogens.

TCR and CD proteins work closely together to identify major histocompatibility complex (MHC) proteins, which are specialized guidepost signals displayed on all cell surfaces. Cells communicate their status to the immune system by using MHC proteins to advertise antigens - processed protein molecules that stimulate an immune response - through which the cells exchange critical information, allowing the immune system to distinguish friend from foe.

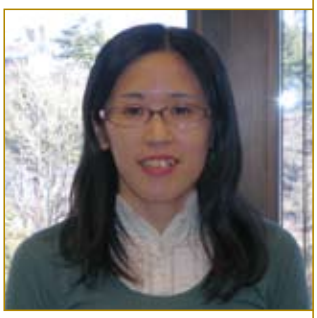
All immature thymocytes express two special CD proteins, CD4 and CD8, but the two main subsets of functionally distinct mature T cells are defined by which one of these proteins is eventually NOT displayed. Hence, $CD4^+CD8^-$ helper T cells - which warn the immune system of pathogen invasions and also help to harmonize a coordinated immune response - only express CD4, whereas $CD4^-CD8^+$ killer T cells - which actually kill cells that have been infiltrated - express only CD8. Two versions of MHC proteins, class I and class II, are important drivers for this critical cell fate determination but much remains unknown.

Th-POK - a DNA-binding protein that regulates gene expression - was recently identified as the main lineage-determining factor controlling T cell differentiation. Cells that engage class II MHC complexes activate *Th-POK* expression, which in turn induces CD4 production while simultaneously suppressing CD8 production, thereby committing the cells to the T helper line. The team has now linked other key mechanistic components to this Th-POK switch.

The group had previously identified another family of DNA-binding proteins, called Runx, which repress CD4 expression in immature and mature killer T cells, as important determinants of T cell fate. Knowing numbers of that killer T cell are dramatically reduced when Runx proteins are absent or inactivated, the scientists sought to understand how this happens by examining genetically engineered mice that expressed mutant Runx protein but not MHC class II proteins. The majority of MHC class I-restricted cells in these special mice differentiated into $CD4^+CD8^-$ T cells that carry the $CD4^+$ T helper signature and were also functionally similar to T helper cells.

Th-POK gene expression levels were elevated in these Runx mutant mice and because Runx protein complexes bound selectively to specific DNA sequences lying upstream of *Th-POK*, the Runx proteins must somehow mediate the repression of *Th-POK*. The team created mutant mice that were missing distinct portions of this putative *Th-POK* regulatory region and found that these mutant mice had much higher *Th-POK* expression levels than normal mice did. The scientists termed this control region the *Th-POK* silencer, concluding that it is a key determinant for regulating *Th-POK* expression pattern, and that this silencer activity is in turn dependent on binding of Runx complexes.

The researchers posit that other molecular players are needed to effect the *Th-POK* silencer activity and that there is a complex antagonistic interplay between *Th-POK* and Runx elements. Future studies will no doubt shed more light on the genetic programming that refines and defines our immune systems.



Ruka Setoguchi

ORIGINAL RESEARCH PAPER

Setoguchi, R., Tachibana, M., Naoe, Y., Muroi, S., Akiyama, K., Tezuka, C., Okuda, T., Taniuchi, I. Repression of the Transcription Factor Th-POK by Runx Complexes in Cytotoxic T Cell Development. *Science*, 319: 816-819, 2008

RIKEN Press release
http://www.riken.jp/engn/r-world/info/release/press/2008/080208_2/index.html

Dampening dangerous inflammation

Immune therapy using unique immune cells significantly reduces inflammation in mouse models of human disease

Katsuaki Sato

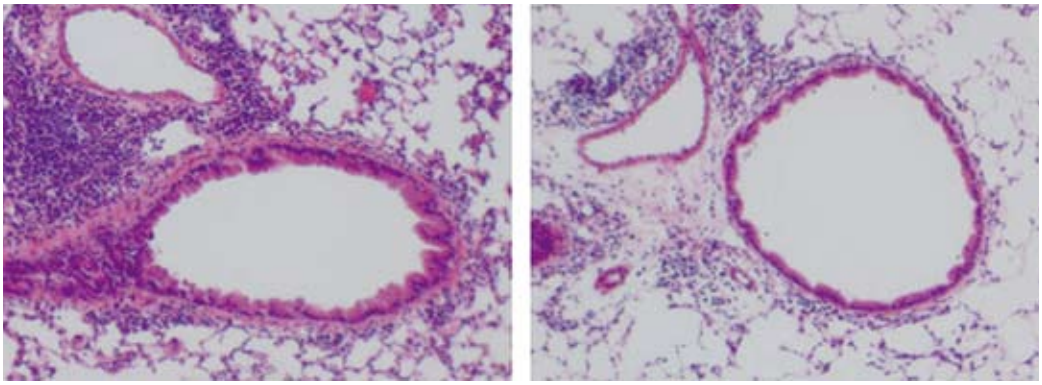


Figure Treatment with dendritic cells clears the airways. A section of an inflamed mouse lung shows numerous immune cells stained in purple that lead to airway constriction (*left*). Treatment with regulatory dendritic cells significantly reduces the inflammation, leading to open airways (*right*).

A team of Japanese researchers is working to find better treatments for chronic inflammation. New work by the team demonstrates that conditions such as lethal disease associated with tissue and organ transplantation, and asthma, can be successfully treated with specialized immune cells to reduce inflammation.

Led by Katsuaki Sato at the RIKEN Research Center for Allergy and Immunology in Yokohama, the team studied dendritic cells of the immune system, so called because of the many 'dendrites', or branched projections, on their surface. Dendritic cells initiate immune responses by directly communicating with other immune cells. One outcome of such communication is the production of 'guardian' lymphocytes that keep the immune system in balance; referred to as 'regulatory T cells', these lymphocytes are increasingly seen as critical players in a myriad of diseases.

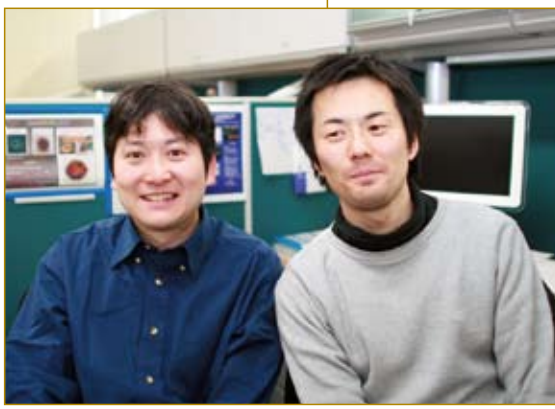
Sato's team has demonstrated that therapeutic treatment of mice with specially conditioned dendritic cells significantly reduces inflammation. Referring to such dendritic cells as 'regulatory dendritic cells' (or simply 'DC_{regs}'), the team—in a series of articles in the past few years—has described the protective effect of immunotherapy with DC_{regs} in mouse models of human disease.

As Sato explains, "to exploit a novel immunotherapeutic strategy using dendritic cells for immunopathogenic diseases, we have tried to establish use of DC_{regs} with a potent immuno-regulatory property even under inflammatory conditions." By focusing on ameliorating inflammation when it is chronically present and especially difficult to treat, the team's work is different from other attempts to maximize the efficacy of immune therapy.

Using mouse models of human disease, Sato's team studied bone-marrow transplantation rejection for graft-versus-host disease (GVHD) and lung-airway inflammation for asthma. DCr_{egs} produced in the laboratory and then injected into mice with these diseases demonstrated significantly reduced inflammation (Figure). Looking closely at the recipient mice, the team noted that the treatment was associated with increased numbers of regulatory T cells—key suppressors of inflammation.

According to Sato, the next key step is to clarify the molecular mechanisms responsible for the production of regulatory T cells by DC_{regs}. Longer term, Sato is clearly set on the goal of treating human diseases with DC_{regs}: "we have recently established the methods to prepare a large number of human DC_{regs} for clinical application."

Although not yet ready for the clinic, immunotherapy with DC_{regs} has clear potential thanks to the work reported by Sato and his team that demonstrates the unique power of regulatory dendritic cells to reign in lethal inflammation.

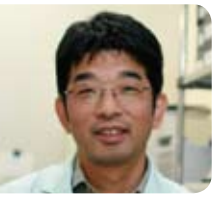


Shigeharu Fujita *(left)* and Tomohiro Fukaya *(right)*

ORIGINAL RESEARCH PAPER

Fujita, S., Sato, Y., Sato, K., Eizumi, K., Fukaya, T., Kubo, M., Yamashita, N. & Sato, K. Regulatory dendritic cells protect against cutaneous chronic graft-versus-host disease mediated through $CD4^+CD25^+Foxp3^+$ regulatory T cells. *Blood* 110, 3793–3803 (2007).

Fujita, S., Yamashita, N., Ishii, Y., Sato, Y., Sato, K., Eizumi, K., Fukaya, T., Nozawa, R., Takamoto, Y., Yamashita, N., Taniguchi, M. & Sato, K. Regulatory dendritic cells protect against allergic airway inflammation in a murine asthmatic model. *Journal of Allergy and Clinical Immunology* 121, 95–104 (2008).



Shin-ichiro Fujii

On the road to a vaccine against tumors

Researchers find how to generate an immune response in mice

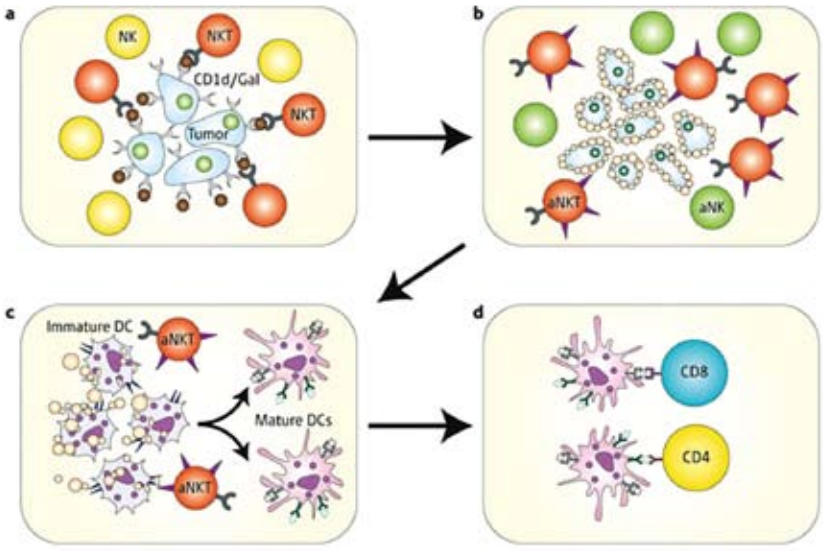


Figure Mechanism of the mouse immune response against tumors. (a) Tumor/Gal activates NKT/NK cells. (b) The activated NKT/NK cells then kill tumor cells. (c) Next, DCs engulf tumor debris and cross-present on CD1d to NKT cells, which matures the DCs. (d) Mature antigen-capturing DCs induce long-lived, adaptive T-cell immunity. Reproduced from Fujii, S. et al. Immunological Reviews 220, 183–198 (2007). Copyright (2007) with permission from Blackwell Munksgaard

A research team from RIKEN's Research Center for Allergy and Immunology (RCAI) in Yokohama has discovered a means of inducing persistent immunity to tumors in mice. In the long term, the work could lead to a vaccine against certain tumors in people.

Unlike infectious organisms and foreign tissue, tumor cells do not elicit a powerful immune response naturally. In particular, tumors do not activate the production of immune $CD4^+$ and $CD8^+$ T-cells geared to fighting them. This typically needs two signals. In addition to a compound known as an antigen which is specific to the tumor and reacts with a T-cell receptor when presented by dendritic cells (DCs), it also requires a co-stimulatory molecule that tumors appear to lack.

In a recent paper in the *Journal of Experimental Medicine*, the research team—comprising members from RCAI and The Rockefeller University in New York—detailed a method for inducing immunity in mice to four common tumors lasting up to 12 months.

A glycolipid compound derived from marine sponges known as α-galactosylceramide (α-GalCer) can activate the immune system's natural killer (NK) and natural killer T-(NKT) cells against tumors, but alone does not protect mice from cancers such as B16 melanoma. The researchers found, however, that a low dose of B16 tumor cells loaded with α-GalCer and injected intravenously will induce a protective immune response to B16 melanoma. The same was true for three other mouse tumors which normally generated a poor immune response.

To trace what was taking place, the team tracked labeled α-GalCer-loaded tumor cells under the confocal microscope. They found that α-GalCer did indeed activate NK and NKT cells to kill tumor cells (Figure a, b). Debris from the dead cells was captured by DCs in the spleen. These DCs then changed or matured to mount an immune response by presenting tumor antigen peptide in such a way as to stimulate the production $CD4^+$ and $CD8^+$ T-cells against that particular tumor (cross-presentation 1) (Figure c). In addition, the mature DCs presented the original α-GalCer to NKT cells again, stimulating further response (cross-presentation 2) (Figure d). Immunity against individual tumors persisted for at least six months.

"We now have a mouse model for generating an immune response to tumors," says project coordinator Shin-ichiro Fujii. "We can use it to focus on questions of basic science, such as what triggers the immunity. We would also like to extend the research into human patients."

Kanako Shimizu

ORIGINAL RESEARCH PAPER

Shimizu, K., Kurosawa, Y., Taniguchi, M., Steinman, R.M. & Fujii, S. Cross-presentation of glycolipid from tumor cells loaded with α-galactosylceramide leads to potent and long-lived T cell-mediated immunity via dendritic cells. *Journal of Experimental Medicine* 204, 2641–2653 (2007).

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http://www.rikenresearch.riken.jp/research/381/

Understanding cancer in mice and men

A new mouse model of human leukemia may provide fresh insights on the genesis of the disease

Fumihiko Ishikawa

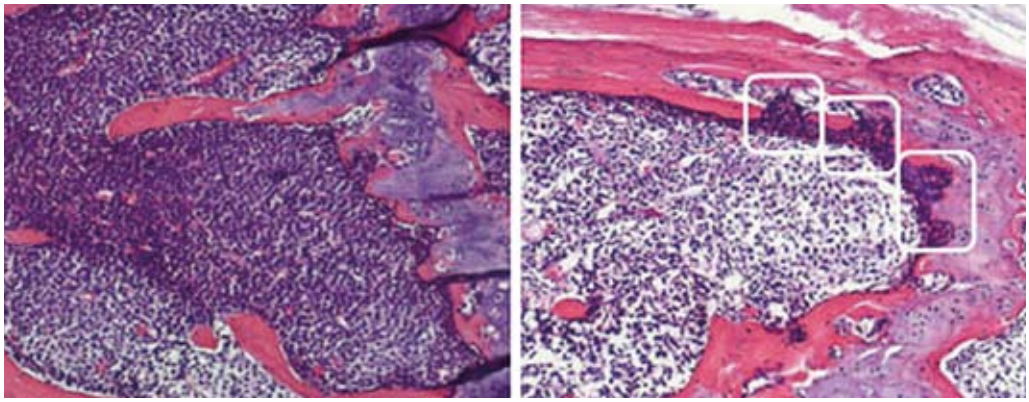

Figure Bone sections derived from AML-engrafted mice before (*left*) and after (*right*) chemotherapy.

Japanese and American researchers have used a mouse model engrafted with human cells to characterize a population of cancerous stem cells that gives rise to acute myelogenous leukemia (AML).

The cells are haematopoietic stem cells, a subset of bone marrow-derived cells that gives rise to essentially all of the cell types in the blood and immune systems. When isolated from patients with AML, the cells are called leukemic stem (LS) cells, and produce the immature leukemic cells characteristic of this disease.

The team, based at the RIKEN Research Center for Allergy and Immunology in Yokohama, developed the mouse model to better study the pathogenic mechanisms leading to the development of human leukemia. The model uses as recipients a strain of mice with a severe immunodeficiency that have been further compromised by a mutation that inactivates a major subset of the immune system's signaling molecules. These mice cannot recognize human cells as 'non-self' and by using newborn mice for the engraftment of the human cells, a more robust and longer-lived model is created.

Initially, the researchers demonstrated that engrafted human LS cells homed to the bone marrow and that these cells were capable of both self-renewal and differentiation into non-stem leukemic cells.

Antibody staining of bone sections showed that the LS cells specifically homed to and engrafted within the micro-environmental niche at the endosteum, a layer of connective tissue on the inner surface of the bone cavity. The presence of the LS cells suppressed normal formation of new blood cells, a phenomenon also observed in AML patients.

In addition, the LS cells were resistant to the cytotoxic agent Ara-C, due to the majority of them being in the quiescent (G0) phase of the cell cycle. The researchers believe this explains why AML relapse after chemotherapy is common—non-stem leukemic cells, but not LS cells, are eliminated by cell cycle-dependent cytotoxic agents used to treat the disease (Figure).

Finally, genetic analysis of the engrafted LS cells showed they retained characteristic gene expression patterns, even after serial transplantation.

According to principal investigator Fumihiko Ishikawa, the retention of phenotype, function and gene expression means the model will be a useful tool. "This xenotransplant model will be helpful for developing a cell-bank for human primary AML stem cells and for testing safety and efficacy of various treatment modalities for AML," he notes. "It has enabled us to identify the major reason and mechanism for AML relapse."

Yoriko Saito (*top left*), Nahoko Suzuki (*top right*), and Shuro Yoshida (*bottom*)

ORIGINAL RESEARCH PAPER

Ishikawa, F., Yoshida, S., Saito, Y., Hijikata, A., Kitamura, H., Tanaka, S., Nakamura, R., Tanaka, T., Tomiyama, H., Saito, N., et al. Chemotherapy-resistant human AML stem cells home to and engraft within the bone-marrow endosteal region. *Nature Biotechnology* 25, 1315–1321 (2007).

Award Winners 2007

Shohei Hori and Sho Yamasaki received the Research Encouragement Award of the Japanese Society for Immunology


01


02

During its 37th annual meeting in November 2007, the Japanese Society for Immunology awarded Research Encouragement Awards to Dr. Shohei Hori (Leader, Research Unit for Immune Homeostasis) (Photo 1) and Dr. Sho Yamasaki (Senior Scientist, Lab. for Cell Signaling) (Photo 2).

This annual award is given to up to five young investigators who conduct distinguished immunological studies. Dr. Hori was awarded for his discovery of Foxp3, the master gene of regulatory T cells, and homeostatic proliferation of regulatory T cells in peripheral tissues. Dr. Sho Yamasaki was awarded for his research on the molecular mechanisms of TCR response mediated by ITAM receptor.

Shigeharu Fujita received the Academic Encouragement Award of Japanese Dendritic Cell Society and the Jury's Special Award of the Tokyo Reseach Forum on Respiratory Organs


03

During its 18th meeting, the Japanese dendritic cell society (May 25, 2007) presented its Academic Encouragement Award to Dr. Shigeharu Fujita (Photo 3) (Junior Research Associate, Lab. for Dendritic Cell Immunobiology) for his oral presentation "Impact of immunostimulatory and regulatory DCs on T cell-polarization in the control of T_H2-mediated allergic immunity". This award is given every year to one society member who has made a significant research contribution in the area of dendritic cells.

Dr. Fujita also received the Jury's Special Award at the 4th Tokyo Research Forum on Respiratory Organs on Nov. 17, 2007.

Masashi Ebisawa received The Melchers' Travel Award of the Japanese Society for Immunology


04

Mr. Masashi Ebisawa (Photo 4), a graduate student in the Lab. for Epithelial Immunology, received The Melcher's Travel Award on Nov. 21 at the 37th meeting of Japanese Society for Immunology. Mr. Ebisawa received the award for his research on "Identification of a $CD11c^+$ $CD19^+$ unique cell population in the mucosa-associated lymphoid tissues: A potential role in M-cell differentiation".

This award supports one or several promising young Japanese scientists who would like to attend the Annual Meeting of the Japanese Society of Immunology, German Society of Immunology, German-Japan Immunology Meeting, or the International Congress of Immunology to present their work. Dr. Fritz Melchers, the former director of Basel Institute of Immunology (BII), and his wife donated this prize in recognition of the outstanding activity of the Japanese alumni at the BII.

Outstanding Contribution of the Year

RIKEN RCAI's Outstanding Contribution of the Year Award was established in 2006 to recognize staff members who made the most outstanding contributions to the Center. The awardees are determined by the Director and receive trophies and an honorarium. In order to celebrate young people's efforts through this award and to develop the potential of young RCAI investigators, the Director and Group Directors agreed this year to exclude themselves from consideration for this award. During the awards ceremony on March 14, Dr. Taniguchi said that it was rewarding for him this year to see the remarkable progress of the young team and unit leaders that is reflected in the number of outstanding publications. Among those young leaders, Drs. Tsuneyasu Kaisho (Photo 1), Ichiro Taniuchi (Photo 2), and Hiroshi Kawamoto (Photo 3) received the award. The Director also mentioned one more area of important progress this year, the launch of the Primary Immunodeficiencies Database Japan by RCAI in collaboration with Kazusa DNA Research Institute and 13 domestic universities. This activity was recognized by the American NPO, the Jeffrey Model Foundation and, as a result of a contribution from this organization, the RIKEN Jeffrey Model Diagnostic and Research Center for Primary Immunodeficiencies was established within RCAI last January. RCAI's IT team lead by Mr. Yasuaki Murahashi (engineer) (Photo 4) received the award for their contribution to establish the database.

Dr. Tsuneyasu Kaisho, the leader of the Laboratory for Host Defense received the award for his research elucidating the signaling mechanisms of plasmacytoid dendritic cells.

The plasmacytoid dendritic cell is a specialized type of dendritic cell that produces large amounts of anti-viral cytokines called type I interferons when it encounters virus. Plasmacytoid dendritic cells sense and bind to virus-derived nucleic acid components through two types of toll-like receptors (TLR), TLR7 and TLR9. It was known that after the activation of TLR7 or 9, a TLR-associated molecule called MyD88 activates a transcription factor, Interferon Regulatory Factor-7 (IRF-7) through an unknown signaling cascade, thus inducing IFNα production. However, what actually activates IRF-7 was unknown.

Dr. Kaisho took note of a cytosolic protein known as I kappa B kinase (IKK). The IKK family plays an important role in the production of interferon and inflammatory cytokines by dendritic cells. Out of four members in the family, three had already been shown to play a role in innate immunity. The other one was IKKα. "IKKα was known to be important for adaptive immunity. People used to think that this was all IKKα did, so they didn't study it in the context of innate immune reactions. However, we thought that IKKα also played a role in innate immune reactions, like the other IKK molecules."

His team isolated plasmacytoid dendritic cells from IKKα knock-out mice and stimulated them with nucleic acids. They found that, although proinflammatory cytokines were induced, much less IFNα was produced, which indicated that IKKα must be especially important in the production of IFNα. The next question was, how is it important?

Photo 1 Dr. Tsuneyasu Kaisho

Dr. Kaisho conducted a series of experiments using an inactive form of IKKα. Through the experiments, he found that inactivation of IKKα inhibits MyD88-induced IFNα gene activation, and he further proved that IKKα actually combines with IRF-7 to control the production of IFNα (Hoshino et al.. *Nature,* 2006). " We discovered that IKKα is the molecule that activates IRF-7, and that the reason less IFNα was produced in the knockout mice was that IRF-7 hadn't been activated."

His team recently identified another important mechanism that regulates production of inflammatory cytokines. They

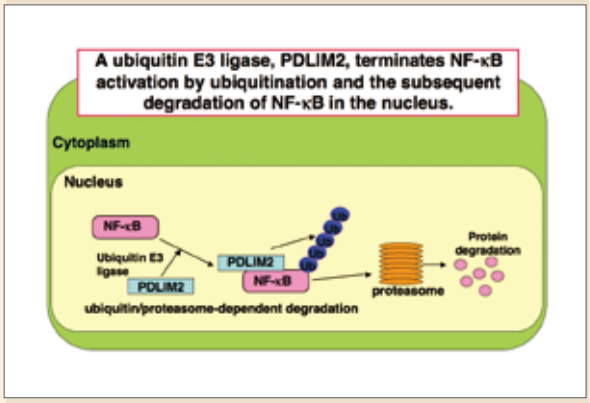

Figure 1 Model of NF-κB activation by PDLIM2

found a factor called PDLIM2 that leads the degradation of Nuclear Factor κB (NF-κB), an important transcription factor for production of inflammatory cytokines. They showed that PDLIM2 attaches ubiquitin to NF-κB and that ubiquitinated NF-κB is transported to distinct sub-nuclear domains called promyelocytic leukemia protein nuclear bodies, where it is ultimately degraded (Figure 1). (Tanaka et al. *Nature Immunol.* 2007)

"It's a pleasure for me to dig up interesting treasure, biologically important truth, among lots of garbage," Dr. Kaisho says. "If possible, I would like to apply my findings to the treatment of autoimmune and allergic diseases. Although it will be a long way to that goal, I am somewhat confident about it."

Dr. Ichiro Taniuchi, the leader of the Laboratory for Transcriptional Regulation, was honored for his studies of Runx transcription factors.

Photo 2 Dr. Ichiro Taniuchi

The common progenitor cells of helper- and cytotoxic-T cells are the $CD4^+CD8^+$ double positive (DP) thymocytes. During their lineage determination, cells expressing MHC I reacting T cell receptors (TCR) develop into cytotoxic-T cells and cease CD4 expression. On the other hand, cells expressing MHC II reacting TCR go to the helper- T cell pathway and cease CD8 expression. Despite a large number of previous studies that attempted to address mechanism underlying lineage determination of helper- and cytotoxic- T cells, the transcription factor network at the heart of this process had remained unclear. Current genetic approaches demonstrated that expression of the Th-POK transcription factor was essential and sufficient for helper-T cell development. However how Th-POK is regulated remained unclear.

Upon identification of Runx transcription factor complexes as essential molecules for activating a silencer for the CD4 gene (Taniuchi et al. *Cell*, 2002), Dr. Taniuchi expand his research on Runx factors. By using a set of mutant mice that lack functional Runx genes, Dr. Taniuchi's team found that $CD8^+$ cytotoxic-T cells were lost when Runx function was eliminated in $CD4^+CD8^+$ DP thymocytes. Furthermore, the *Th-POK* gene was abnormally pre-activated in DP cells. "These results suggested that Runx represses the expression of Th-POK in DP cells, but we wanted to know how," said Dr. Taniuchi. In order to provide molecular insight, the team took advantage of ChIP on chip analyses, and identified two Runx Binding Sequences (RBS) in the *Th-POK* locus. *In vivo* functional characterization of these DNA elements in transgenic

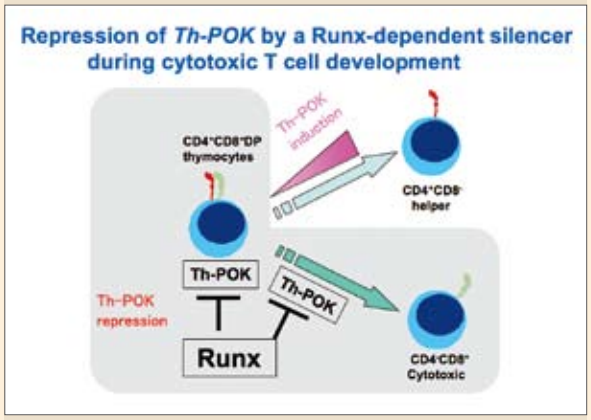

Figure 2 Scheme of Th-POK repression by a Runx-dependent Silencer during cytotoxic T cell development

reporter mice demonstrated a transcriptional silencer activity in the distal RBS region. To further confirm *in vivo* relevance, they generated mutant mice lacking this silencer region, and proved that repression of Th-POK by the Runx-dependent silencer is essential for cytotoxic- T cell development (Figure 2) (Setoguchi et al. *Science,* 2008). Discovery of a transcription factor network regulating the lineage determination of DP thymocytes revealed a prominent role for Runx genes in the development and regulation of the immune system (See Research Highlights section).

Dr. Hiroshi Kawamoto, the leader of the Laboratory for Lymphocyte Development, was honored for validation of a new hematopoietic cell lineage model.

During blood cell formation, the progeny of pluripotent hematopoietic stem cells become sequentially restricted in their developmental potential to give rise to a variety of lineage committed progenitors. The classical model of hematopoiesis postulated that in the first step of differentiation, the hematopoietic stem cell generates common myelo-erythroid progenitors and common lymphoid progenitors (CLPs) (Figure 3A). However, using a clonal analysis of lympho-hematopoietic cells in the fetal liver of mice, Dr. Kawamoto previously demonstrated that myeloid potential is maintained even as the lineage branches segregate towards T and B cells *(Trends in Immunol,* 2006). The team has therefore proposed the "myeloid-based" model of haematopoiesis in which the stem cell initially generates common myelo-erythroid progenitors and common myelo-lymphoid progenitors (Figure 3B). T and B cell progenitors subsequently arise from

Photo 3 Dr. Hiroshi Kawamoto

common myelo-lymphoid progenitors through myeloid-T and myeloid-B stages, respectively. However, it has been unclear whether this myeloid-based model is also valid for adult haematopoiesis. To determine this, he decided to critically examine whether the CLP stage exists or not in the developmental pathway from the stem cell to T cells during adult haematopoiesis. His team developed a new clonal assay system, which supports the generation of both T cells

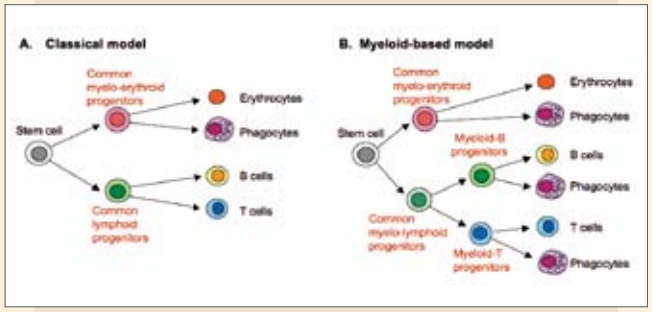

Figure 3 Models of hematopoiesis: classical model (A) and myeloid-based model (B)

and myeloid cells. As a source of progenitor cells, they used green fluorescent protein (GFP)-transgenic mice whose macrophages and T-cell progenitors express GFP whereas T cells do not. When 192 thymic progenitor cells were individually cultured with stromal cells, they found 13 wells containing both T cells and macrophages. This indicated that T-cell progenitors retain substantial macrophage potential after shutting off B-cell potential. They further investigated whether thymic T-cell progenitors can generate macrophages within the thymic environment. They injected thymic progenitors from green mice directly into the recipient's thymus. Two weeks later, they confirmed that GFP^+ macrophages could develop from T-cell progenitors in the adult thymus.

Thus, his new "Myeloid-based Model" settled the long controversy in the field of hematopoiesis. "Our results showed that the classical model often drawn in textbooks is not correct. It may be revised in the near future," said Dr. Kawamoto.

RCAI's IT-team lead by Mr. Yasuaki Murahashi received the special prize from the Outstanding Contribution of the Year Award for their contribution to establishing the Primary Immunodeficiencies Database Japan (http://pidj.rcai.riken.jp/index.html). "Although this award primarily targets researchers, we found that their contribution was extremely important for the Center this year." Dr. Taniguchi commented.

Primary Immunodeficiencies (PID) cause disorders in immune functions due to congenital abnormalities, making these patients very susceptible to infection by pathogens such as bacteria, viruses, and fungi. These are extremely serious disorders, which at times are accompanied by malignant tumors at a young age, autoimmune diseases, and allergies. It is estimated that about 10,000 people in Japan suffer from these syndromes. RCAI, Kazusa DNA Research Institute, and 13 Japanese universities are currently collaborating in an effort to elucidate the pathology of PIDs, establish methods of early diagnosis, and find effective treatments (See Collaborative Networks section).

For this purpose, it is extremely important to have an informational network that allows retrieval and analysis of accurate data for hospital clinicians, basic researchers, clinical PID experts, and PID patients. Mr. Murahashi and the IT-team,

Photo 4 Mr. Yasuaki Murahashi

in collaboration with clinical immunologists and Kazusa DNA Research Institute, worked to construct a clinical information and DNA analysis data repository of PID patients to provide a framework for accurate PID diagnosis. Consequently the clinical information repository, designated PIDJ, is now open to the public and more than 40 patient samples were subjected to DNA analytical diagnosis in 2007. "We first thought of outsourcing the construction of this database. However, we realized that we would have to do it by ourselves to make it the best fit for everyone's purposes. It was not easy to do this big project along with all of the IT-related work at the Center," commented Mr. Murahashi. "We made our best effort for this platform, and we will continue to support patients and clinicians who are fighting PID".

Figure 4 PIDJ home page

Related publications

Hoshino, K. et al. IκB kinase-α is critical for interferon-α production induced by Toll-like receptors 7 and 9. ***Nature.*** 440, 949-953 (2006).

Tanaka, M. et al. PDLIM2-mediated termination of transcription factor NF-κB activation by intranuclear sequestration and degradation of the p65 subunit. ***Nature Immunol.*** 8, 584-591 (2007).

Taniuchi et al. Differential requirements for Runx proteins in CD4 repression and epigenetic silencing during T lymphocyte development. ***Cell*** 111, 621-633 (2002)

Setoguchi R. et al. Repression of the Transcription Factor Th-POK by Runx Complexes in Cytotoxic T Cell Development. ***Science*** 319: 816-819 (2008)

Kawamoto, H. A close developmental relationship between the lymphoid and myeloid lineages. ***Trends in Immunol.*** 27: 169-175 (2006)

Wada H. et al. Adult T-cell progenitors retain myeloid potential. ***Nature*** 452:702-703 (2008)

2007 Excellent Paper Award and Excellent Poster of the Year


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The RCAI Award for Excellent Paper was established in 2004 with donations from Dr. Masaru Taniguchi and Dr. Toshio Hirano. The annual award aims to recognize exceptional publications by RCAI scientists. This year, five excellent papers were selected from 16 candidates for this prestigious award.

- **Drs. Ruka Setoguchi and Ichiro Taniuchi**, for their *Science* paper "Repression of the Transcription Factor Th-POK by Runx Complexes in Cytotoxic T Cell Development". They studied cell fate decisions using CD4/CD8 lineage commitment in the thymus as a model system and found that the Runx transcription factor complex actively suppresses the expression of another transcription factor required for CD4 T cell development, thus allowing for differentiation of cytotoxic T cells.
- **Drs. Shuro Yoshida, Yoriko Saito and Fumihiko Ishikawa**, for their paper published in *Nature Biotechnology* "Chemotherapy-resistant Human AML Stem Cells Home to and Engraft Within the Bone-marrow Endosteal Region". They used a humanized mouse model to identify and characterize the leukemic stem (LS) cells in acute myelogenous leukemia and found that the quiescencent state of the LS cells may be a mechanism underlying their resistance to cell cycle-dependent cytotoxic therapy, and validated the humanized mouse as a potentially useful model for development of novel therapeutic strategies.
- **Drs. Takashi Tanaka and Tsuneyasu Kaisho**, for their work on "PDLIM2-mediated termination of transcription factor NF-κB activation by intranuclear sequestration and degradation of the p65 subunit" (*Nature Immunology*). In this paper, they identified a novel nuclear ubiquitin ligase that terminates NF-κB activation through intranuclear sequestration and subsequent degradation.
- **Drs. Yoshihiro Baba and Tomohiro Kurosaki** for their *Nature Immunology* paper "Essential function for the calcium sensor STIM1 in mast cell activation and anaphylactic responses". They discovered that the calcium-binding endoplasmic reticulum protein STIM1 is vital to mast cell function: it critically regulates FceRI Ca influx, activation of the NF-κB and NFAT transcription factors, degranulation, and *in vivo* anaphylaxis responses.
- **Drs. Hiroshi Watarai and Masaru Taniguchi**, for their paper "PDC-TREM, a plasmacytoid dendritic cell-specific receptor, is responsible for augmented production of type I interferon" (*Proc. Natl. Acad. Sci., USA*). They discovered a novel TREM family receptor expressed only on TLR activated plasmacytoid dendritic cells, which plays a crucial role in Type I interferon production after TLR activation.

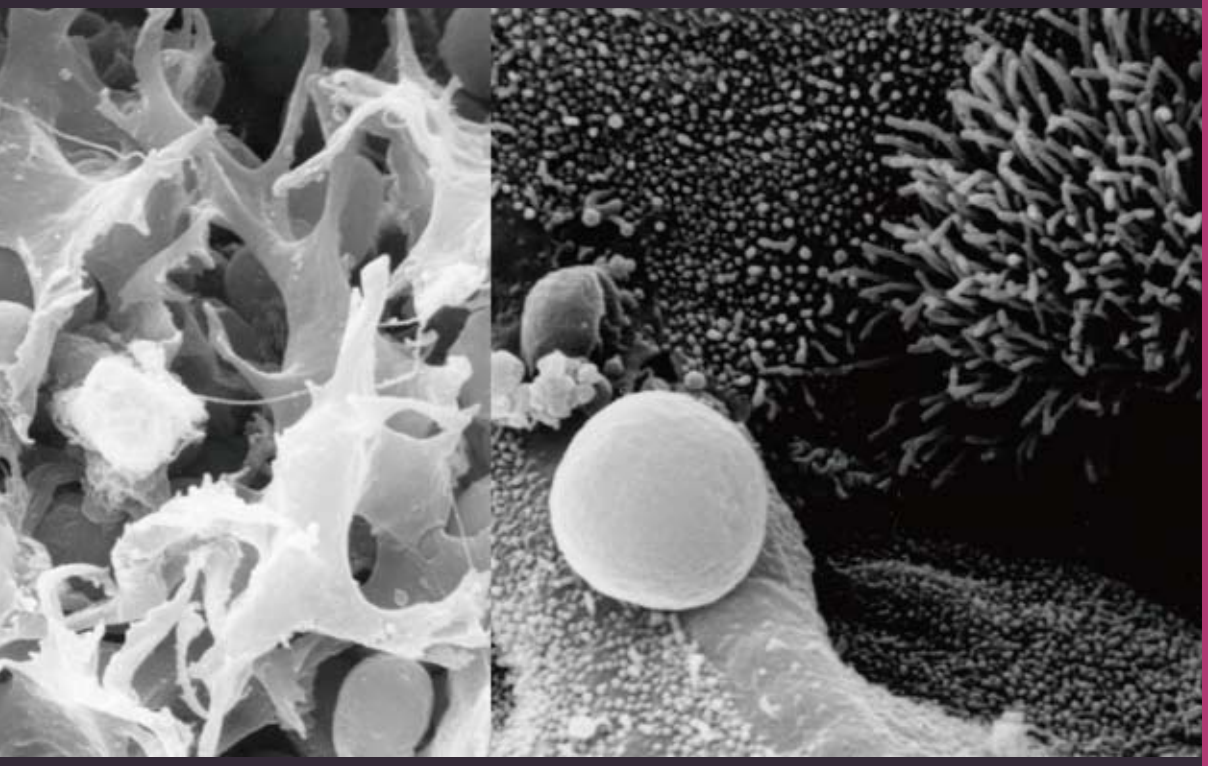
The award ceremony was held on May 9, 2008 at RCAI. The awardees described the difficulties they had encountered and expressed appreciation for the support of their colleagues.

On September 3rd, RCAI held two poster presentation sessions during its retreat meeting in Chiba. Among eighty-six posters presented, thirteen posters were selected for a "2007 Award for Excellent Poster" by vote of laboratory heads. Winning the award was not only a happy surprise for the awardees, but also allowed each of them to introduce his/her project to an audience of ~150 in a 10-minute English oral presentation held the next day. "This year, we tried to select unpublished results for the award. I hope the young researchers were stimulated and motivated for their research through this opportunity" the organizer, Dr. Udono explained.

The awardees were:

Toshiyuki Fukada (Lab. for Cytokine Signaling) (01)
Kazuya Kawano (Lab. for Epithelial Immunobiology) (02)
Noriko Komatsu (Research Unit for Immune Homeostasis) (03)
Yasutaka Motomura (Lab. for Signal Network) (04)
Mari Ohmura-Hoshino (Lab. for Infectious Immunity) (05)
Rika Ouchida (Lab. for Immune Diversity) (06)
Yoriko Saito (Research Unit for Human Disease Model) (07)
Rumi Satoh (Lab. for Lymphocyte Development) (08)
Keiichiro Suzuki (Lab. for Mucosal Immunity) (09)
Takashi Tanaka (Lab. for Host Defense) (10)
Ichiro Taniuchi (Lab. for Transcriptional Regulation) (11)
Asuka Terashima & Hiroshi Watarai (Lab. for Immune Regulation) (12)
Masayuki Tsuji (Lab. for Mucosal Immunity) (13)

2007 **Research Activities**



Laboratory for Developmental Genetics

Group director

Haruhiko Koseki

Researchers : **Mitsuhiro Endo, Yûichi Fujimura, Shûichi Hiraoka, Kyôichi Isono, Yuki Takada, Jun Shinga**

Technical Staffs : **Takanori Hasegawa, Midori Iida, Iyo Kataoka, Naoko Ohnaga, Hiroshi Kajita, Yasuaki Murahashi, Shinobu Mochizuki, Masashi Matsuda, Tomoyuki Ishikura, Tamie Morisawa, Atushi Kajiwara, Yôko Koseki, Rie Suzuki, Kayoko Katsuyama, Naomi Ootsuka, Isamu Hisanaga, Momoko Ogoshi, Natsumi Saito,**

Student Trainee : **Masayo Harada** (JRA)

Visiting Scientist : **Mutô Masahiro**

Assistants : **Ryôko Moriizumi, Hiroko Iwamoto**

The Developmental Genetics Research Group fulfills a double role within RCAI. A large portion of the manpower and financial resources of the group is devoted to the maintenance of a high-standard mouse facility at RCAI. 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 the epigenetic regulation mediated by Polycomb group (PcG) proteins in development. PcG genes were first identified in *Drosophila melanogaster* as a cluster of genes required for maintenance of segmental identity and were subsequently shown to be structurally and functionally conserved in mammals. Genetic analyses of mammalian PcG proteins revealed their roles not only in anterior-posterior specification but also in cellular proliferation, differentiation, and senescence. In particular, significant impact of PcG functions on lymphocyte and lymphoid organ development have frequently been reported. PcG gene products form at least two different multimeric protein complexes; one mediates histone H3-K27 trimethylation and the other H2A-K119 ubiquitinylation on the chromatin. It is, however, unknown how PcG complexes mediate transcriptional repression and, consequently, exert their biological functions. To elucidate the molecular nature of PcG-mediated transcriptional regulation, we have been focusing on the following issues; (1) regulatory mechanisms of PcG binding to their targets, and (2) regulation of PcG functions by interacting proteins.

Regulatory mechanisms of PcG binding to their targets (Endoh et al., Submitted)

To address the molecular mechanisms underlying PcG-mediated heritable silencing of developmental regulators, we have focused on the role of these proteins in embryonic stem (ES) cells. The Polycomb repressive complexes-2 (PRC2) has been shown to share target genes with the core transcription network, including Oct3/4, Sox2 and Nanog, to maintain ES cells in an undifferentiated state. However, it is still unclear whether and how PcG and the core transcription network are functionally linked. We have identified an essential role for the core components of Polycomb repressive complexes-1 (PRC1), Ring1A and Ring1B, in

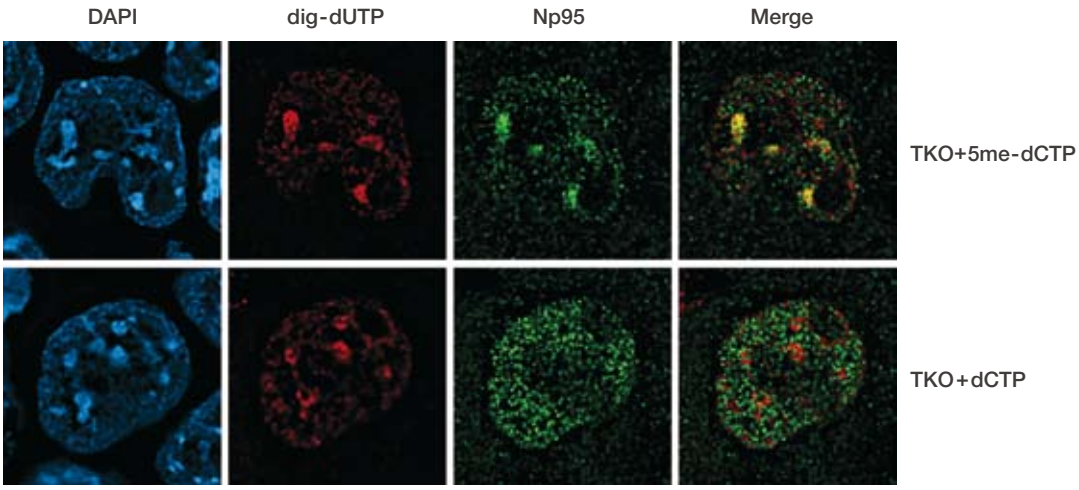


Figure Recognition of hemimethylated DNA by Np95.
Dnmt1/3a/3b triple knockout (TKO) ES cells were labeled by either methylated dCTP (TKO+5me-dCTP) or unmethylated (TKO+dCTP) tother with dig-dUTP. Note the accumulation of Np95 to newly replicated heterochromatic regions (marked by colocalization of DAPI and dig-dUTP) in TKO cells that incorporated methylated dCTP.

repressing developmental regulators in ES cells and thereby in maintaining ES cell identity. A significant proportion of the PRC1 target genes are also repressed by Oct3/4. We could show that engagement of PRC1 at target genes is Oct3/4-dependent whereas engagement of Oct3/4 is PRC1 independent. Moreover, upon differentiation induced by Gata6 expression, most of the Ring1A/B target genes are derepressed and the binding of Ring1A/B to their target loci is also decreased. Collectively these results have allowed us to conclude that Ring1A/B-mediated Polycomb silencing functions downstream of the core transcriptional regulatory circuitry to maintain ES cell identity.

Regulation of PcG functions by interacting proteins (Sharif et al., Nature)

We identified a SET and RING finger-associated (SRA) domain protein Np95 (also called UHRF1 or ICBP90) as an interacting protein for Mel18, a PRC1 component. Purification of Np95 complexes revealed its association with not only PRC1 components but also with DNA (cytosine-5-)-methyltransferase 1 (Dnmt1), which is the principal enzyme responsible for maintenance of CpG methylation and is essential for the regulation of gene expression, silencing of parasitic DNA elements, genomic imprinting, and embryogenesis. This year, we focused on the functional link between Np95 and Dnmt1. Dnmt1 is needed in S-phase to methylate newly replicated CpGs occurring opposite methylated residues on the mother strand of the DNA, and this process is essential for the epigenetic inheritance of methylation patterns in the genome. Despite an intrinsic affinity of Dnmt1 for such hemi-methylated DNA, the molecular mechanisms that ensure the correct loading of Dnmt1 onto newly replicated DNA *in vivo* are not understood. The Np95 protein binds methylated CpG through its SRA domain. We have shown that localization of Np95 to replicating heterochromatin is dependent on the presence of hemi-methylated DNA. Np95 forms complexes with Dnmt1 and mediates the loading of Dnmt1 to replicating heterochromatic regions. By using Np95 deficient embryonic stem (ES) cells and embryos, we could show that Np95 is essential *in vivo* to maintain global and local DNA methylation and to repress transcription of retrotransposons and imprinted genes. The link between hemi-methylated DNA, Np95, and Dnmt1 thus establishes key steps of the mechanism for epigenetic inheritance of DNA methylation.

Recent publications

Sharif J., Muto M., Takebayashi S., Suetake I., Iwamatsu A., Endo T.A., Shinga J., Mizutani-Koseki Y., Toyoda T., Okamura K., Tajima S., Mitsuya K., Okano M., Koseki H. (2007) The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. *Nature* 450:908-912.

Hiraoka S., Furuichi T., Nishimura G., Shibata S., Yanagishita M., Rimoin D.L., Superti-Furga A., Nikkels P.G., Ogawa M., Katsuyama K., Isono K., Toyoda H., Kinoshita-Toyoda A., Ishida N., Sanai Y., Cohn D.H., *Koseki H. *Ikegawa S. (* Corresponding authors) (2007) Nucleotide-sugar transporter SLC35D1 is critical to chondroitin sulfate synthesis in cartilage and skeletal development in mouse and human. *Nat Med.* 13:1363-1367.

Takada Y, Isono K, Shinga J, Turner J.M.A, Kitamura H, Ohara O, Watanabe G, Singh P.B, Kamijo T, Jenuwein T, Burgoyne P.S, Koseki H,, (2007) Mammalian Polycomb Scmh1 mediates exclusion of Polycomb complexes from the XY body in the pachytene spermatocytes *Development* 134:579-590.

Fujimura Y, Isono K, Vidal M, Endoh M, Kajita H, Mizutani-Koseki Y, Takihara Y, van Lohuizen M, Otte A, Jenuwein T, Deschamps J, Koseki H (2006): Distinct roles of Polycomb group gene products between transcriptionally repressed and active domains of Hoxb8 *Development* 133:2371-2381

Isono K., Mizutani-Koseki Y., Komori T., Schmidt-Zachmann M.S., Koseki H (2005) Mammalian Polycomb-mediated repression of Hox genes requires the essential spliceosomal protein sf3b1. *Gene Dev.* 19, 536-541.

Research Unit for Lymphocyte Cloning

Unit leader

Andrei Rybouchkin

Research Scientist : **Menzorov Aleksey**

Technical Staff : **Sakoda Raul**

As a physical entity, this Unit has existed since September 2007. The mission of the Unit is to provide expertise in reprogramming of somatic cells to the pluripotent state and in animal cloning to benefit immunological research. Analysis of the differentiation of ES cells derived from immune cell nuclei, as well as analyzing the immune system of the animals cloned from immune cells, may provide valuable insight into the development and function of the immune system. Additionally, some human immune system cells, e.g. NKT and HSC, may be difficult to expand to the quantities necessary for cell therapy approaches. In principal, these cells could be converted to the pluripotent state, propagated to the needed quantities, and then re-differentiated into the appropriate cell type. Our Unit will attempt to develop this technology.

In our recent research we found that serial nuclear transfer improves derivation of ES cell lines from NKT cells of inbred [C57BL/6 (B6)] mice. Previously NKT derived ES cell lines were obtained only from hybrid strains of mice, for example B6/129 F1 (*Inoue et al., 2005 Current Biology 15:1114*). Derivation from inbred strains was a significant challenge, likely due to the generally low viability of inbred embryos which exacerbated the already low development of somatic nuclei-derived clones. This problem was especially acute for the B6 strain of mice that is most commonly used in immunological studies. The early preimplantation embryos of this strain are very sensitive to *in vitro* culture conditions and respond to culture stress by increased levels of the p53 pro-apoptotic transcription factor and reduced cell number in the blastocysts (*Li et al., 2007 Biol. Reprod. 76:362*). Indeed, we have found that NKT derived cloned embryos from this strain of mice rather successfully develop to the 4 cell stage (60%), but do not generate good quality blastocysts (less than 1% in comparison to 15% for B6D2 F1 NKT cloned embryos), thus making the derivation of B6 ES cell lines a very difficult task. As a new approach to this problem, we used Sendai virus induced fusion to transfer nuclei of 2 cell stage NKT cloned embryos to the timed matched cytoplasm of *in vivo* fertilized *in vivo* cultured B6D2F2 embryos (*Liu et al., J Reprod Dev. 2007, 53(4):785*) and obtained greatly improved development of clones. About 40% of cloned embryos (2 cell stage) obtained by our serial transfer technique reached the

Figure Usual morphology of ntES colonies derived from B6 NKT cells by our serial NT approachfeeder cells.

blastocyst stage with a fully expanded blastocoele and well developed inner cell mass. About 10% of these blastocysts went on to give rise to NKT derived ntES cell lines (Figure).

In another study we found that NK cell nuclei are almost as resistant to reprogramming as T cell nuclei and that both of these cell types are far less efficient as nuclear donors compared to NKT cells.

It has been known since 2002 (*Hochedlinger and Jaenisch, 2002 Nature, 415:1035*) that T cell nuclei are very resistant to reprogramming following exposure to oocyte cytoplasm. Only 20-30% of the 2-cell stage embryos cleave to the 4 cell stage, and only 4-5% of these reach the blastocyst stage, compared to 60 and 30% for cumulus cells. By contrast, in 2005 it was shown that NKT cell nuclei respond to nuclear transfer very readily and after remodeling in oocyte cytoplasm support a high rate of clone development (around 60% to the 4 cell-stage and 30% to the blastocyst stage) (*Inoue et al., 2005 Current Biology 15:1114*). We hypothesize that a comparison of gene expression regulatory networks and epigenetic properties of T cells and the closely related NKT cells will give valuable insight into the mechanisms of reprogramming. As a first approach to deciphering this riddle, we compared the reprogramming efficiency of NK cells to that of NKT and T cells in B6 mice. NKT cells are thought to have many properties closeer to the innate NK cells rather than to adaptive CD4T cells, and therefore are sometimes classified as "innate-like" lymphocytes having a specific "innate signature" in their pattern of gene expression (*Yamagata et al., 2006 Immunol. Reviews, 210:52*). Innate responses are less specific, but faster than adaptive responses and do not have a memory component. Therefore, a comparison of the reprogramming response of NK cells to that of NKT and T cells should provide an indication of the relation of the "innate signature" of gene expression to the reprogramming response.

The major difference between NKT and T cells was observed at the second cleavage division of cloned embryos. We found that NKT derived embryos cleave from the 2 to the 4-cell-stage at a much higher efficiency than T cell derived embryos (roughly 60% versus 20%, respectively). We have found that this feature is very specific for NKT cell nuclei, as NK cell nuclei are only slightly better responders (roughly 30% second cleavage division rate) than T cell nuclei. This model system will likely allow us to identify an NKT-specific reprogramming signature after analysis of the NKT regulatory networks and chromatin patterns. This may aid in the general understanding of the mechanisms of reprogramming of somatic nuclei to the pluripotent state.

Laboratory for Lymphocyte Development

Team leader

Hiroshi Kawamoto

Research Scientists : **Tomokatsu Ikawa**
Kiyokazu Kakugawa
Technical Staff : **Rumi Satoh**
Student Trainee : **Yuuki Hayashi**
Assistant : **Misa Uenoyama**
Visiting Scientists : **Nagahiro Minato**
Yoshimoto Katsura

During hematopoiesis, pluripotent hematopoietic stem cells (HSC) become sequentially restricted in their developmental potential to give rise to a variety of lineage-committed progenitors. The major aim of the Laboratory for Lymphocyte Development is to elucidate the molecular mechanisms that regulate cell fate decisions in the process of lineage restriction from HSC to unipotent progenitors. We have previously established a clonal assay system that makes it possible to examine the developmental potential of individual progenitor cells toward T, B and myeloid cell lineages. This work has led to a fundamental redefinition of the nature of lymphoid progenitors and the ontogeny and phylogeny of T- and B-cell development.

Process of lineage commitment in hematopoiesis

The classic dichotomy model of hematopoiesis postulates that the first step of differentiation from the HSC generates the common myelo-erythroid and common lymphoid progenitors (CLP) (Figure 1A). Our previous studies in fetal mice, however, indicated that the first step of lineage restriction of HSC is the generation of myelo-lymphoid lineage progenitors and myelo-erythroid lineage progenitors (Figure 1B). Most notably, the myeloid potential is retained even after the segregation of myelo-lymphoid progenitor towards T and B cell lineages. Thus, each process of specification towards T, B and erythroid lineages appears to proceed accompanying the prototypical myeloid program. Recently we have termed this model the “myeloid-based model”. The concept of the CLP has, however, persisted in models of adult hematopoiesis, since several groups have provided experimental results supporting the presence of CLP in the bone marrow. We have been analyzing the developmental potential of progenitors in adult mice, and have recently obtained evidence indicating that the myeloid-based model is also applicable to adult hematopoiesis (Wada et al, *Nature*, 2008).

Progenitors for thymic epithelial cells in adult thymus

We are also interested in the development of thymic epithelial cells, and are now focusing on the homeostasis of thymic epithelial cells in adult mice. Although the early steps in the generation of thymic epithelial cells have recently been documented, the postnatal development

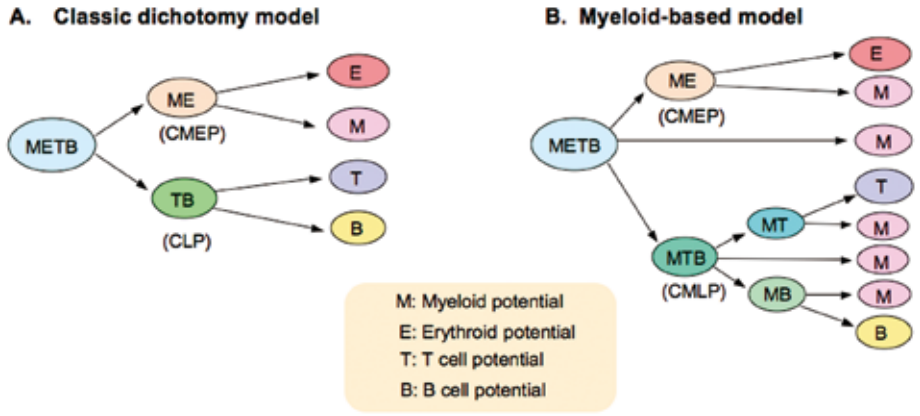


Figure 1 Models of lineage commitment during hematopoiesis.
A, Classic dichotomy model. Hematopoietic stem cells (HSC) diverge into CMEP and CLP. Note that CMEP are sometimes referred to as common myeloid progenitors (CMP). The findings reported by Dr. Weissman's group that CLP are present in adult BM has provided support for this model.
B, Myeloid-based model. In this model, the first branch point generates CMEP and CMLP, and the myeloid potential persists in the T and B cell branches even after these lineages have diverged. This model postulates that specification towards erythroid, T and B cell lineages proceeds on the basis of a prototypical myeloid program (Kawamoto H., Trends in Immunol., 2006).

of the thymic microenvironment still remains obscure. In collaboration with the Unit for Thymic Environment, we have identified a novel subset of cortical thymic epithelial cells (cTEC), responsible for the maintenance of microenvironments in the thymic cortex of adults (Figure 2). These cells emerge in the deep thymic cortex, and eventually replace the entire cortex over time. Importantly, this cell type has the capacity to replace damaged cTECs.

Besides the above-mentioned two projects, we are currently interested in the issue of whether environmental factors instructively induce lineage commitment or selectively support autonomously committed progenitors during lymphopoiesis. To address this issue, it is important to establish an experimental system in which lineage commitment of progenitors can be monitored with real-time imaging. We have previously shown that the earliest T cell progenitors in the thymus retain the potential to generate NK cells and dendritic cells, and that the termination of this tri-potentiality with clear commitment to the T cell lineage occurs before the initiation of TCRβ chain gene rearrangement. In order to directly visualize this differentiation step, we are using GFP transgenic mice in which the expression of GFP is controlled by the proximal promoter of the T cell-specific tyrosine kinase lck.

Our research activities also include the study of human lympho-hematopoiesis. Cord blood cells are used as a progenitor source, and the basic culture system is a modification of the co-culture with murine stromal cells.

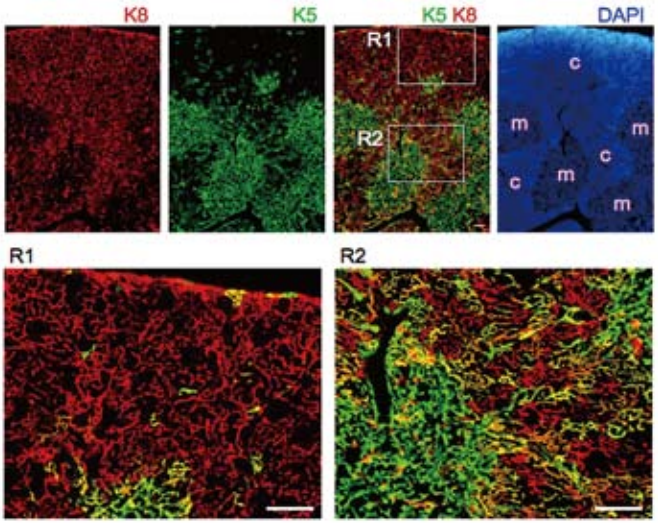


Figure 2 Second generation cTECs emerging postnatally in the deep thymic cortex.
A cryostat section of the thymus from a 7 week old mouse was stained with anti-cytokeratin (K) 5 (green) and anti-K8 (red) antibodies. Whereas cTECs in the outer cortex are almost exclusively $K5^{-}K8^{+}$ (R1), 60 percent of cTECs located between medullary islets in the deep cortex displayed a $K5^{+}K8^{+}$ phenotype (R2). These $K5^{+}K8^{+}$ cTECs are thought to represent the second wave cTECs. c: cortex, m: medulla. Bar; 100 μm.

Recent publications

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Laboratory for Transcriptional Regulation

Team leader

Ichiro Taniuchi

Research Scientist : **Yoshinori Naoe**

Technical Staff : **Sawako Muroi**
Kaori Akiyama
Chizuko Suzuki

Student Trainee : **Masashi Tachibana**

Assistant : **Chieko Tezuka**

One of 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 progenitor cells sense external or intrinsic stimuli and turn on a genetic program for regulating lineage choice and (b) how an established gene expression pattern is maintained by epigenetic mechanisms. We are addressing these questions by studying transcription factors network regulating the lineage decision of $CD4^+CD8^+$ double-positive (DP) thymocytes differentiating into either $CD4^+$ helper - or $CD8^+$ cytotoxic-lineage cells as a model system. Our previous findings had shown that the Runx transcriptional factor complexes play an essential role in CD4 gene silencing during CD8 T cell development by binding to the intronic silencer in the CD4 gene. Further studies during the past three years have revealed a critical regulatory pathway for cytotoxic T cell development, in which expression of the Th-POK transcription factor, a central regulator for helper T cell development, is repressed thorough Runx-dependent silencer activity. Furthermore, during differentiation of T helper type 1 (Th1) cells, Runx complexes play an essential role to repress interleukin (IL)-4 production by direct binding to the IL4 silencer.

Transcription factors networks in the helper- versus cytotoxic-lineage decision

T lymphocytes expressing the $\alpha\beta$TCR exist as two distinct lineages, helper and cytotoxic, and are differentiated in the thymus from common progenitor $CD4^+CD8^+$ DP thymocytes. Cells expressing class II-restricted TCR differentiate primarily into the helper lineage and cease to express CD8, whereas cells expressing class I-restricted TCR differentiate into the cytotoxic linage and silence CD4. However, molecular pathways in the nucleus that regulate cell fate determination of $CD4^+CD8^+$ DP thymocyte remain poorly understood. Current genetic approaches have identified a central regulator for helper-lineage development, the Th-POK transcription factor. $CD4^+CD8^+$ DP thymocytes differentiate into $CD4^+$ helper lineage cells upon expression of Th-POK, but commit to the $CD8^+$

cytotoxic lineage in its absence. An understanding of how cell fate determination of DP thymocytes is regulated therefore requires elucidation of the mechanisms that regulate helper-lineage specific expression of Th-POK. By using conditional inactivation of Runx gene family members, we found that loss of Runx complex function in thymocytes results in de-repression of Th-POK and in redirected differentiation of class I-restricted thymocytes into $CD4^+CD8^-$ helper-like T cells. Furthermore we identified a Runx-binding sequence within the Th-POK locus that acts as a transcriptional silencer. Removal of this newly identified element, termed the Th-POK silencer, from the Th-POK locus demonstrated that this silencer is essential not only for Th-POK repression in both pre-selection $CD4^+CD8^+$ DP thymocytes and $CD8^+$ cytotoxic-lineage cells but also for development of $CD8^+$ T cells. These results demonstrate that Th-POK expression, and thus the genetic programming of helper T cell development, is actively repressed by Runx complex-dependent Th-POK silencer activity, allowing for differentiation of cytotoxic T cells (Figure). Identification of the transcription factors networks in CD4/CD8 lineage choice provides insight into how distinct T cell subsets are developed for regulating adaptive immune responses.

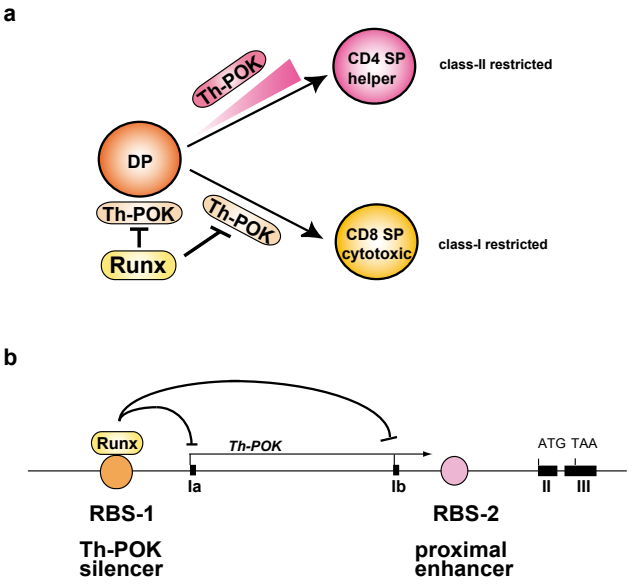

Figure Model of Th-POK gene regulation by the Th-POK silencer. (a) The Th-POK gene is expressed as a function of CD4 lineage commitment but is actively repressed by the Runx-dependent Th-POK silencer in DP thymocytes and in thymocytes developing toward the CD8 lineage. (b) Runx complexes associate with the Th-POK locus at two regions, Runx-binding sequences (RBS)-1 and RBS-2. RBS-1 acts as transcriptional silencer that is essential to repress Th-POK expression in DP thymocytes and in CD8 linage cells, while RBS-2 functions as a transcriptional enhancer. Thus, the helper-lineage specific expression of the Th-POK gene is regulated by the RBS-1 silencer (Th-POK silencer), whose activity depends on binding of Runx complexes

Role of Runx complexes in IL-4 silencing during T helper cell differentiation.

Upon encountering antigen, naïve $CD4^+$ T helper cells differentiate into several effector cell subsets including T helper type 1 (Th1) cells expressing IFNγ and T helper type 2 (Th2) cells expressing IL-4. It is unclear how the exclusive expression pattern of each hallmark cytokine is established. We observed that T cell-specific inactivation of the Cbfβ gene, which encodes the common binding partner for all Runx family members, led to a spontaneous elevation of serum IgE and airway infiltration by lymphoid and eosinophilic cells, two features that resemble what is seen in human asthma mediated by enhanced Th2 responses. To understand the molecular basis for this phenotype, we examined the *in vitro* differentiation of Th1/Th2 cells and found de-repression of IL4 in Runx-deficient Th1 cells producing IFNγ. Furthermore, binding of Runx complexes to the Il4 silencer was detected in naïve $CD4^+$ T cells and Th1 cells, but not in Th2 cells. Transduction of Th1 cells with the Gata-3 transcription factor released the Runx from the Il4 silencer and induced IL-4 production. These results demonstrate the critical role of Runx complexes in regulating immune responses, at least in part through the repression of the Il4 gene during T helper cell differentiation.

Thus Runx complexes are involved in the regulation of CD4, Th-POK and Il-4 genes by activating silencers. Further studies on the regulatory pathways that determine lineage specificity of silencers in those genes would shed light on how signals initiated by external stimuli are converted into genetic programs in the cell nucleus.

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Laboratory for Cell Signaling

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Akiko Furuno (Leica)

RCAI Central Facility mAb Laboratory
Shinji Seki (MBL)**, Tomomi Aoyama, Hajime Inamochi**

The long-term goal of the Cell Signaling group is to determine the molecular mechanisms of activation and homeostasis of T cells in order to be able to modulate T cell activation/function in immunological disorders. Therefore, the group is involved in a range of projects related to the basic mechanisms of antigen recognition, activation, differentiation, and functional regulation of T cells from the viewpoint of signal transduction. Particularly, the group has recently been using real-time imaging analysis, which has allowed us to gain novel insight into the dynamic regulation of the assembly of the TCR signalsome and the immunological synapse, as well as related downstream signaling pathways upon antigen recognition. The events that diversify the antigen recognition signals into differential functions by differentially utilizing the adaptor CARMA1 and CARD9 complexes and the transcription factors NF-AT versus NF-κB in both T cells and myeloid cells have been investigated for innate and adaptive immunity. The group also analyzes the regulation of cell migration as a consequence of T cell activation to establish the peripheral antigen-specific repertoire for effector functions. *In vivo* imaging analysis will allow us to integrate the mechanisms of cell signaling with *in vivo* cell movement and function.

Dynamic regulation of T cell recognition and activation

The group has studied the dynamic movement of signaling molecules in the process of the formation of the immunological synapse and T cell activation upon antigen recognition at the single-cell level. Using a novel single-molecule imaging technique developed by the Single-molecule Immuno-imaging Unit, the group could visualize the behavior of single molecules during the dynamic process of T cell activation.

The group visualized the dynamics of CD3ζ, ZAP-70 and SLP-76 as representatives of the T cell receptor, and associated kinase and adaptor molecules, respectively, in the TCR signaling complex, and analyzed the entire process of synapse formation. The group found that microclusters containing TCR, kinases and adaptors are the structures responsible for the initial and sustained activation of T cells and that the classical cSMAC is not the site for signaling. The discovery of microclusters as the fundamental units of antigen recognition and activation lead to a major revision in current

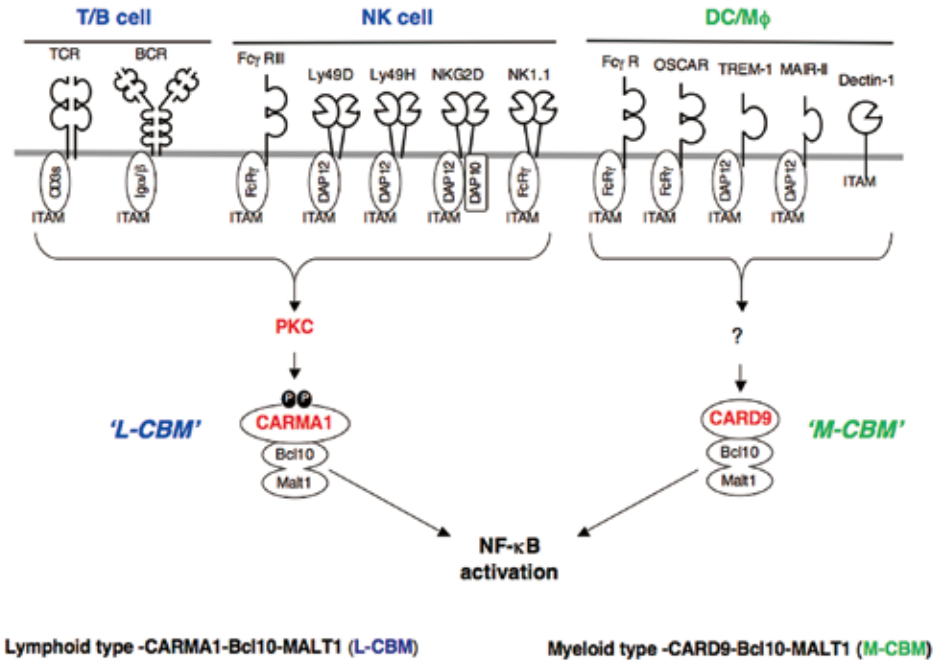

Figure Cell type-specific PKC-dependent NF-κB activation through ITAM-receptors.

models on T cell activation, including the regulation of co-stimulation signals, the function of lipid rafts, and the functional involvement of self-peptide recognition. In more recent studies, the dynamic regulation of the co-stimulation signals has been analyzed. The major co-stimulation receptor CD28 was also found to be recruited to TCR microclusters at the initiation phase, but later it accumulated in the outer region of the cSMAC. CD28 recruits PKCθ to the microclusters for initial stimulation but to the outer region of cSMAC at later time, possibly for sustained co-stimulation. Thus, TCR signals and CD28-mediated co-stimulation signals are dynamically regulated by spatially different signalsomes.

Regulation of innate receptor-mediated cell activation

The group has analyzed activation signals through ITAM-containing adaptor molecules, such as CD3 and FcRγ, that are associated with receptors on T cells and myeloid cells. ITAM-mediated signal transduction is a prototypic lymphoid-specific signaling mechanism. TCR mediated signal transduction leads to several signaling pathways including activation of NFAT and NF-κB. The CARMA1-Bcl10-MALT1 (CBM) complex mediates NF-κB activation upon TCR stimulation. However, genetic analysis revealed that a CARMA1-related Bcl10 binding molecule CARD9 functions for ITAM receptor-mediated NF-κB activation in myeloid. CARD9-deficient DCs showed defects in cytokine production due to impaired NF-κB activation upon signaling through these ITAM- receptors. The analysis indicates that stimulation of ITAM-containing receptors induces two distinctly regulated CBM complexes; lymphoid CBM (CARMA1-Bcl10) functions only in lymphocytes upon TCR and BCR stimulation, whereas myeloid CBM (CARD9-Bcl10) mediates signal transduction to NF-κB activation only in DC and macrophages. The requirement of these adaptor complexes in NK cell activation was also analyzed. Although the ITAM-receptors on NK cells are related to those on DCs, all NK ITAM-receptors utilized lymphoid CBM, similar to T cells. These results showed cell type-specific regulation of the usage of the signal adaptors CARMA1 and CARD9.

Signals for NF-κB activation upon crosslinking the surface receptors associated with ITAM-bearing adaptors (FcRγ, DAP12) are mediated through the CARMA1-Bcl10-MALT1 complex (Lymphoid CBM) in T/B cells and NK cells, whereas these receptor signals are mediated through the CARD9-Bcl10-MALT1 complex (Myeloid CBM) in myeloid cells such as DCs and macrophages.

The group also analyzed the function of TLR expressed on various naïve and effecter T cell subsets, and found that Th1 cells are directly activated to secrete IFN-γ by TLR2 stimulation through NF-κB activation. None of the other TLRs were able to stimulate Th1 cells, and naive and Th2 cells cannot be activated by direct TLR stimulation. Th1 can thus be activated through three stimuli, TCR, IL-18, and TLR2. As a result, microbial infection may directly affect T cell development and function through TLRs. In future studies, the *in vivo* function of TLR2-mediated Th1 activation and the function of other TLRs for T cells will be analyzed.

Recent publications

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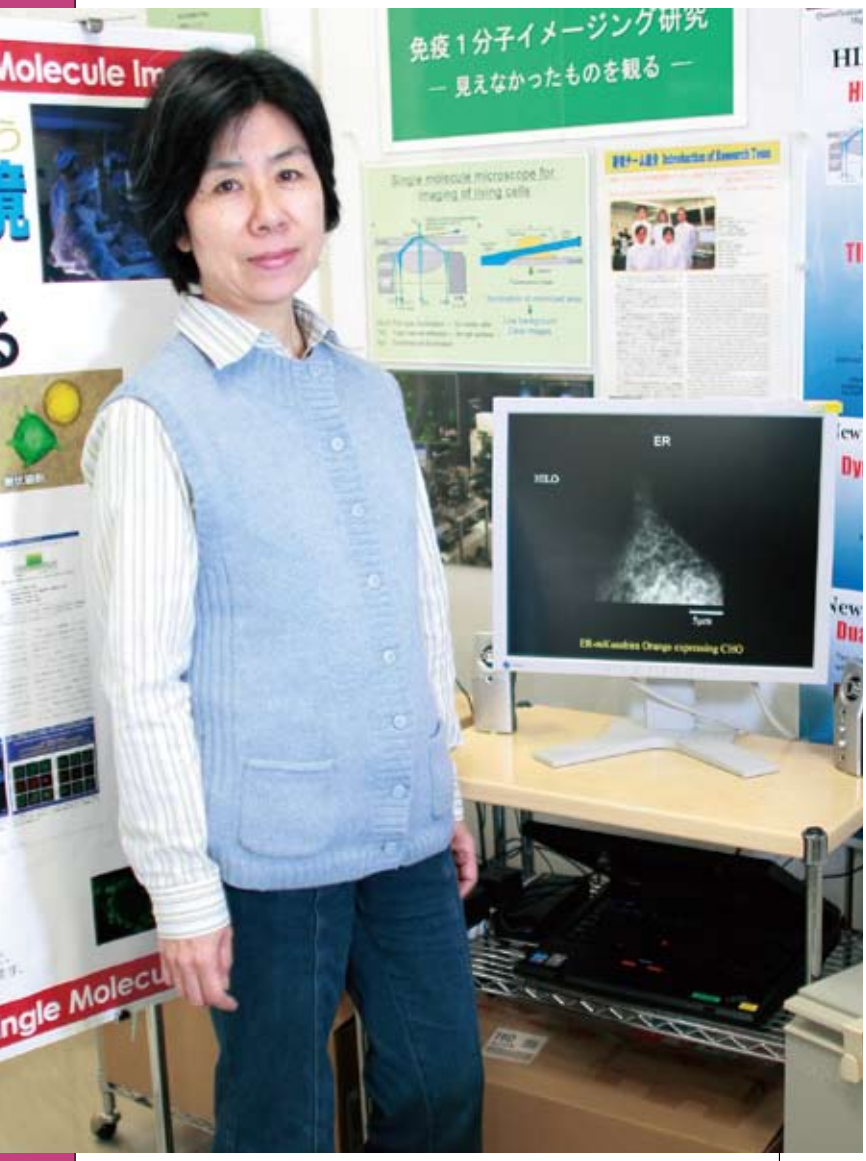
Yokosuka T., Sakata-Sogawa K., Kobayashi W., Hiroshima M., Hashimoto-Tane A., Tokunaga M., Dustin M.L., Saito T. Newly generated T cell receptor microclusters initiate and sustain T cell activation by recruitment of Zap 70 and SLP-76. *Nat Immunol.* 6, 1253-1262 (2005)

Research Unit for Single Molecule Imaging



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Assistant : **Chieko Tsukiyama**

Single molecule approaches enable us to capture transient intermediates and heterogeneous behavior, thus avoiding ensemble averaging. This ability is particularly powerful in elucidating mechanisms of cellular functions: which molecule interacts with what, when, where, and how it works inside living cells. The goal of the research unit for single molecule imaging is to elucidate immunological responses and signaling processes with the technique of single molecule imaging and quantification. To this end we installed a microscope system (Fig. ure 1) and we have focused on the development of novel types of fluorescence microscopy for use in single cell/single molecule studies. In addition, we are developing analysis software tools for imaging and quantitative analysis. Our unit has a close collaboration with the Research Unit for Molecular Systems Immunology for development of new microscopy techniques and analysis software.

Single molecule microscope

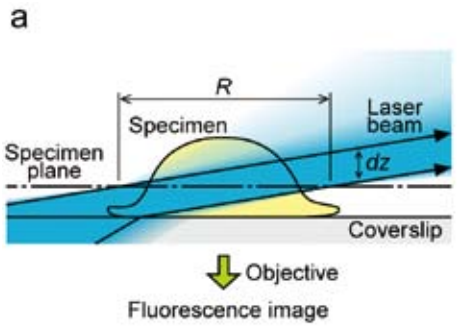
We have been involved in the development of total internal reflection fluorescence (TIRF) microscopy, a light-microscopic technique that uses evanescent light to illuminate single molecules. TIRF has become a widespread technique for single-molecule imaging at cell surfaces, but cannot be used for single-molecule imaging inside cells due to the very limited depth of evanescent light. To overcome this limitation, a new microscopy termed "Highly Inclined and Laminated Optical Sheet" (HILO) microscopy has been developed. Illumination by a highly inclined and thin beam increases image intensity and decreases background intensity, yielding a signal/noise ratio about eightfold greater than that of epi-illumination. A high signal/noise ratio yields clear single-molecule images. Further, there was much lower photobleaching than in conventional confocal microscopy because of the lower intensity and nonfocused nature of the illumination. These features allow us to observe and trace single molecules inside cells. Thus, HILO is also useful for quantitative studies of the distribution, dynamic movement, or interaction of large numbers of molecules. Further, using TIRF and HILO as a combination system, we can expand single molecule imaging and quantification from the cell surface to the interior of the cell. To this end, we have developed various microscopy techniques. For example, the

Figure 1 Single molecule microscope installed in a temperature-controlled room in RCAI. Several lines of laser beams are introduced into the microscope for illumination of the fluorescent molecules.

computer-controlled illumination system enables us to select the best illumination system for the sample among epi, TIRF and HILO during real time observation. For single cell/single molecule imaging and quantification, several research collaborations including external collaborations are ongoing.

Molecular dynamics of transcription factors in the nucleus

Transcription factors are known to be involved in regulation of target genes downstream of specific signaling pathways. Stimulation of immune cells via receptors on the cell membrane is transmitted through signaling pathways to the nucleus. Transcription factors bind to specific short sequences in DNA and associate with other activator or inhibitory proteins to regulate transcription initiation. As an application of HILO microscopy, we are focusing on visualization of the dynamic properties of transcription factors inside the nucleus. We constructed Sp1-EGFP, a fusion protein of EGFP and the Sp1 transcription factor and prepared CHO cells stably expressing Sp1-EGFP at low levels. HILO microscopy yielded clear images of single Sp1-EGFP molecules interacting with DNA or other proteins (Figure 2). Quantitative analysis of the fluorescence images revealed the existence of either multimer or monomer Sp1-EGFP molecules.



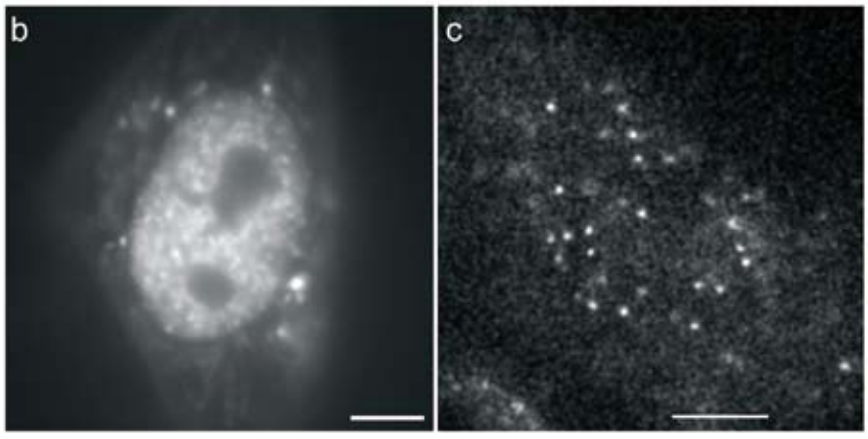


Figure 2 Imaging in the nucleus by HILO microscopy. (a) Specimens are illuminated with a thin sheet of laser beam. The thickness (dz) of illumination by the laser beam was determined to be 7 µm at the diameter R below 20 µm. (b) An EGFP-Sp1 fusion protein was stably expressed in CHO cells and observed. To show the distribution of Sp1-EGFP in the nucleus, fluorescence images were averaged over 8 sec. (c) Each spot depicts monomers or multimers of single Sp1-EGFP molecules interacting with DNA or with other protein molecules (Frame rate: 30 msec). Scale bars: 5 µm.

Recent publications

Tokunaga, M., Imamoto, N., Sakata-Sogawa, K.: Highly inclined thin illumination enables clear single-molecule imaging in cells. *Nat Methods.* 5, 159-161 (2008).

Yamasaki, S., Sakata-Sogawa, K., Hasegawa, A., Suzuki, T., Kabu, K., Sato, E., Kurosaki, T., Yamashita, S., Tokunaga, M., Nishida, K., *Hirano, T.: Zinc is a novel second messenger. *J. Cell Biol.* 177, 637-645 (2007)

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Yamasaki S., Ishikawa, E., Sakuma, M., Ogata, K., Sakata-Sogawa K., Hiroshima, M., Wiest, D. L., Tokunaga M., Saito, T. Mechanistic basis of pre-T cell receptor-mediated autonomous signaling critical for thymocyte development. *Nature Immunol*. 7, 67-75 (2006)

Yokosuka T., Sakata-Sogawa K., Kobayashi, W., Hiroshima, M., Hashimoto-Tane, A., Tokunaga M., Dustin, M. L., Saito, T. Newly generated T cell receptor microclusters initiate and sustain T cell activation by recruitment of Zap70 and SLP-76. *Nature Immunol*. 6, 1253-1262 (2005)

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Signals propagated through the pre B cell (preBCR) and B cell (BCR) receptors are crucial for the development of B lymphocytes, their subsequent antigen-triggered differentiation into memory B cells and antibody secreting plasma cells, and for the maintenance of B cell tolerance. The outcomes of these signaling events, for example proliferation, differentiation, or apoptosis, are dependent on the developmental stage of the cell and the quality of the signal. The latter is dictated by multiple factors including which co-receptors are engaged during preBCR/BCR signaling and which downstream effector molecules are recruited to the signaling complexes. Our laboratory has focused on understanding the molecular composition of these signaling complexes and the mechanisms of signaling pathway crosstalk that lead to crucial cell fate decisions during B lymphocyte differentiation. We have also applied insights gained from our studies of B cells to another important immune effector cell, the mast cell.

Phosphoinositide 3-kinase (PI3K) activation mechanisms in B lymphocytes

During BCR signaling, non-receptor protein tyrosine kinase phosphorylate multiple substrates including the coreceptor CD19 and cytoplasmic adaptor proteins such as B cell linker protein (BLNK) and B cell adaptor for PI3K (BCAP), which then recruit effector enzymes including PI3K and phospholipase Cγ2 that are essential for signal propagation. CD19 is phosphorylated on multiple tyrosines upon BCR engagement and two YXXM motifs in its cytoplasmic domain are recruitment sites for the p85α subunit of PI3K. However, the defect in B cell development in p85α$^{-/-}$ mice is more severe than in CD19$^{-/-}$ mice, leading us to propose that other signaling molecules are involved in PI3K activation. BCAP seemed a likely candidate, since it too can be phosphorylated and recruit p85α following BCR stimulation. We have used CD19$^{-/-}$, BCAP$^{-/-}$, and CD19/BCAP double knockout mice to analyze this hypothesis. Our results indicate that BCAP and CD19 have overlapping functions in BCR-mediated PI3K activation.

Fine tuning of the NF-κB signaling pathway

The NF-κB signaling pathway regulates essential aspects of both innate and adaptive immune responses. In the resting state, NF-κB complexes are retained in the cytosol due to their association with inhibitors of NF-κB (IκB).

Activation of this signaling pathway occurs when IκB is phosphorylated by the IκB kinase (IKK) complex, resulting in IκB degradation and translocation of NF-κB to the nucleus where it regulates gene transcription. In B cells, a macromolecular signaling complex consisting of the adaptor proteins CARMA1 [caspase recruitment domain (CARD)11, Bimp3], Bcl10, and MALT1 (mucosal-associated lymphoid tissue 1) appears to be involved in IKK activation. CARMA1 is a molecular scaffold that organizes the complex formation and its phosphorylation by protein kinase Cβ (PKCβ) correlates with IKK activation through unknown mechanisms. We have found that PKCβ phosphorylates CARMA1, a critical event in IKK activation. Unexpectedly, we also found that activated IKKβ can amplify the NF-κB signal by phosphorylating, enhancing the assembly of the complex and increasing its activity. This feedback mechanism optimizes the strength and duration of the NF-κB signal.

An essential role for the calcium sensor STIM1 in mast cell activation and anaphylactic responses

Mast cells are the main effector cells in IgE-mediated allergic diseases. They express the high affinity IgE receptor, FcεRI, and crosslinking of FcεRI-bound IgE by multivalent antigen induces mast cell activation. This results in the immediate secretion of mediators such as histamine that are stored in cytoplasmic granules, as well as the *de novo* synthesis of proinflammatory lipid mediators and cytokines. The importance of calcium influx in mast activation and degranulation has been appreciated for many years. The calcium flux triggered by FcεRI occurs in two waves, first a rapid but transient release of Ca from ER stores, and then a sustained influx of extracellular Ca across the plasma membrane (PM). Initiation of this second wave is thought to result directly from the emptying of ER Ca stores, which activates Ca channels in the PM, a process referred to as "store-operated Ca" (SOC) influx. Until recently, the mechanism of SOC influx was a mystery, but the identification of several molecules including STIM1 (sensor stromal interaction molecule 1) has provided long-awaited insight. STIM1 has an amino terminal Ca sensor that resides in the lumen of the ER. Depletion of ER Ca stores results in STIM1 relocation to puncta beneath the PM and activation of SOC channels. The necessity for SOC influx in mast cell activation and degranulation has, however, been debated, as both SOC-dependent and -independent pathways may coexist. To directly asses to involvement of SOC influx, we generated STIM1-deficient mice. We found that STIM1 critically regulated FcεRI Ca influx, activation of the NF-κB and NFAT transcription factors, degranulation, and *in vivo* anaphylaxis responses. Thus our data provide direct genetic evidence for the importance of SOC influx multiple functions of activated mast cells.

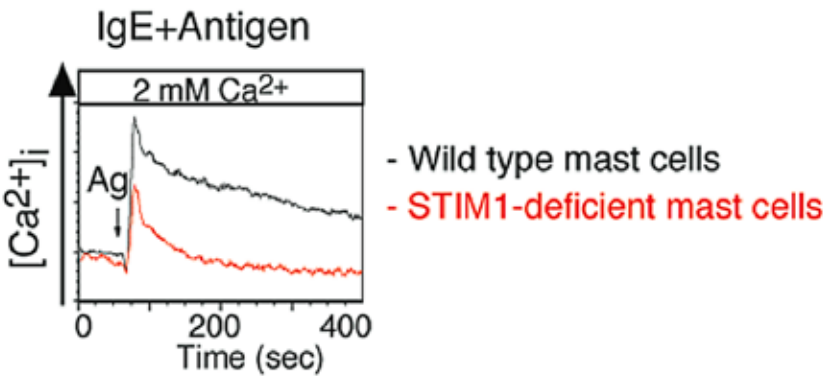

Figure Ca^{2+}-mobilization profiles in the presence of 2 mM Ca^{2+} in anti-DNP IgE sensitized wild type and STIM1-deficient mast cells after stimulation with DNP-HSA antigen. Ca^{2+}-mobilization was impaired in STIM1-deficient mast cells. $[Ca^{2+}]$ i : intracellur calcium concentration

Recent publications

Aiba, Y., Kameyama, M., Yamazaki, T., Tedder, T.F. and Kurosaki, T. Regulation of B cell development by BCAP and CD19 through their binding to phosphoinositide 3-kinase. (2008) *Blood* 111, 1497-1503

Baba, Y., Nishida, K., Fujii, Y., Hirano, T., Hikida, M. and Kurosaki, T. Essential function for the calcium sensor STIM1 in mast cell activation and anaphylactic responses. (2008) *Nat. Immunol.* 9, 81-88

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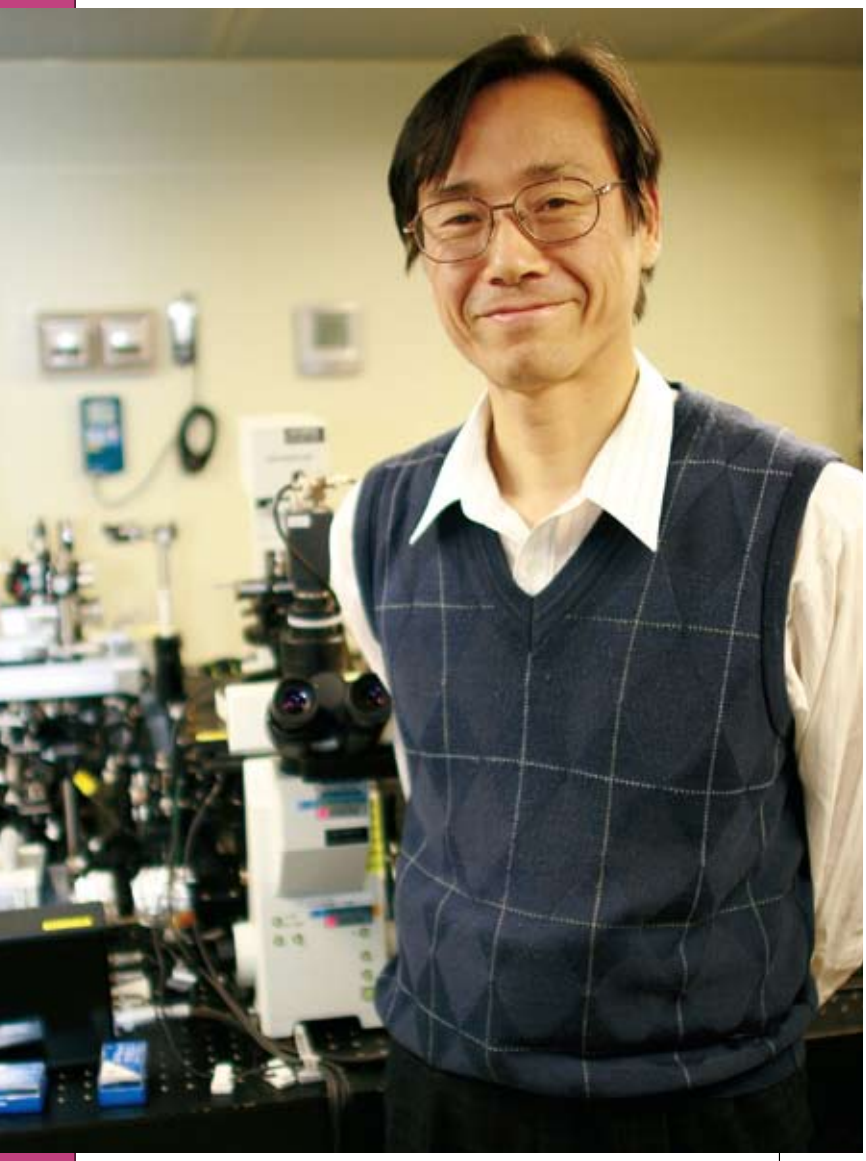
Sanjo, H., Hikida, M, Aiba, Y., Mori, Y., Hatano, N., Ogata, M. and Kurosaki, T. Extracellular signal-regulated protein kinase 2 is required for efficient generation of B cells bearing antigen-specific Immunoglobulin G. (2007) *Mol. Cell. Biol*. 27, 1236-1246

Research Unit for Molecular Systems Immunology



Unit leader

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Technical Staff : **Yoshiyuki Sasaki**
Tatsuki Yasuda
Makiko Ino

Assistant : **Yukiko Tamate**

Our laboratory has developed technologies that allow immunological 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 for Molecular Quantification

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 that uses evanescent light to illuminate single molecules. TIRF has become a widespread technique for single-molecule imaging at surfaces, but cannot be used for single-cell imaging due to the very limited depth of evanescent light. We have achieved notable success in overcoming this limitation by inclining the illumination beam and by minimizing the illumination area.

Highly inclined and laminated optical sheet (HILO) microscopy is built upon the TIRF approach (Fig. 1). Illumination by a highly inclined and thin beam increases image intensity and decreases background intensity, yielding a signal/background ratio about eightfold greater than that of epi-illumination. Most importantly, HILO can be used to visualize single molecules not only on cell surfaces but also inside living cells.

Single molecule microscopy using HILO and TIRF can be used for observations within living cells of molecules that are sensitive to the illuminated light, and for time-lapse observation over long periods since only weak illumination is required. Using HILO, the dynamic movements of large numbers of single molecules inside a cell can be traced. Thus, HILO is also useful for quantitative studies of the distribution, dynamic movement, or interaction of large numbers of

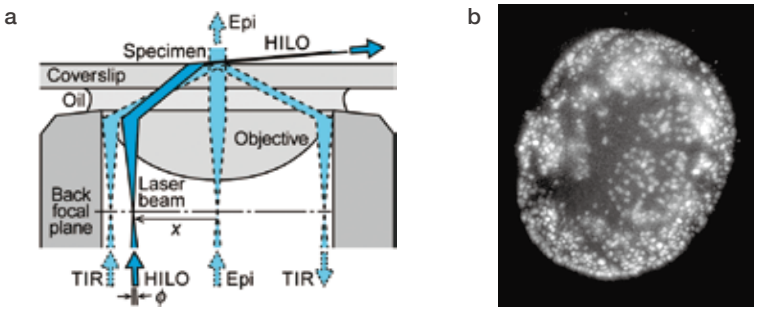

Figure 1 Molecular imaging enables one to visualize and quantify molecular dynamics, interactions, and kinetics in cells for molecular systems biology. (a) HILO microscopy for molecular imaging in cells. Illumination by a highly-inclined and thin beam increases image intensity and decreases background 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 reconstructed a three-dimensional image from serial images of nuclear pore complexes labeled with EGFP-importin β in an MDBK cell. Scale bar, 5.0 μm.

molecules. During the first phase of development we achieved single-color HILO, but now we have installed a new multi-color system to observe intermolecular interactions in ever greater detail.

Quantification of Molecular Kinetics and Interactions in Cells

To explore potential new uses of this technology, we performed quantitative analysis on nuclear import to demonstrate its application to kinetic studies (Fig. 2 left). 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 at a concentration at or below the nanomolar range. The interaction time of single molecules with nuclear pore complexes (NPCs) was determined base on the duration of the fluorescent spots.

At greater than nanomolar concentrations of GFP-importin β, individual NPCs were clearly visualized as fluorescent spots (Fig.1b). The number of GFP-importin β molecules bound to a single NPC could be estimated by determining the ratio of the fluorescence intensities of single NPC images to that of single molecule images. We determined the number of bound molecules as a function of the GFP-importin β concentration. Importin β in the absence of RanGTP exhibited two types of binding with the NPC. The higher affinity binding showed a dissociation constant of approximately 0.3 nM.The maximum number of bound molecules was calculated to be approximately 7 molecules/NPC, although the actual number is probably 8 molecules/NPC since the NPC exhibits an 8-fold symmetry. The lower affinity binding exhibited almost the same kinetic parameters irrespective of the presence or absence of cargo, with a dissociation constant of 70+50/-30 nM and a maximum bound number of 110 +60/-40 molecules/NPC.

We then examined the rate of translocation into the nucleus of cargo or importin β at the single-cell level. The rate of nuclear import was obtained as the maximum slope in the time course of nuclear accumulation. The import rate was plotted as a function of the cargo-importin β concentrations, and it was fitted to a Michaelis-Menten equation with a Michaelis constant of 54 ±16 nM. The import rate showed a significant correlation with the inverse of the retention time, which represents the frequency of translocations per second of single molecules. The correlation coefficient was approximately 8 molecules/NPC, which exhibits the number of the translocation sites into the nucleus per NPC. This corresponds well with the 8-fold symmetry of the NPC structure.

"*In silico*" Modeling and Simulation

As shown above, 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 (Fig. 2 left). 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.

As demonstrated herein, the combination of single molecule quantification and modeling opens new approaches for developing molecular system biology.

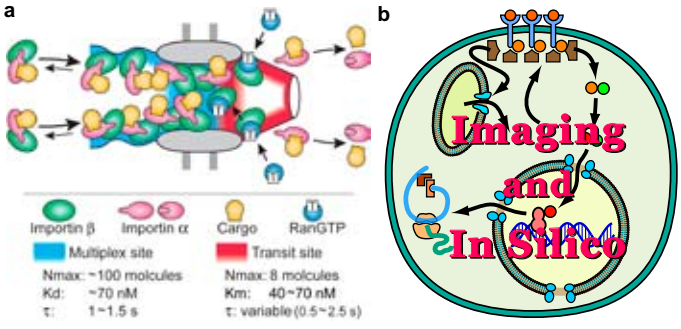

Figure 2 The combination of single molecule quantification and "in silico" modeling opens new approaches for developing molecular systems biology. (left) Molecular interactions with the assembled NPC were quantified by single molecule analysis. Retention times, the number of associated molecules, the dissociation constant, and stoichiometry of import were all determined. Simulation based on a model with two types of multi-binding sites using these parameters fit well with the molecular kinetic features in cells. (right) Aiming at understanding immune cells as molecular systems, we are going 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. dynamics, interactions, and kinetics in cells for molecular systems biology.

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Laboratory for Epithelial Immunobiology

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Assistant : **Yuuki Yamada**

The mucosal epithelium that lines the inner surfaces of the body, especially within the intestine, is exposed to various antigens, such as food-borne macromolecules and microorganisms, whose efficient uptake is vital for maintaining appropriate immune response. Epithelial cells that overlay the gut-associated lymphoid tissue (GALT), such as Peyer's patches (PPs) and isolated lymphoid follicles, are distinct from normal absorptive epithelial cells of the villi and are termed follicle-associated epithelium (FAE). The FAE itself 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. Despite their significance, the study of M cells has remained in its infancy until recently, mainly because the low frequency of M cells and the lack of specific surface markers has made it difficult to purify the M cells for molecular/biochemical analyses. Accordingly, 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 the mucosal and systemic immune system. These studies may lead to the development of novel and more efficient mucosal vaccination protocols/drug delivery systems as well as functional foods/preventive medicine based on host-microbiota interactions.

Identification of M cell-specific molecules

Although M cells are believed to play an important role in mucosal immunity, the molecular mechanisms of antigen uptake and transport remain undiscovered. Notably lacking is the identification of endocytic receptors on the apical membrane of M cells. Our transcriptomic analysis has identified several M-cell-specific genes. One of them, glycoprotein-2 (GP-2) was originally reported to be abundantly expressed in pancreatic acinar cells and secreted in the pancreatic fluid, although its physiological role remains elusive. We found that GP-2 was expressed on the apical plasma membrane of M cells of both mice and humans, and propose that GP-2 is a novel, universal M-cell marker. Our data further suggest that GP-2 may function as an endocytic receptor for a subset of intestinal bacteria and could serve as a potential target for M-cell-mediated oral vaccination

and/or drug delivery.

Another M-cell-specific gene identified in our screen encodes a novel protein with some homology to one of the Sec family members, and was thus designated M-sec. The Sec family molecules are involved in the vesicular transport among the organelles of the secretory pathway in eukaryotic cells, originally identified with yeast genetic screenings. In the intestinal epithelium, M-sec was exclusively expressed in M cells and was also detected in splenic macrophages and dendritic cells. Exogenous expression of M-sec in HeLa cells resulted in the formation of numerous membrane tubules extending from the plasma membrane. These eventually fused with the plasma membrane of nearby cells to form intercellular membrane tubules reminiscent of tunneling nanotubules (TNT). RNAi-mediated knockdown of M-sec in a macrophage cell line significantly suppressed TNT formation, suggesting that M-sec is a key regulator of TNT formation. We have recently established M-sec knockout mice, and the physiological role(s) of M-sec in M cells and macrophages/dendritic cells are under investigation.

Psg18 is specifically expressed in FAE

Pregnancy-specific glycoproteins (Psgs) secreted by the placenta regulate the immune system to ensure survival of the fetal allograft by inducing IL-10, an anti-inflammatory cytokine. However, it is unknown whether Psgs are involved in more general aspects of the immune response. We discovered that the Psg18 gene was highly expressed in the FAE. Bioinformatics analysis with RefDIC (http://refdic.rcai.riken.jp/welcome.cgi), as well as RT-PCR analysis demonstrated that Psg18 mRNA was exclusively expressed in FAE in adult mice, in contrast to other Psg family member genes that were either not expressed or only expressed at low levels in FAE. Psg18 gene expression was observed in FAE of germ-free mice, and was slightly upregulated after bacterial colonization. In situ hybridization analysis revealed that Psg18 was widely expressed throughout the FAE. Furthermore, Psg18 protein was deposited on the extracellular matrix in the subepithelial dome region (SED), beneath the FAE, where antigen-presenting cells accumulate. Taken together, these results suggest that Psg18 is an FAE-specific marker protein that could promote the interplay between FAE and immune cells in mucosa-associated lymphoid tissues by inducing IL-10 production from macrophages and/or dendritic cells in the SED for the maintenance of anti-inflammatory/immunosuppressive condition of GALT.

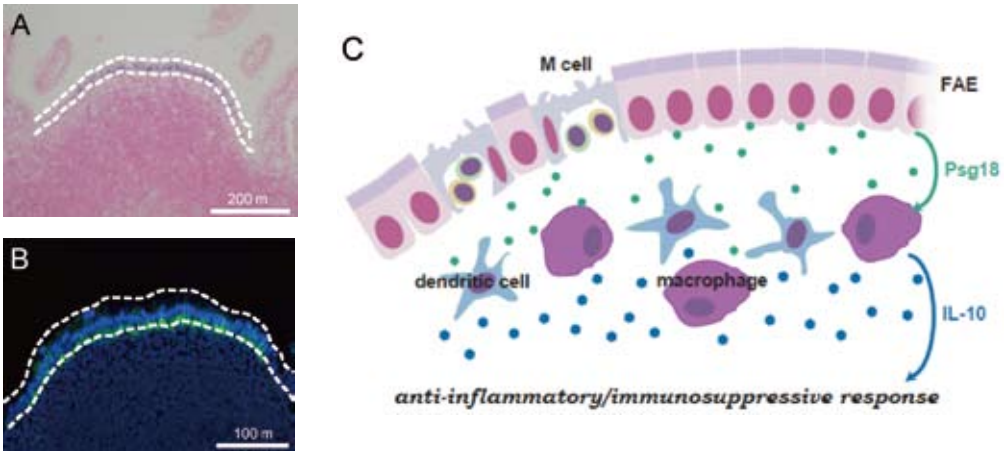

Figure Psg18 is specifically expressed in FAE
(A) In situ hybridization demonstrates FAE-specific expression of Psg18.
(B) Immunofluorescence staining of Psg18 indicates that Psg18 is secreted basolaterally by FAE toward the SED region.
(C) Model depicting the possible role of Psg18 in the induction of IL-10 secretion by dendritic cells/macrophages leading to an anti-inflammatory/immunosuppressive response.

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Takatsu H., Hase K., Ohmae M., Ohshima S., Hashimoto K., Taniura N., Yamamoto A., Ohno H. CD300 antigen like family member G: A novel Ig receptor like protein exclusively expressed on capillary endothelium. *Biochem. Biophys. Res. Commun.* 348, 183-191 (2006)

Laboratory for Mucosal Immunity

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* until June, 30th

Vertebrates coexist with an extraordinarily dense and diverse bacterial community that provides metabolic traits, competes with the growth of pathogenic microorganisms, and ensures the development and fitness of the immune system. To peacefully coexist with more than 500-1000 bacterial species, the host has developed highly sophisticated and efficient regulatory mechanisms that prevent chronic inflammation. Our laboratory aims to understand commensal host-bacterial relationships in the gut, with multiple feed-back and feed-forward controls linking the bacteria and immune cells, and to characterize cellular and molecular interactions responsible for tolerance and immunity in the intestinal mucosa.

Requirement for adult RORγt+LTi cells in the formation of isolated lymphoid follicles (ILF) and T-independent generation of IgA in the gut

Lymphoid tissues-inducer (LTi) cells were first identified as hematopoietic-derived cells that are essential for lymph node and Peyer's patch formation during embryonic development. Recently, RORγt$^+$ cells with a phenotype similar to that of embryonic LTi cells (Lin$^-$CD4$^{+/-}$IL7Rα$^+$) were also identified in the gut of adult mice. However, their role remained totally unknown.

We found that formation of isolated lymphoid follicles (ILFs) is regulated by interactions between RORγt$^+$LTi cells and stromal cells (SCs). Activation of SCs by RORγt$^+$LTi cells through LTβR and simultaneously by bacteria through TLRs induces recruitment of dendritic cells (DCs) and B cells, and formation of ILFs (Figure 1A).

We further found that ILFs are sites where induction of AID and IgA class switching take place in the absence of T cells. This is a novel finding, against the prevalent view that IgA synthesis in all organized lymphoid structures is T cell-dependent. We demonstrate that TNFα, produced by the local dendritic cells (DCs) and RORγt$^+$LTi cells, induces IgA class switching by mediating conversion of TGFβ from its latent to active form (Figure 1B). Our findings provide insight into the crosstalk between bacteria, RORγt$^+$LTi cells, SCs, DCs, and B cells required for ILF formation and establish a critical role of ILFs in T cell-independent IgA synthesis in gut.

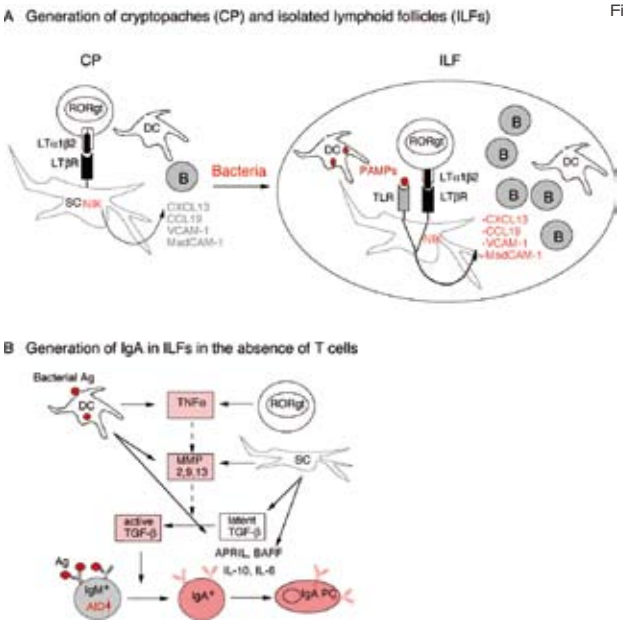

Figure 1. Proposed model for CP and ILF formation and generation of IgA in ILFs in the absence of T cells. (A) The formation of CPs requires the presence of RORγt⁺LTi cells and their interaction with SCs through LTβR and NIK. This interaction leads to activation of SCs and production of adhesion molecules and chemokines, which are involved mainly in the recruitment of DCs. CPs receive signals from gut bacteria, most likely through the DCs. Concomitant signals from RORγt⁺LTi cells through LTβR and bacteria through TLRs further augment the activation of SCs, leading to recruitment of B cells and organization of ILF. (B) The recruited B cells are then activated by antigen presenting DCs or by polyclonal stimulation by the captured microbes. Activated B cells up-regulate AID and undergo class switching to IgA, even in the absence of T cells. TNFα secreted by both Lin-RORγt⁺ cells as well as activated DCs induces production of matrix metalloproteinases (MMPs) that activate latent TGFβ, leading to preferential class switching to IgA in the gut. APRIL, BAFF, IL6, and IL10 produced by SCs or DCs further enhances the survival of IgA⁺ B cells and their differentiation into IgA plasmablasts/plasma cells.

Role of TLR-MyD88 signals for B cell recruitment, activation and class switch recombination in Peyer's patch germinal centers.

Germinal centers (GCs) are recognized as the main sites for generation of IgA in the Peyer's patch (PP), yet the mechanisms that control B cell recruitment, activation, and IgA production in GCs are not completely understood.

We found that bacterial stimulation through TLR-MyD88 regulates follicular maturation and GC reaction in the gut immune system. Unexpectedly, we found that TLR-stimulation of both the BM-derived compartment (B cells and DCs) and the stromal cell compartment (follicular dendritic cells FDC) is required for induction and maintenance of GCs in PPs. Stimulation of FDCs through the TLR-MyD88 pathway partly regulated PP GCs formation by recruitment of B cells and activated helper T cells to the PP follicles through enhanced secretion of the specific B cell attractant chemokine CXCL13. We further demonstrated that PP FDCs support preferential switching of activated B cells to IgA through abundant production of TGFβ (Figure 2).

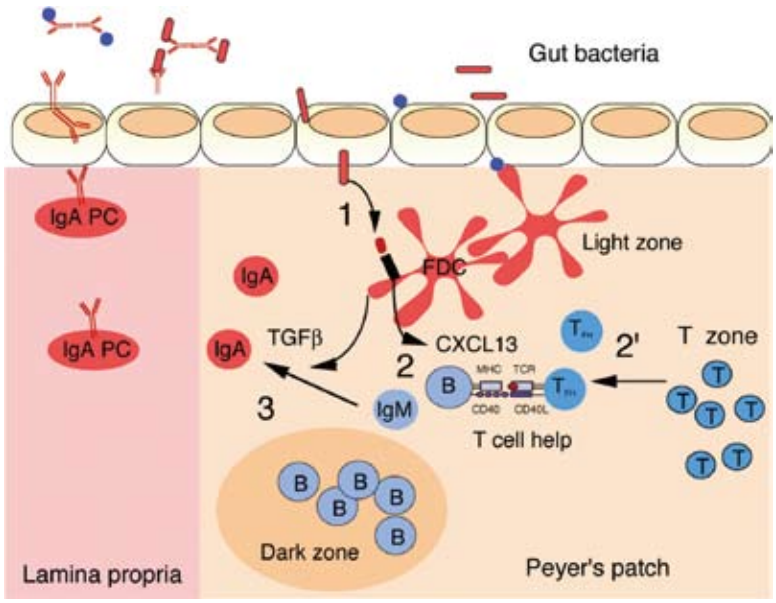

Figure 2. Role of TLR stimulation of FDCs in GC formation and IgA synthesis in the gut. FDCs located in the light zone of the PP follicles express TLRs. They respond to stimulation through TLRs by secreting factors, such as like CXCL13, that promote migration and survival of B cells and CD40L expressing T cells (Follicular helper T_{FH} cells). B cell-T cell interactions through MHC II-TCR are critical for the GC reaction in PPs. Other factors secreted by FDCs, like TGFβ, facilitate preferential class switching of B cells from IgM to IgA. IgA B cells generated in GC of PPs migrate to the lamina propria, where they further differentiate into IgA-secreting cells. IgA transported and secreted into the intestinal lumen prevents bacterial access to the epithelium.

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Laboratory for Immunological Memory

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Junko Taka

Assistant : **Tomoko Takahashi**

The traditional view supports the concept that high-affinity B cell variants generated in the germinal centers (GCs) differentiate into long-lasting antibody-forming cells and memory B cells. This scenario is based primarily on the observation of affinity maturation and the accumulation of a large number of somatic mutations in antigen-specific hybridoma cell lines established from the secondary response. Furthermore, a rapid reduction in the number of Ig^+ GC B cells during the second week of the response coincided with the appearance of memory phenotype B cells, leading to the idea that GC B cells convert to memory B cells late in the immune response. In contrast, we have previously suggested that at least some of the memory B cells are generated during the early immune response, probably prior to the development of germinal centers. However, the population dynamics during memory B cell development remain largely unknown, making the experimental resolution of this important issue difficult.

Establishment of memory lineage precursors during the early phase of the immune response

To address this question, we have used a 6-color FACS system to analyze the origin, selection, function and gene expression profiles in memory B cells at different time points after immunization. We observed that, in contrast to the traditional view, antigen-specific memory precursors were identified in the spleen concurrently with GC lineage cells from day 5 to day 6 after immunization, accompanying efficient proliferation and isotype-class switch from IgM to IgG1 . Memory precursors are subsequently recruited into the $IgG1^+$ memory compartment and sustained for a long period, although these cells appear to attain full functional development as the immune response progresses. Cluster analysis for all microarrays demonstrate that memory B cell pools at different time points after immunization are a population independent from GC B cell and plasma cell populations and naïve B cells. NP-specific $IgG1^+$ memory B cells established a unique VH gene repertoire during the early immune response, a repertoire distinct from that of GC B cells, raising the question of whether GC B cell progeny are in fact recruited into the memory compartment. We concluded from these results that memory precursor cells

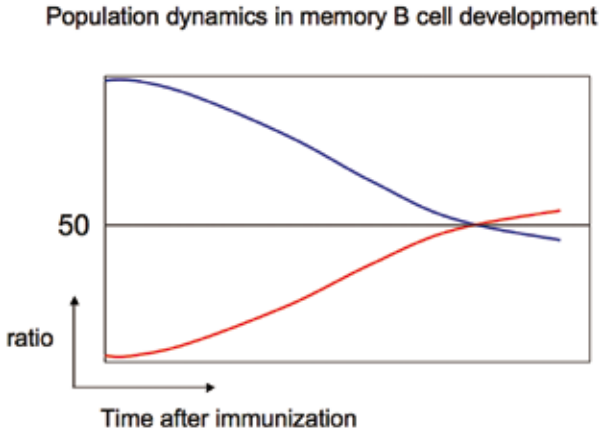

Figure The population dynamics underlying memory B cell development. Antigen-specific memory precursor cells with no accumulation of somatic mutations develop within the first wk of the response (blue line) and establish the IgG1 memory B cell compartment. However, owing to their limited lifespan, the number of these cells is gradually reduced as the immune response progresses. Meanwhile, the memory compartment is slowly and persistently replaced by newly arising memory cells, probably originated from GCs, that have an accumulation V gene mutations (red line),.

are established prior to GC development during the early phase of the immune response. There is an accompanying expression of transcripts unique in this population, which appears to be the major contributor to the long-term memory compartment.

Molecular mechanism underlying memory lineage commitment

To understand the intrinsic differences in IgG1$^+$ memory B cells and other type of B cells at different time points after immunization, we characterized gene expression profiles by using the Affymetrix GeneChip technology. Q-PCR revealed that memory B cells expressed a group of transcripts selectively enriched in this population, with similar time-dependent changes in their expression patterns. The first group of transcripts was maintained throughout the immune response from day 7 to day 40 post-immunization, whereas the levels of the second group of transcripts increased or decreased in memory phenotype B cells as the immune response progressed. Thus it appears that time-independent and time-dependent transcriptional regulatory mechanisms affect memory B cell commitment and development, probably under the influence of antigen encounter, T-cell interactions, and microenvironmental influences. According to the expression pattern and expected revitalization, we have finished the construction of targeting vectors for three of these genes to explore their roles in memory B cell development and have established one mutant mouse line deficient in a signaling molecule that we have found to be enriched in memory B cells.

Deficiency in immunological memory

The survival of motor neuron (SMN) gene is expressed in all mammalian tissues but at particularly high levels in α-motor neurons during embryonic development and in activated B cells. The human SMN gene is duplicated on chromosome 5q13 as one telomeric copy (*SMN1*), which is composed of seven exons and encodes an intact SMN protein, and the centromeric *SMN2*, which predominantly encodes a truncated isoform lacking exon 7 due to a silent non-polymorphic nucleotide transition in the exon 7.

Disease-causing missense mutations in SMN1 mostly localize in the C-terminal region encoded by exons 6 and 7, which is essential for SMN activity. Accordingly, homozygous mutations or a loss of SMN1 result in the predominant production of a differentially spliced form of SMN2 lacking the 16 C-terminal amino acids (SMNΔ7). Expression of this truncated protein triggers spinal muscular atrophy (SMA), which is characterized by degeneration of α-motor neurons in the spinal cord and severe recurrent infections in the severe form, resulting in the death of the affected children before the age of 18 months. Based on the assumption that deletions or missense mutations in the C-terminal region of SMN might affect B cell responses, including B cell memory, in SMA patients, we have analyzed B cell activity in mutant mice with a deletion of SMN exon7. We observed that conditional deletion of SMN exon 7 in Igα$^+$ B cells diminished the number of pre-BCR$^+$ and BCR$^+$ B lineage cells, suggesting am essential role for SMN in B cell development and activation, probably mediated through its pro-survival effect. In agreement with this hypothesis, we demonstrated that high levels of SMN prolonged cell survival by enhancing mitochondrial activity; however, this activity was compromised by deletion of AIF-binding domains or AIF-knockdown in host cells and thus identified SMN-AIF as a functionally active complex. We conclude that SMN forms a complex with AIF and contributes to cell survival, probably through the enhancement of mitochondria activity.

Laboratory for Immune Diversity

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Hiromi Mori

Assistant : **Kanae Fukui**

Germinal center (GC) B cells undergo somatic hypermutation (SHM) and class switch recombination of the immunoglobulin (Ig) genes and can ultimately differentiate into antibody-producing plasma cells or memory B cells. Dysregulation of this terminal differentiation pathway can lead to immunodeficiency, autoimmune diseases and B cell malignancies. The goal of the current research is to understand the mechanism of Ig gene SHM and to obtain new insights into the molecular basis of B cell terminal differentiation.

SHM is initiated by the activation-induced cytidine deaminase (AID); however, the activity of multiple DNA polymerases is required to ultimately introduce mutations. For the past several years, we have been analyzing the roles of a number of low fidelity DNA polymerases, including POLQ and POLH, in the generation of different types of base substitutions during SHM of Ig genes. In parallel, by using a lacZ-transgenic system where there is no positive or negative selection of mutations, we have found that among normal tissues/cell types, the GC B cells are a unique cell population that has an intrinsic property to generate A:T mutations. We aim to clarify the mechanism of A:T mutations and to isolate potential factors that are required to generate these mutations.

The differentiation of GC B cells into antibody-producing plasma cells or memory B cells is a highly regulated complex process but the molecular mechanisms still remain poorly understood. Taking advantage of the large scale microarray experiments carried out at RCAI, we have conducted extensive analysis of gene expression profiles in over 100 different immune cells and have identified a number of uncharacterized genes that are selectively expressed in GC B cells. Although GC B-specific expression of these genes does not necessarily guarantee that they will have specific functions in GC B cells, we hope to obtain new insights into the molecular events that control GC B cell differentiation by elucidating their physiological roles.

Role of the low-fidelity DNA polymerases in the somatic hypermutation of Ig genes

We found that the absence of POLQ resulted in ~20% reduction of both C:G and A:T mutations. Others have shown that POLH deficiency caused an ~80% reduction of A:T mutations. To investigate whether the residual

Pathways for A:T mutations in Ig and non-Ig genes in GC B cells

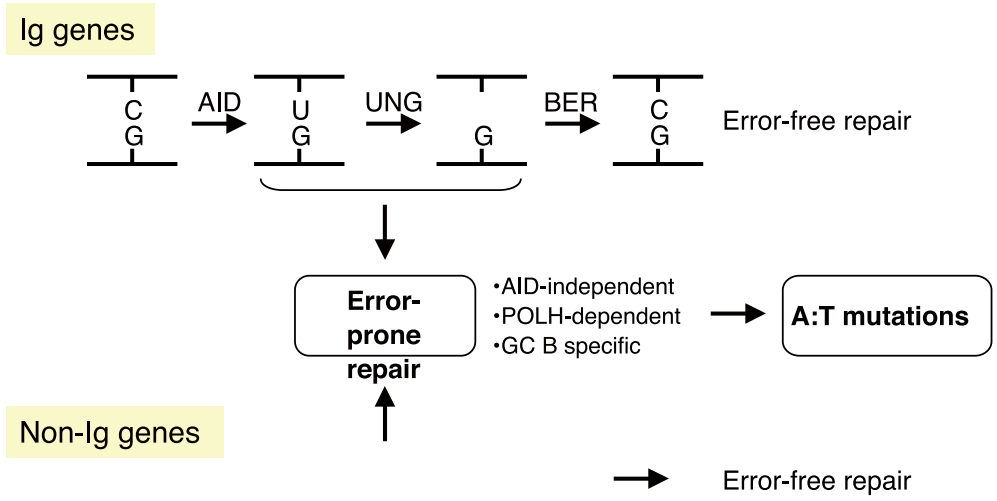

Figure GC B cells have an intrisic property to generate A:T mutations in Ig and non-Ig genes. In Ig genes, AID deaminates cytosine (C) to uracil (U) and generates a U:G lesion. Under normal situations, this U:G lesion is correctly repaired by the error-free base excision repair (BER) pathway. During Ig gene SHM, however, the intermidiates of the BER are resolved by error-prone repair pathways, leading to the mutations at non-damaged A:T pairs. Using a lacZ-trangenic system, we found that GC B cells also efficiently generate A:T mutations in non-Ig genes in an AID-independent but POLH-dependent manner.

A:T mutations observed in the absence of POLH are generated by POLQ and how these two polymerases might cooperate or compete with each other to generate A:T mutations, we have established and analyzed mice deficient for both POLH and POLQ. *Polq*$^{-/-}$*Polh*$^{-/-}$ mice, however, did not show a further decrease of A:T mutations as compared to *Polh*$^{-/-}$ mice, suggesting that POLH and POLQ function in the same genetic pathway in the generation of these mutations. Frequent misincorporation of nucleotides, in particular opposite a template T, is a known feature of POLH, but the efficiency of extension beyond the misincorporation differs significantly depending on the nature of the mispairing. Remarkably, we found that POLQ catalyzed extension more efficiently than POLH from all types of mispaired termini opposite A or T. Moreover, POLQ was able to extend mispaired termini generated by POLH albeit at relatively low efficiency. These results reveal genetic and biochemical interactions between POLH and POLQ and suggest that POLQ might cooperate with POLH to generate some of the A:T mutations during SHM of Ig genes.

Mechanism of increased A:T mutations in GC B cells

To understand why AID-triggered U:G lesions lead to mutations at non-damaged A:T pairs in GC B cells, we decided to compare mutation patterns in non-Ig genes in GC B cells with those in other cells/tissues. For this purpose, we utilized a sensitive lacZ-transgenic (lacZ-Tg) system in which genome mutations can be detected in an unbiased way since there is no selective pressure to obscure the lacZ mutations. We found that approximately half of the mutations in the lacZ gene occurred at A:T pairs in GC B cells, which was in striking contrast to naïve B and non-GC B cells where mutations occurred predominantly at C:G pairs. To examine whether the increase of A:T mutations is a specific feature of GC B cells, we further analyzed mutation patterns in different tissues. Mutations occurred exclusively at C:G pairs in the brain, heart and liver, and only a small fraction of the mutations occurred at A:T in the small intestine. Importantly, the increased A:T mutations in GC B cells were also observed in mice lacking AID, indicating that the induction of A:T mutations is a process independent of AID-mediated Ig gene SHM. These results suggest that GC B cells have an intrinsic propensity to mutate A:T pairs in both Ig and non-Ig genes (Figure). The GC B cells likely express a factor(s) or provide a special environment that allows efficient generation of A:T mutations not only in resolving AID-triggered U:G lesions but also during repair of endogenous DNA damage.

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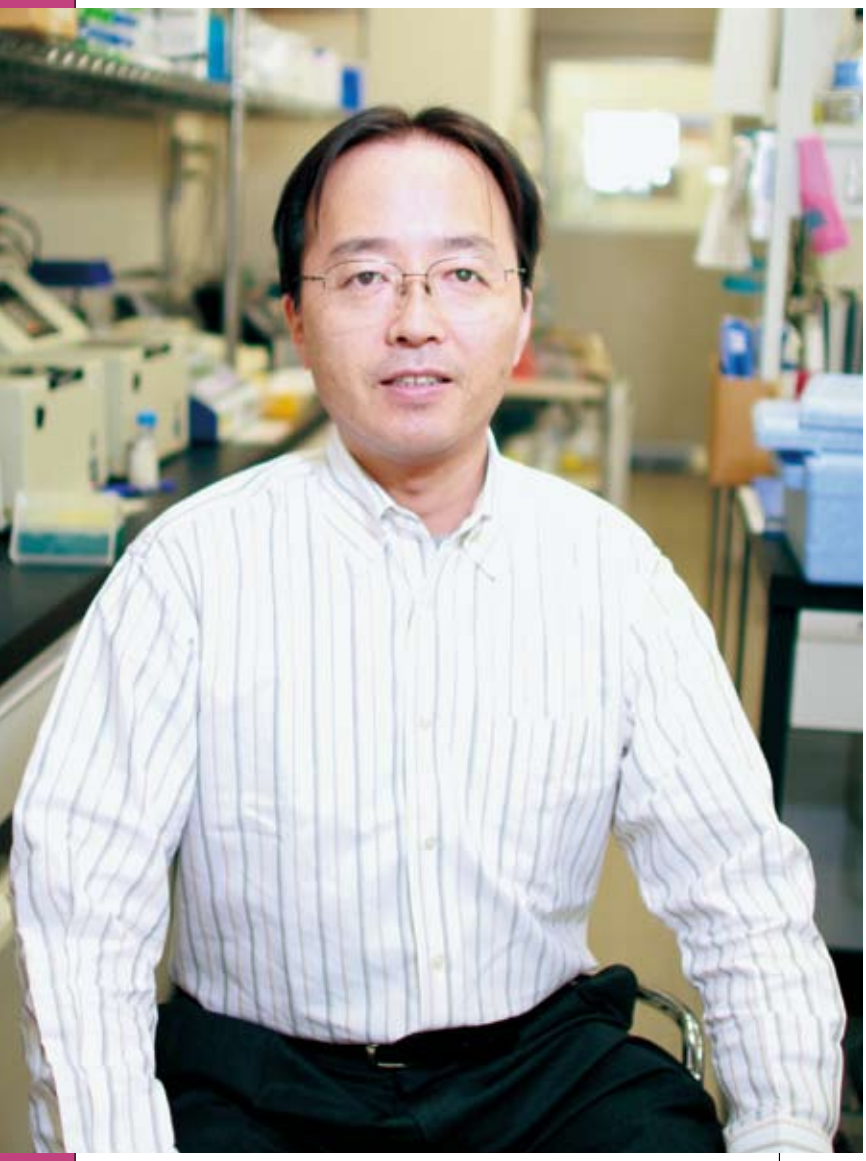
Laboratory for Host Defense



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Host defense in mammals consists of innate and adaptive immunity. Innate immunity functions as a hard wired pathogen sensor and eradicator. Furthermore, innate immunity contributes both to the establishment and the features of an adaptive immune response. Dendritic cells (DCs) are antigen presenting cells critically involved in regulating these immune responses. DCs sense various pathogen-derived molecules and exert their immunostimulatory functions by producing inflammatory cytokines and/or upregulating expression of costimulatory molecules. The pathogen-derived components, termed immune adjuvants based on their DC activating abilities, 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 strategies. We are attempting to clarify how DCs are activated through pattern recognition receptors and to obtain essential information for effectively manipulating the immune response. Various immune adjuvants, TLR ligands, and gene targeted mice are important tools for this purpose.

Immunoadjuvant effects of poly (A:U)

A variety of immune adjuvants, which can be broadly categorized as lipids, proteins, or nucleic acids, can exert their own unique functions. Poly(I:C) is a synthetic mimic of double-stranded RNA (dsRNA) and a known ligand for TLR3 and cytosolic sensors. Another type of dsRNA, poly(A:U), can also act as an immune adjuvant, but it has been unclear how it exhibits its adjuvant effects. We have evaluated the effects of poly(A:U) on a various types of DCs from several gene targeted mice. Poly(A:U) could induce production of both IFN-α and IL-12p40 by murine bone marrow (BM) DCs. Poly(A:U)-induced IFN-α production depended on a particular DC subset, the plasmacytoid DC (pDC), and required TLR7. IL-12p40 was also produced by poly(A:U)-stimulated pDC in a TLR7-dependent manner. In addition to pDC, conventional DC (cDC) also produced IL-12p40 in response to poly(A:U). This IL-12p40 was derived from two cDC subsets, $CD24^{high}$ and $CD11b^{high}$, in a TLR3- and TLR7-dependent manner, respectively. Furthermore, *in vivo* injection of poly(A:U) with antigen led to $CD8^{+}$ T cell responses, which were shown to

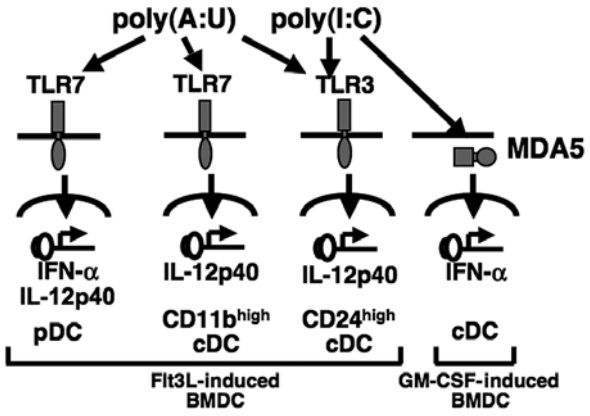

Figure 1 Molecular mechanisms of poly(A:U)-induced adjuvant effects.
Murine BM DCs can be generated in the presence of Flt3L or GM-CSF. Flt3L-induced BM DCs contain both pDC and cDC, the latter of which can be further divided into two subsets. By contrast, GM-CSF-induced BM DCs contain only cDC. Notably, all of these DC subsets are distinct in the expression pattern of and responsiveness to TLRs and cytosolic sensors. poly(A:U) fails to activate cytosolic sensors but can stimulate DCs through TLR3 or TLR7.

result in clonal expansion and IFN-γ production by antigen-specific $CD8^+$ T cells. Consistent with the previous *in vitro* findings, TLR3 and TLR7 were both required for the clonal T-cell expansion. Notably, TLR3 was more critical than TLR7, for generating IFN-γ-producing $CD8^+$ T cells. Interestingly, type I IFNs, which are thought to play a major role in the generation of $CD8^+$ T-cell responses, were dispensable for responses induced by poly(A:U). Our results demonstrate that poly(A:U) is an *in vivo* and *in vitro* immunoadjuvant functioning mainly through TLR3 and TLR7. The next important issue is to clarify the mechanisms by which TLR3 signaling induces $CD8^+$ T-cell responses

The regulation of innate immune responses

A nuclear ubiquitin E3 ligase, PDLIM2/SLIM, interacts with STAT4, the transcription factor essential for IL-12-mediated T-helper 1 (Th1) cell differentiation (Tanaka et al. Immunity 22, 729-736, 2005). PDLIM2 contains a PDZ domain at the N-terminus and a LIM domain at the C-terminus and belongs to a large family of LIM proteins. In $CD4^+$ T cells, PDLIM2 can interact with STAT proteins in the nucleus and promote their polyubiquitination and subsequent proteasomal degradation. Thus, PDLIM2 negatively regulates STAT-dependent signaling. Consistent with these observations, Th1 cells from PDLIM2-deficient mice have increased levels of STAT protein and enhanced IFN-γ production

Activation of the NF-κB transcription factor in innate immune cells is also tightly regulated to prevent excessive inflammatory responses. How NF-κB activation is terminated, however, is not fully understood. After stimulation with various TLR ligands, NF-κB activation is first upregulated, then downregulated and terminated. During the downregulating phase, an NF-κB subunit, p65, is ubiquitinated and degraded in the nucleus. Expression of PDLIM2 suppressed NF-κB activity. PDLIM2 bound to p65 and promoted p65 polyubiquitination in the nucleus. In addition, PDLIM2 targeted polyubiquitinated p65 to discrete intranuclear compartments where it was degraded in a proteasome-dependent manner. This intranuclear translocation of p65 depended on the PDZ domain of PDLIM2. Furthermore, PDLIM2-deficiency led to defective p65 ubiquitination and an increase in nuclear p65 levels. Consistent with these findings, PDLIM2-deficient DCs showed enhanced production of proinflammatory cytokines in response to TLR4 or TLR9 signaling and the mutant mice were hypersensitive to endotoxin shock. Our findings define a pathway by which PDLIM2 terminates NF-κB activation through nuclear sequestration and subsequent degradation.

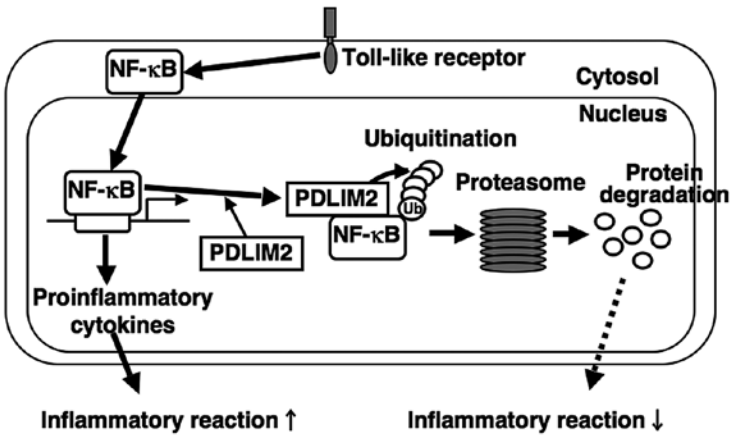

Figure 2
PDLIM2-mediated regulation of NF-κB activity. PDLIM2 can bind and polyubiquitinate the p65 subunit of NF-κB in the nucleus. The p65 is then degraded in specialized nuclear regions in a proteasome-dependent manner.

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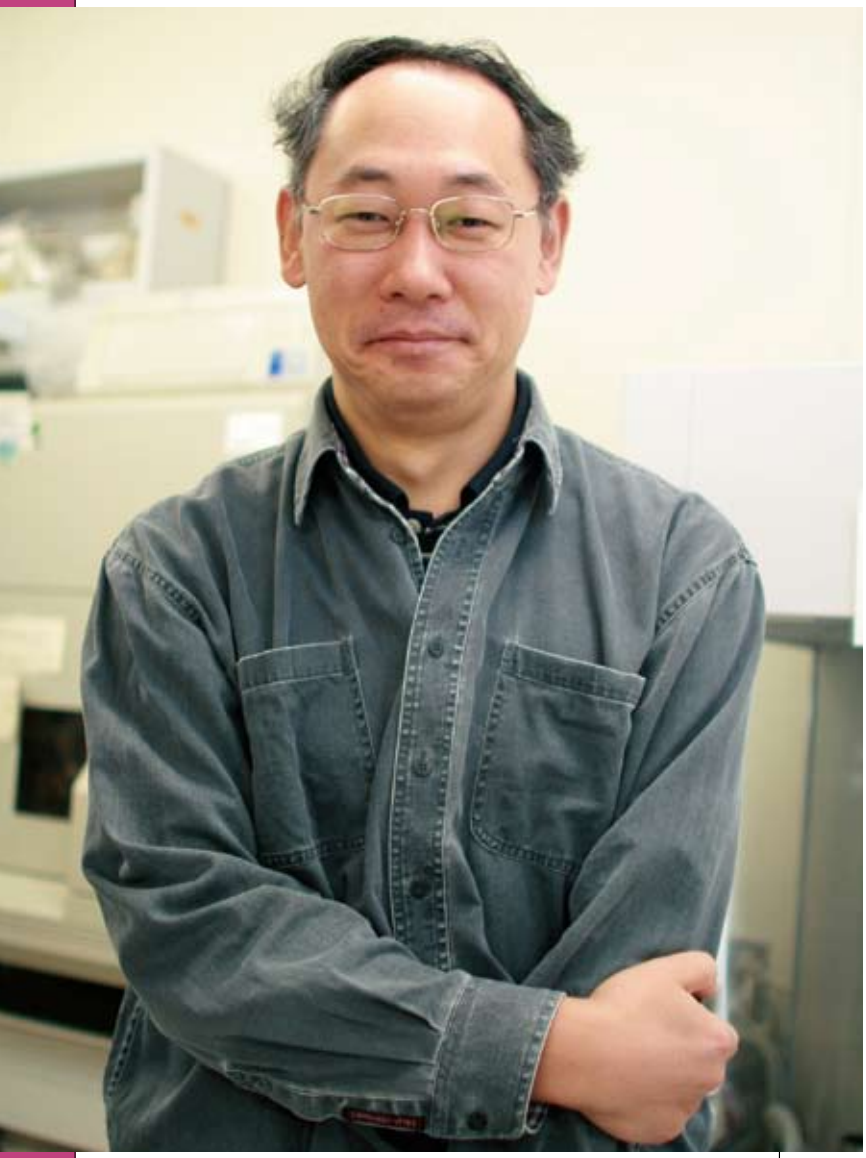
Laboratory for Infectious Immunity



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Recently, we have discovered a novel E3 ubiquitin ligase family, the MIR family, which consists of viral E3 ubiquitin ligases (E3) and their mammalian homologues. These novel E3s are membrane-bound molecules that all have a similar secondary structure and a catalytic domain for E3 activity. All family members have two transmembrane regions in the middle of the molecule and a RING-CH domain at the amino terminus. Forced expression of these novel E3s has been shown to reduce the surface expression of various membrane proteins through ubiquitination of the target molecules. Initial examples of viral E3s were identified in Kaposi's sarcoma associated herpesvirus (KSHV) and murine g-herpesvirus 68 (MHV-68) and have been designated as Modulator of Immune Recognition (MIR) 1, 2 and mK3, respectively. MIR 1, 2 and mK3 down-regulate MHC class I molecule expression, and mK3 is required to establish an effective latent viral infection *in vivo*. The first characterized mammalian homologue of MIR 1, 2 and mK3 is called c-MIR. Forced expression of c-MIR down-regulates B7-2, a co-stimulatory molecule important for antigen presentation. Subsequently, several mammalian molecules related to c-MIR have been characterized and named the MARCH family. However, the physiological function of MARCH family members and the precise mechanism by which they down regulate target proteins is unknown.

Major observations

At present, ubiquitination is thought to play an important role in the degradation of membrane proteins through the induction of endocytosis. In yeast, an E3, Rsp5p, has been reported to induce ubiquitination of the cytoplasmic tail of substrate proteins, a step that is necessary for their endocytosis and degradation. During this year, we were able to show that c-MIR, one of the MIR family members, utilizes a similar mechanism to that of Rsp5p for substrate internalization. To analyze the molecular mechanism of c-MIR-mediated down-regulation, we have generated T-REx-c-MIR, a novel mammalian Tet-on B cell line, where c-MIR expression is induced by adding doxycycline (Dox). By applying the surface biotinylation method to T-REx-c-MIR, we could monitor the fate of target cell surface molecules after initiation of ubiquitination process by doxycycline (Dox)-induced c-MIR expression. Target molecules that pre-existed

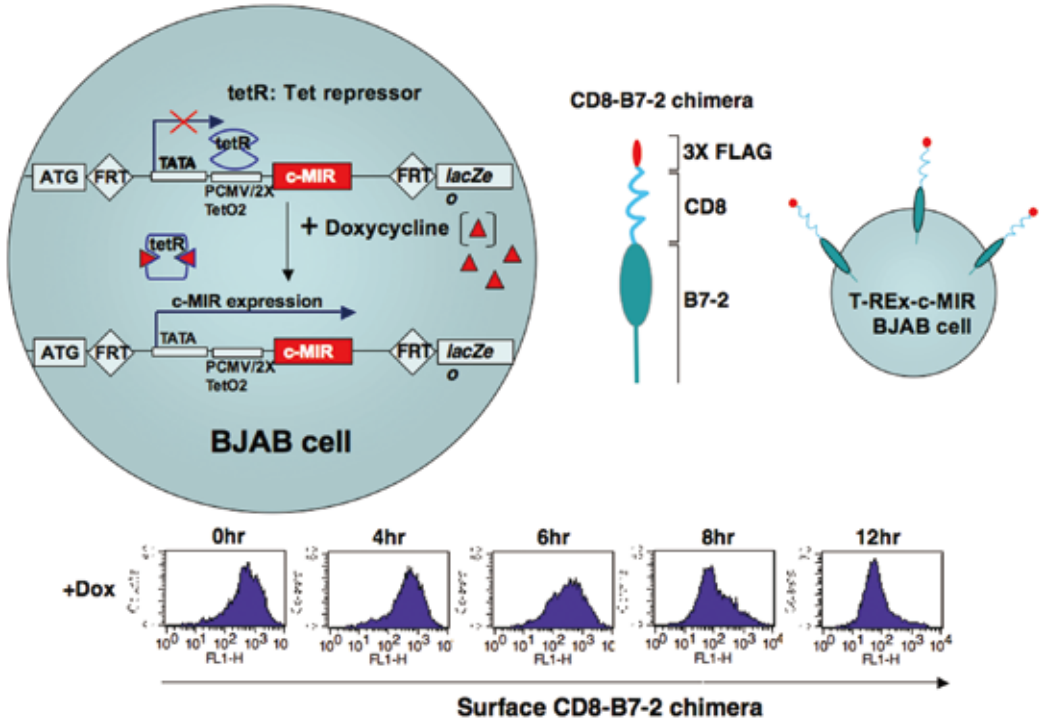

Figure 1 Establishment of the T-REx-c-MIR B cell line

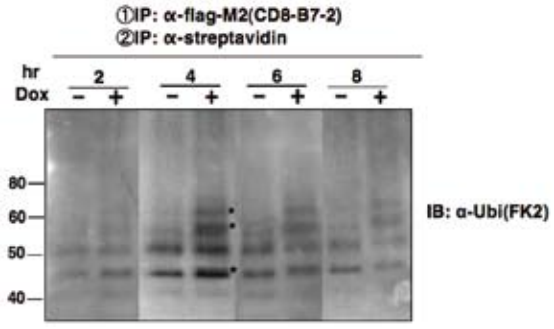

Figure 2 Induction of surface ubipuitination by c-MIR

at the plasma membrane before induction of c-MIR expression were oligo-ubiquitinated and degraded following Dox-induced c-MIR expression. Dox-induced c-MIR expression initiated the rapid internalization of target molecules, and blockage of the internalization caused the accumulation of the surface target molecules that were newly ubiquitinated by c-MIR. Inhibition of the ubiquitination of cell surface proteins by down-regulating the ubiquitin conjugating enzyme E2 impaired the internalization of target molecules. Finally, a complex of c-MIR and the target molecule was detected at the plasma membrane. These results demonstrate that c-MIR induces the internalization of cell surface proteins from the plasma membrane through ubiquitination of their cytoplasmic tails. In addition, the same T-REx-c-MIR approach can be used in transgenic mice to analyze how surface ubiquitination regulates internalization *in vivo*.

We have also explored the physiological functions of MIR family members, and could demonstrate that in APCs, the surface expression of MHC II is regulated through ubiquitination by MARCH-I, but that ubiquitination does not contribute to the internalization of surface MHC II in B cells. In parallel with the stabilization of surface MHC II, we found that MARCH-I-deficient APCs highly expressed exogenous-antigen-loaded MHC II on their surface and had an enhanced ability to present exogenous antigens. Currently we are analyzing MARCH-I deficient mice in order to understand the physiological roles of MARCH-I in the immune system.

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Laboratory for Dendritic Cell Immunobiology

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Dendritic cells (DCs), the most potent of the antigen (Ag)-presenting cells (APCs), were originally defined by their dendritic morphology but are now known to be heterogeneous, both in terms of lineage, myeloid or lymphoid, as well as maturity in both lymphoid and peripheral tissues. Immature DCs (iDCs) sense the presence of invading pathogens via various pattern recognition receptors (PRRs) and process the pathogens intracellularly in inflamed tissues, developing into mature DCs (mDCs) characterized by the upregulation of major histocompatibility complex (MHC), and costimulatory molecules. Subsequently, mDCs home into secondary lymphoid tissues where they present the processed Ags to naïve T cells to generate effector T cells. Thereby, DCs play a crucial role in the link between innate and adaptive immunity. Accumulating indirect evidence suggests that iDCs are involved in the induction of peripheral tolerance under steady-state conditions *in vivo*. On the other hand, the modification of iDCs with certain immunosuppressive molecules generates tolerogenic DCs, which not only show a reduced T-cell stimulatory capacity but also induce anergic T cells and regulatory T (T_R) cells. We have previously identified modified DCs that have a capacity to produce anergic T cells as well as T_R cells than previously characterized tolerogenic DCs. We have designated this unique DC subset regulatory DCs (DC_{regs}). Our goal is: (1) to clarify the molecular mechanisms underlying the T-cell immunoregulatory function of DC_{regs}, (2) to develop immunotherapy with DC_{regs} for immunopathogenic diseases, and (3) to characterize the specific DC subsets involved in immune regulation.

Regulatory dendritic cells protect against cutaneous chronic graft-versus-host disease mediated through $CD4^+CD25^+Foxp3^+$regulatory T cells

Chronic graft-versus-host disease (cGVHD) is a common cause of morbidity and mortality following allogeneic bone marrow transplantation (alloBMT), however effective strategies for the treatment of cGVHD have not been established. In this study, we examined the therapeutic utility of DC_{regs} for cGVHD in a model of alloBMT in which there is MHC compatibility but incompatibility at multiple minor histocompatibility Ags. Treatment of recipient mice following alloBMT with recipient-type DC_{regs} lead to greater

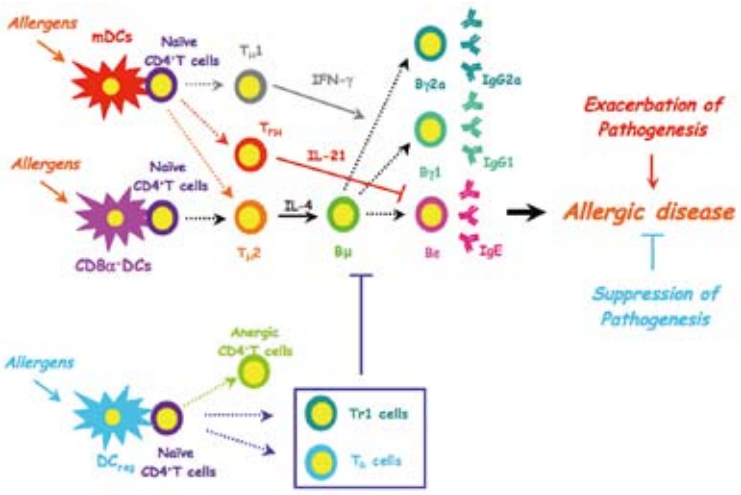

Figure 1 Regulation of allergic response by dendritic cells. DCs capture and process allergen in connective tissues, and subsequently present the processed peptides to naïve $CD4^+$T cells. $CD8\alpha^-$DCs instruct Ag-specific T_H2 cell differentiation in secondary lymphoid tissues. These T_H2 cells not only promote the differentiation of naïve B cells to Bε cells in an Ag-specific manner but also induce local inflammation. These aberrant T_H2 immune responses might initiate and promote allergic diseases. The mDCs not only generate Ag-specific IFN-γ-producing T_H1 cells and IL-21-producing T_{FH} cells but also cause further activation of Ag-specific IL-4- and IL-10-producing T_H2 cells in the T_H2-biased immune response. This activation of the diverse types of T_H response exacerbates allergic diseases despite the inhibition of IgE responses. In contrast, DC_{regs} suppress production of all Ig isotypes and allergic inflammation via the induction of Ag-specific anergic T_H2 cells as well as $CD4^+CD25^-$Tr1 cells and $CD4^+CD25^+T_R$ cells. Thus, an immunotherapeutic strategy using Ag-pulsed DC_{regs} might be beneficial for treatment of TH2-driven allergic diseases.

suppression of both the incidence and severity of cutaneous cGVHD than did rapamycin, a commonly used immunosuppressant. By contrast, treatment with the recipient-type mDCs promoted cGVHD pathogenesis. Analysis of the recipient mice suggested that the protective effect of the DC_{regs} involved the peripheral generation of alloreactive $CD4^+CD25^+Foxp3^+T_R$ cells from donor-derived $CD4^+CD25^-Foxp3^-$ T cells. Thus, immunotherapy with DC_{regs} is a promising strategy for the treatment of cGVHD in alloBMT and appears to be mediated through the induction of dominant tolerance involving $CD4^+CD25^+Foxp3^+T_R$ cells.

Regulatory dendritic cells protect against allergic airway inflammation in a murine asthma model.

DCs are crucial for the induction of immunity and tolerance. There is an a relatively good understanding of how DCs control of T type 1 helper (T_H1)-biased immunity, but little is known about how they regulate T_H2-mediated immunity. Here we report the effects of immunostimulatory mDCs and DC_{regs} on T_H2-driven allergic responses involving IgE production. Ag-pulsed mDCs inhibited Ag-specific IgE production but enhanced the production of Ag-specific IgG1 and IgG2a. Analysis of $Ifng^{-/-}$mice and $Il21r^{-/-}$mice revealed that the inhibitory effect of Ag-pulsed mDCs on Ag-specific IgE production involved IL-21-producing T follicular helper (T_{FH}) cells, but not IFN-γ-producing T_H1 cells. In contrast, Ag-pulsed DC_{regs} impaired the production of Ag-specific IgE, IgG1, and IgG2a. *In vivo* blockade experiments showed that Ag-specific $CD4^+CD25^+Foxp3^+T_R$ cells were the main mediators of the suppressive effect on Ag-specific IgE production by the Ag-pulsed DC_{regs}. Ag-pulsed mDCs promoted airway inflammation, whereas Ag-pulsed DC_{regs} markedly suppressed this pathologic response.

Taken together, the results of our studies indicate that DC_{regs} are able to abolish T_H2-mediated IgE production and allergic inflammation by an Ag-specific dominant tolerance mechanism whereas mDCs exacerbate the pathogenesis, despite inhibiting the IgE response, through the activation of diverse types of T_H cell-responses.

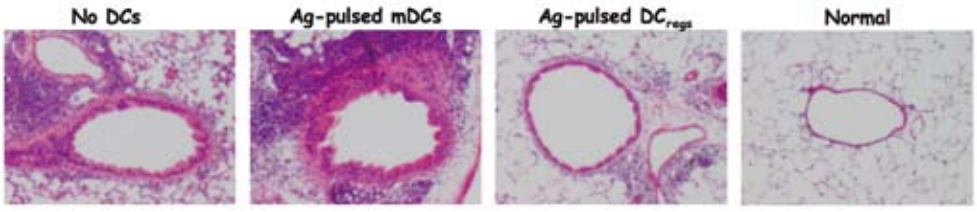

Figure 2 Regulation of asthma-like phenotype by different subsets of dendritic cells. Histology of lung tissues from asthmatic and normal mice (H&E staining).

Recent publications

Fujita S., Yamashita N., Ishii Y., Sato Y., Sato K., Eizumi K., Fukaya T., Nozawa R., Takamoto Y., Yamashita N., Taniguchi M., Sato K. Regulatory dendritic cells protect against allergic airway inflammation in a murine athmatic model. *J. Allergy Clin. Immunol.* 121, 95-104 (2008)

Fujita S., Sato Y., Sato K., Eizumi K., Fukaya T., Kubo M., Yamashita N., Sato K. Regulatory dendritic cells protect against cutaneous chronic graft-versus-host disease mediated through $CD4^+CD25^+Foxp3^+$T cells. *Blood* 110, 3793-3803 (2007)

Sato K., Fujita S. Dendritic cells-Nature and classification. *Allergol. Int.* 56, 183-191 (2007). Review.

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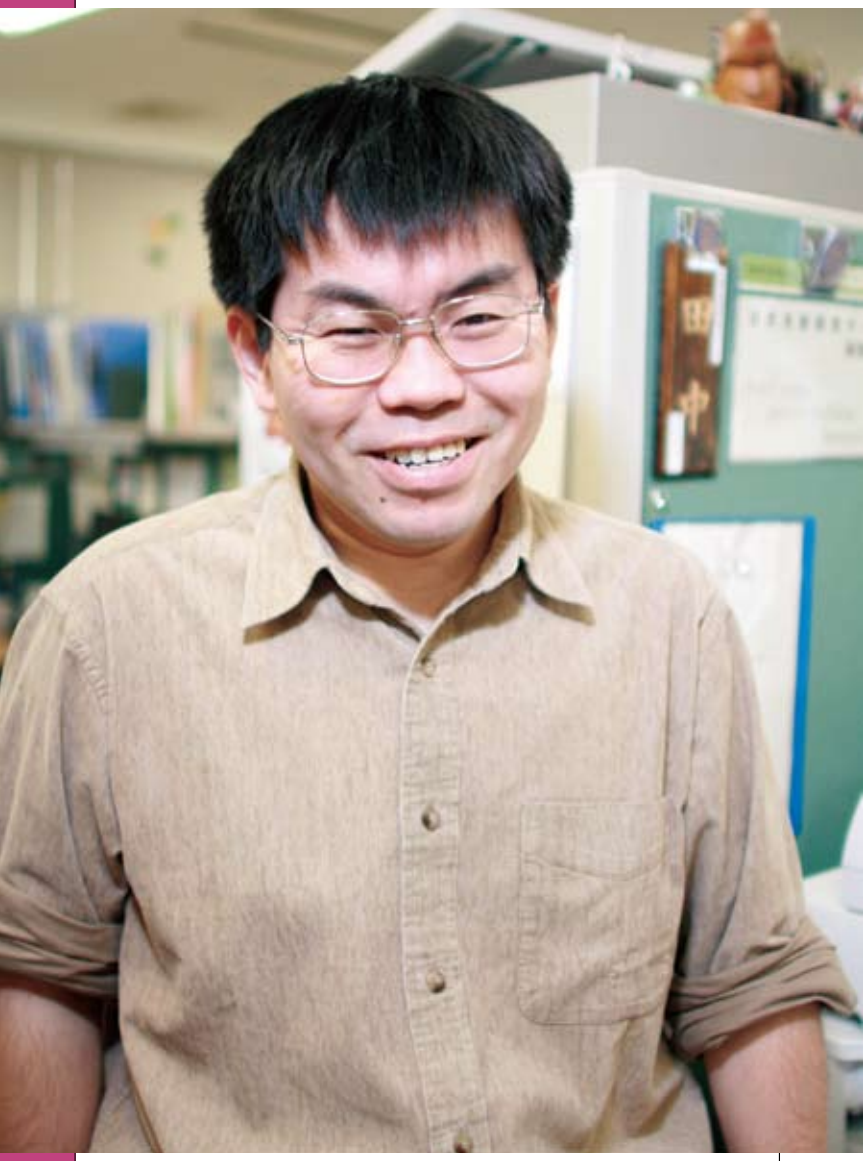
Fujita S., Seino K., Sato K., Sato Y., Eizumi K., Yamashita N., Taniguchi M., Sato K. Regulatory dendritic cells act as regulators of acute lethal systemic inflammatory response. *Blood* 107, 3656-3664 (2006)

Laboratory for Innate Cellular Immunity



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Assistant : **Hiromi Matsuda**

The immune system has evolved to protect against invasion and proliferation of pathogenic microorganisms and for this purpose it is necessary to discriminate "self" from "non-self." To recognize "enemy" non-self, phagocytic cells, such as macrophages and dendritic cells (DCs), possess a wide variety of molecules that bind cellular components found on the surface of microorganisms. These cells ingest microorganisms and digest and kill them in lysosomes. In the process, peptides derived from the enemy are presented in an MHC-dependent manner to T cells for induction of acquired immunity.

On the other hand, the immune system should know what "self" is to avoid attacking host cells and organs. Self antigens are presented by antigen-presenting cells (APCs) in thymus, and developing T cells reactive to these self antigens are eliminated by apoptosis. Theoretically, almost all self-reactive T cells are eliminated in the thymus and never appear in peripheral tissues. However, a small population of self-reactive T cells escapes selection in the thymus and appears in peripheral circulation and tissues. To avoid self-destruction caused by the attack of these autoreactive T cells, they have to be eliminated in peripheral tissues. APCs, such as DCs localized in tissues and organs can collect self peptides by engulfing dead cell corpses and present these peptides in the context of the MHC. When self-reactive T cells that have escaped selection in thymus encouter these APCs, T cell deletion or anergy is induced.

Our laboratory is investigating the molecular mechanisms for recognition and phagocytosis of dying cells by phagocytes, and the pathological relevance of impaired phagocytosis to inflammatory disorders including autoimmune diseases.

Phagocytosis of apoptotic cells by dendritic cells in spleen and tolerance induction to cell-associated antigens

Circulating dying cells are cleared in spleen and tolerance to self antigens expressed in these cells is induced. We have established an experimental model for tolerance induction to cell-associated antigens using experimental autoimmune encephalomyelitis (EAE), a mouse model of human multiple sclerosis. EAE is induced by immunization with myelin oligodendrocyte glycoprotein (MOG) peptide in adjuvant. We found that intravenous injection of apoptotic

cells expressing the MOG fragment suppressed the development of EAE. This was accompanied by a reduction in MOG-specific T cell responses, indicating that tolerance to cell-associated antigens was specifically induced by apoptotic cell injection. Since injected apoptotic cells first accumulated in the marginal zone (MZ) of spleen, we studied the role of MZ macrophages in the induction of immune tolerance by generating transgenic mice in which macrophages in MZ could be transiently deleted by injection of diphtheria toxin (DT). We found that tolerance induction to cell-associated antigens failed in the MZ macrophage-depleted mice, demonstrating a role for these macrophages in tolerance induction. To understand the cellular mechanisms of failure of tolerance induction, we examined phagocytosis of injected apoptotic cells by DC subsets in spleen. $CD8\alpha^+$ DCs preferentially engulf injected apoptotic cells in wild-type mice and can present cell-associated antigens and stimulate proliferation of T cells *in vitro*. On the other hand, the deletion of macrophages in MZ caused aberrant phagocytosis of injected apoptotic cells by $CD8\alpha^-$ DCs. These results indicate that $CD8\alpha^-$ DCs have the potential to phagocytose apoptotic cells *in vivo*, and suggest that the aberrant phagocytosis by these $CD8\alpha^-$ DCs interferes with tolerance induction. We are now investigating the relationship between phagocytosis of apoptotic cells by DC subsets and subsequent immune responses.

Necrotic products enhance migration of dendritic cells

In the early phase of apoptotic cell death, integrity of the plasma membrane is intact; therefore, intracellular materials are not released if the apoptotic cells are rapidly engulfed by phagocytes, as observed under physiological conditions. In contrast, when the rapid removal of apoptotic cells by phagocytes is impaired, the cells undergo secondary necrosis, releasing intracellular materials. In this case, the presence of endogenous immunostimulatory molecules may stimulate DCs and subsequently interfere with the tolerance induction to cell-associated antigens. Endogenous immunostimulatory molecules, such as HMGB1 and heat shock proteins have been reported to induce production of inflammatory cytokines and expression of costimulatory molecules such as CD80 and CD86 by DCs. We recently found that necrotic products also enhance migration of DCs *in vitro*. Necrotic products and LPS synergistically stimulated CCL19- and CCL21-driven migration activity of bone marrow-derived DCs. Expression of CCR7 was not increased by treatment of the bone marrow-derived DCs with necrotic products, suggesting that necrotic products may exert their effect by a novel mechanism, which we are currently attempting to identify.

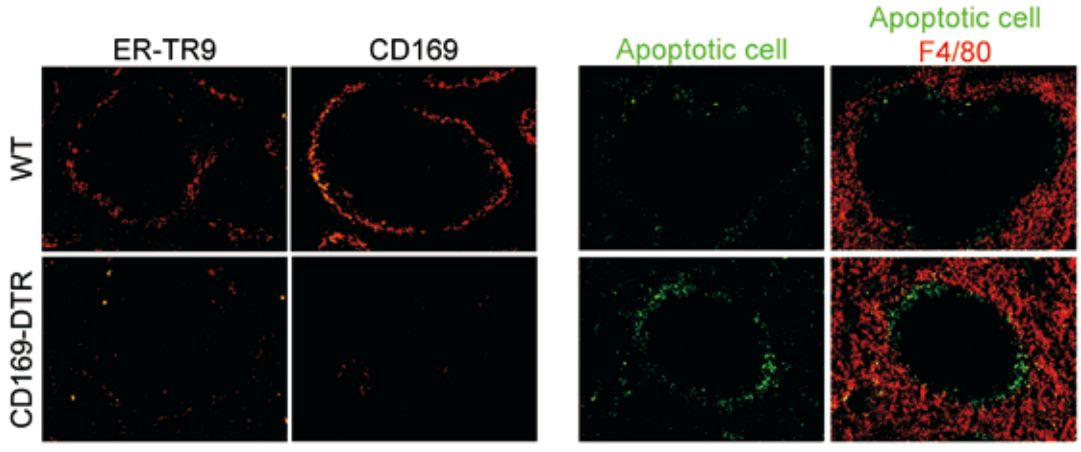

Figure Critical role of marginal zone macrophages in the clearance of intravenously injected apoptotic corpses. The injection of DT induced the deletion of both marginal metallophilic macrophages (CD169 cells), and marginal zone macrophages (ER-TR9 cells) in CD169-DTR mice (*left*). Fluorescently-labeled apoptotic cells were injected intravenously, and the spleens were analyzed by immunohistochemistry. The injected cell corpses accumulated in the marginal zone and rapidly disappeared in wild-type mice. On the other hand, clearance of injected apoptotic cells was severely delayed in DT-treated CD169-DTR mice (*right*).

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Research Unit for Therapeutic Model

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Kanako Shimizu

Assistant : **Kaori Aki**

The goal of our laboratory is to develop a novel therapeutic model for cancer therapy that will lead to clinical application. Our unit has focused on the *in vivo* adjuvant effects of dendritic cells (DCs).

During the last two years, we have used *in vitro* loading experiments to analyze the capacity of different types of antigen presenting cells to present α-GalCer . We found that as long as different types of CD1d expressing leukocytes were able to capture glycolipid *in vitro*, they could stimulate NKT cells. These findings suggested that the costimulatory properties of DCs are not essential in inducing innate immunity, and that perhaps any cell able to present glycolipids might stimulate NKT cells. As a result of this insight, we conducted experiments using α-GalCer-loaded tumor cells instead of DC/Gal and found that these cells could also augment NK cell activity due to “adjunct effects”.

In addition, we have recently demonstrated, by administering ligand-loaded tumor cells, that *in vivo* matured DCs can elicit potent and long-lived adaptive immunity. In this case we took advantage of the ability of DCs to selectively phagocytose and cross-present dying cells to deliver various tumor antigens into DCs. We have continued to evaluate and develop the antitumor response by comparing other approaches.

Tumor cells loaded with α-GalCer induce innate NKT and NK cell-dependent resistance to tumor implantation in mice

DCs loaded with α-GalCer are known to be active APCs for the stimulation of innate NKT and NK cell responses *in vivo*. In this study, we evaluated the capacity of non-DCs to present α-GalCer *in vitro* and *in vivo*, particularly α-GalCer-loaded tumor cells (tumor/Gal). Even though the tumor cells lacked expression of CD40 and the CD80 and CD86 costimulatory molecules, the intravenous injection of tumor/Gal resulted in IFN-γ secretion by NKT and NK cells. These innate responses to tumor/Gal, including the induction of IL-12p70, were comparable to or better than those induced by DC/Gal. B16 melanoma cells that were stably transduced to express higher levels of CD1d showed an increased capacity relative to wild type B16 cells to present α-GalCer *in vivo*. Three different tumor cell lines, when loaded with α-GalCer, failed to establish tumors upon i.v. injection, and the mice survived for at least 6 months. The resistance

against tumor cells was independent of CD4 and CD8 T cells but dependent upon NKT and NK cells. Mice were protected from the development of metastases if the administration of live B16 tumor cells was followed 3 h or 3 days later by the injection of CD1d^{hi}-B16/Gal with or without irradiation. Taken together, these results indicate that tumor cells loaded with α-GalCer are effective APCs for innate NKT and NK cell responses, and that these innate immune responses are able to resist the establishment of metastases *in vivo*.

Cross-presentation of glycolipids from tumor cells loaded with α-galactosylceramide leads to potent and long-lived T cell mediated immunity via dendritic cells.

Mice given tumor/Gal intravenously developed innate NKT cell-dependent immunity against subsequent i.v. tumor challenge. Furthermore, these mice also became resistant to subcutaneous challenge with tumor cells. We propose the following *in vivo* mechanistic scenario. Tumor/Gal cells are initially killed by innate lymphocytes, mainly NKT and NK cells. Subsequently, neighboring DCs capture dying tumor/Gal cells and then are induced to mature by activated NKT cells via CD40-CD40L interactions and inflammatory cytokines. The DCs then were able to induce an adaptive immune response by presenting tumor antigen peptides in such a way as to stimulate the production CD4$^+$ and CD8$^+$ T-cells against that particular tumor (cross-presentation 1). After tumor regression, a variety of T cells responding to specific tumor antigens, such as Trp2, Tyrp, Dct and gp100, persist as memory T cells. In addition, the mature DCs are again able to present the original α-GalCer to NKT cells, stimulating further responses (cross-presentation 2).

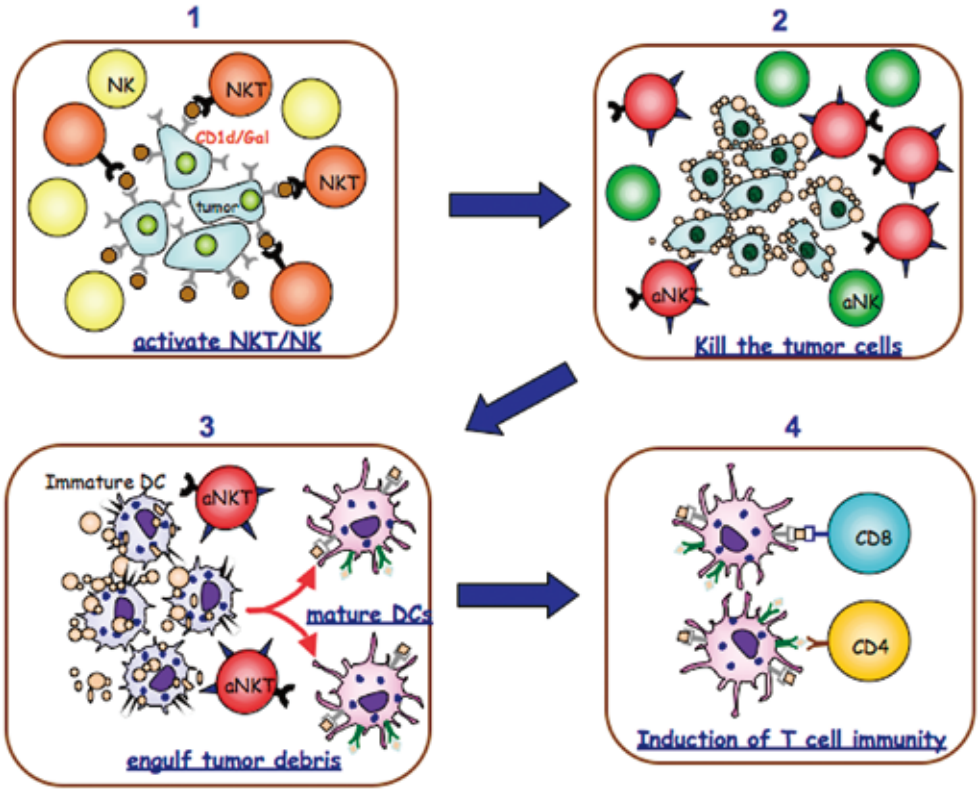

Figure **The sequence of innate and adaptive immunological events after administration of tumor/Gal.**
Four steps are shown.
1: Tumor/Gal activate NKT/NK cells in a CD1d-dependent manner.
2: Activated NKT/NK cells kill the tumor cells including tumor/Gal cells.
3: DCs engulf tumor debris and activated NKT cells mature them at the same time.
4: Mature antigen presenting DCs induce long lived adaptive CD4 and CD8 T cell immunity.

Recent publications

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Laboratory for Autoimmune Regulation

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Type 1 diabetes mellitus is an autoimmune disease caused by the destruction of pancreatic beta cells by autoreactive T cells. In both human and animal models of type I diabetes, class II major histocompatibility (MHC) antigens are the most important genetic factors for disease susceptibility. Furthermore, various environmental factors such as infection and active immunization are known to influence disease development both positively and negatively. The goals of our research group are to understand the disease process and to develop means to modulate this process. Our current projects are: (1) to identify the antigen(s) responsible for the initiation of the disease, (2) to elucidate the biochemical and molecular mechanisms by which certain MHC genotypes confer susceptibility to type I diabetes, and (3) to establish an effective method to regulate disease development *in vivo*. We are also attempting to establish a series of methods to visualize each step, from submolecular, molecular, cellular to whole animal level, in collaboration with different groups in RIKEN.

Screening of diabetes antigens

We have established a number of pancreatic beta cell tumor cell lines expressing diabetogenic antigen(s). Using *in vitro* mutagenesis and selection by T cell mediated killing of antigen positive cells, we have established antigen negative variants of the tumor cell lines. Using these positive and negative cell lines, we will attempt to identify the genes encoding the antigen(s) recognized by diabetogenic T cells.

Class II MHC and presentation of diabetogenic antigens

Based on the previous findings concerning the biochemical nature of diabetes prone class II MHC molecules, we hypothesize that i) poor peptide-MHC interaction generates an unstable complex. ii) this unstable complex exhibits multiple transitional conformations and iii) T cells recognize this transitional conformation to form a stable trimolecular TCR/MHC/peptide complex for activation. To test this hypothesis, we are using diffracted X-ray tracking (DXT) to visualize movement of peptide in the MHC groove.

Regulation of the diabetogenic T cell response by genetic and environmental factors

We have shown that the diabetogenic

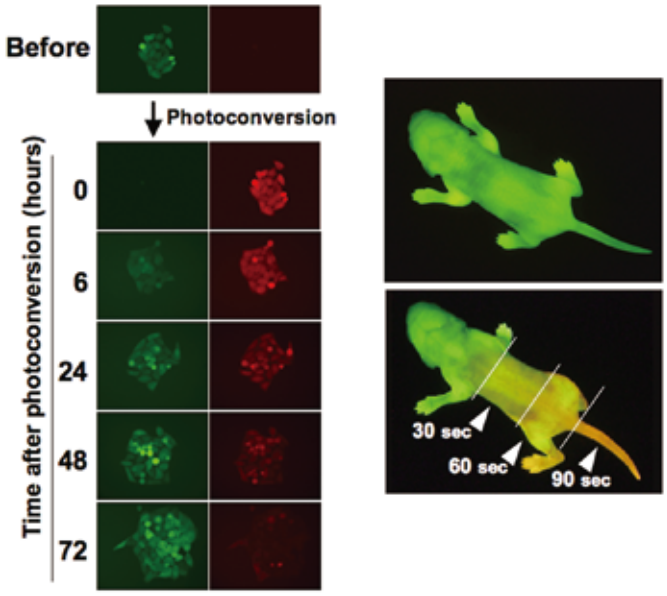

Figure Photoconversion of Kaede in cells and transgenic mouse

process is regulated by the balance of pathogenic T cells and regulatory T cells *in vivo*. We also demonstrated that both genetic and environmental factors differentially affect the generation and function of pathogenic T cells and regulatory T cells. Currently, we are testing whether *in vitro* generated beta cell antigen-specific regulatory T cells can regulate ongoing autoimmune diseases as well as inhibit rejection of transplanted pancreatic islets in the NOD mouse model.

We are currently planning to establish a human TID model. Our basic strategy is as follows: make ES cell lines from pancreatic islet infiltrating lymphocytes (from a DQ8 positive patient), differentiate the ES cells into lymphoid precursor cells, reconstitute NOD. SCID, common cytokine gamma chain deficient, MHC class II deficient and human DQ8 transgenic mice (we already have a mouse line with this genotype in hand) with the lymphoid precursor cells and test T cells from these mice for their reactivity to the beta cell antigen. As a proof of principle, we have successfully carried out experiments using mouse lymphocyte derived ES cell lines. To make human ES cell lines from lymphocytes, we have just begun to collaborate with Dr. Yamanaka's group (Kyoto University) to use lymphocytes as a source of iPS.

Visualization of immune / autoimmune response *in vivo*

1) Visualization of pancreatic beta cell mass *in vivo*

Monitoring beta cell mass is the best method to predict the onset of type I diabetes. In collaboration with different centers in RIKEN, we developed a method to determine the actual bata cell mass with 3D visualization of pancreatic islets in the pancreas (Manuscript in preparation).

2) Monitoring cellular migration *in vivo*

Using transgenic mice expressing the photoconvertible fluorescence protein "KAEDE" (Figure), we have established a method to monitor cellular movement *in vivo* with minimum external manipulations.

3) Functional fluorescence probe

For *in vivo* functional imaging with a two photon fluorescence microscope, we are collaborating with Dr. Miyawaki (BRI Riken Wako) and testing the following fluorescence probes.

SCAT-1: This fluorescence protein can detect active Caspase-3

Cameleon: Similar to SCAT-1, this fluorescence protein detects changes in intracellular calcium levels and is a very good indicator of the initial signal in TCR mediated stimulation of T cells *in vitro*.

Cell cycle dye: These are a series of new fluorescence protein probes that detect cell cycle progression in living cells.

4) Single molecule analysis

Using the single molecule fluorescence microscope, we have tested the role of N domain dimerization in STAT-4 signaling. Our findings provide new insight into STAT signaling by demonstrating that the steady state interaction between dimerized STAT-4 and a surface receptor is required for the signaling mediated by ligand-receptor binding. We are preparing cell lines to investigate how IL-2 and IL-15 mediate different biological outcomes while sharing the same signaling receptor chains.

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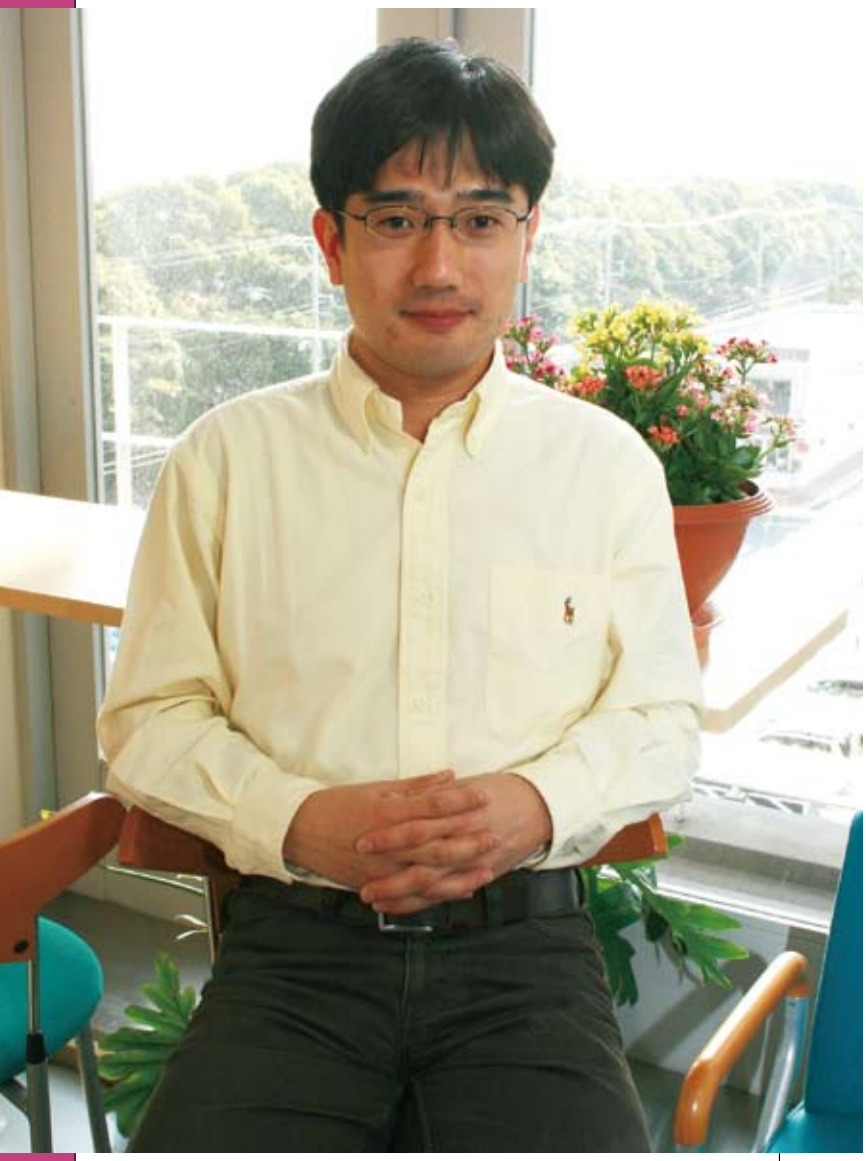
Research Unit for Immune Homeostasis



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Technical Staff : **Takahisa Miyao**
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Student Trainee : **Noriko Komatsu** (JRA)

A small subpopulation of T lymphocytes known as regulatory T cells (T_{reg}) plays a central role in preventing pathological immune responses such as autoimmunity, inflammation and allergy, and thus ensures dominant tolerance to self and innocuous environmental antigens. This has been well illustrated by our previous finding that the development and function of T_{reg} is controlled by the transcription factor Foxp3, whose deficiency leads to the development of a fatal autoimmune pathology in a spontaneous mouse mutant strain called *scurfy* and in human patients with the IPEX syndrome.

The identification of Foxp3 as the "master regulator" of T_{reg} differentiation and function has provided a key to a number of outstanding and as yet unresolved questions concerning the role T_{reg} cells in tolerance and immune regulation, and the physiology of these cells including their origins, the mechanisms controlling their development and function, and their antigen specificity. Resolving these issues is the goal of this laboratory.

In vivo function of T_{reg} in tolerance and immune regulation

Since both scurfy and Foxp3-deficient mice fail to generate T_{reg}, it is now believed that a T_{reg} deficiency is the primary cause of the immune dysregulation in Foxp3-mutant mice and humans. It has also been proposed that Foxp3 inactivation in non-hematopoietic tissues, particularly in thymic epithelium, is required for pathogenesis, since *scurfy* bone marrow cells fail to transmit the disease to lethally irradiated wild-type hosts. To determine the relative contributions of these two proposed pathways to the autoimmune pathology in *scurfy* mice, we repeated this radiation chimera experiment and found that the lack of pathology is due to the presence of radioresistant endogenous $Foxp3^+$ T_{reg} of the host. In addition, chimeras carrying the *scurfy* mutation only in non-hematopoietic cells had no evidence of autoimmune pathology. Thus, Foxp3 deficiency in non-hematopoietic cells does not contribute to the *scurfy* disease. Furthermore, our analyses of radiation chimeras revealed that the peripheral T_{reg} pool is fully and specifically restored and maintained by radioresistant endogenous T_{reg} or adoptively transferred exogenous T_{reg} through "homeostatic" proliferation in the absence of T_{reg} production from *scurfy* donor bone marrow cells. These results thus provide evidence that

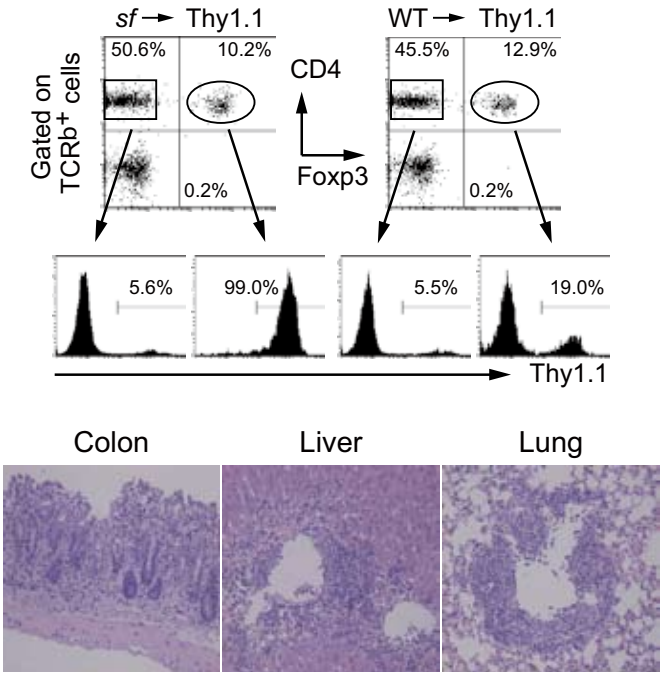

Figure Lethally irradiated wild-type host mice reconstituted with *scurfy* (sf) bone marrow cells fail to develop a fatal autoimmune disease, since the peripheral Foxp3$^+$ T cell pool is fully reconstituted from radioresistant host Thy1.1$^+$ T cells (upper panels). Upon elimination of these host Foxp3$^+$ T cells, the chimeras develop a catastrophic autoimmune disease associated with multiple organ inflammation (lower panels).

the autoimmune pathology in scurfy mice results indeed from a T_{reg} deficiency and illustrate a robust homeostatic mechanism that strictly controls the size of peripheral T_{reg} pool by fine tuning of homeostatic proliferation (Komatsu and Hori, PNAS, 2007). The molecular and cellular mechanisms controlling T_{reg} homeostasis are currently under investigation.

To determine the function of T_{reg} in tolerance and immune regulation in normal individuals, we have recently established a "knock-in" mouse model in which a human CD2-CD52 chimeric reporter was knocked into the *foxp3* locus. Our analyses showed that the Foxp3$^{hCD2\text{-}CD52}$ allele is a highly sensitive reporter of Foxp3 transcription, by virtue of cell surface hCD2 expression. The advantage of our dual reporter is that we can also specifically deplete Foxp3-expressing T cells in intact animals by injecting the well characterized anti-human CD52 monoclonal antibody CAMPATH-1. These mice will be a valuable tool not only to track Foxp3 expression but also to establish the functions of Foxp3$^+$ T_{reg} in tolerance and immune regulation in normal non-lymphopenic animals.

Impact of IPEX mutations on T_{reg} differentiation and function

Having established that Foxp3-deficient mice develop immune pathology due to defective T_{reg} development, we then asked whether and how Foxp3 mutations that had been identified in IPEX patients impact on T_{reg} development and function. To this end, we generated Foxp3 retroviral constructs carrying individual IPEX mutations and transduced them into conventional T cells. Our analyses revealed that all the mutations examined were amorphic or hypomorphic in that T cells expressing these mutants failed to exert full suppressive activity *in vitro* and *in vivo*. Most of the mutations also affected the expression of T_{reg} phenotypic markers, suggesting defective T_{reg} development in patients carrying these mutations. Interestingly, T cells transduced with one of the IPEX mutations expressed T_{reg} phenotypic markers normally, despite their impaired suppressive activity, suggesting that the patients carrying this particular mutation develop IPEX syndrome due to impaired T_{reg} effector function rather than defective T_{reg} development. To test this possibility *in vivo*, we have recently established mice in which this particular IPEX mutation is knocked into the endogenous *foxp3* locus. Preliminary analyses demonstrated that the knock-in animals develop an autoimmune disease similar to *scurfy* disease, indicating that the IPEX syndrome in the patients is indeed caused by this Foxp3 mutation. Furthermore, T_{reg} cells expressing this mutant Foxp3 develop in the thymus and periphery and are phenotypically similar to wild-type Foxp3-expressing T cells, a result consistent with our retroviral transduction studies.

One of the key questions regarding T_{reg} cell biology is how Foxp3 controls T_{reg} differentiation and function. By learning from experiments of nature, i.e. naturally occurring *foxp3* gene mutations identified in IPEX patients, we hope to dissect the molecular mechanisms by which Foxp3 controls T_{reg} differentiation and function *in vivo*.

Recent publications

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Hori S, Takahashi T, Sakaguchi S. Control of autoimmunity by naturally arising CD4$^+$ regulatory T cells. *Adv Immunol* 81, 331-371, 2003

Laboratory for Immune Regulation

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Student Trainees	:	**Asuka Terashima** **Naomi Hongo** **Tomoki Chiba**
Assistant	:	**Norie Takeuchi**

CD1d-restricted natural killer T (NKT) cells express both a single invariant Vα14 antigen receptor and NK receptors such as NK1.1, and are therefore known as Vα14 NKT cells. Because of this unique combination of receptors, the NKT cells occupy a distinctive intermediary position between innate and acquired immunity. Furthermore, because of their ability to quickly release large amount of cytokines, such as interferon (IFN)-γ and IL-4, and their apparent self-reactivity, NKT cells have been hypothesized to play important roles in the initiation and regulation of many types of immune responses. In fact, NKT cells mediate strong adjuvant activity by their IFN-γ production and augment anti-tumor immune responses. Therefore, we have started phase I/IIa clinical trial on NKT cell-targeted therapy for advanced lung cancer patients. On the other hand, NKT cells have been implicated in various autoimmune diseases development such as SLE, type I diabetes, systemic sclerosis, and in allergic diseases such as asthma. Our group studies the regulation of immune functions and responses, both beneficial and harmful, mediated by NKT cells. We hope to contribute to the development of new strategies for human immune therapy.

Identification of IL-17RB as a novel marker for the IL-13 producing *i*NKT subset important for respiratory syncytial virus-dependent airway hyperreactivity development

Asthma is an immunological disease that has increased dramatically in prevalence over the past half a century. In industrialized countries, the incidence of asthma has nearly doubled since 1980 and one in five to ten individuals is affected. Asthma is caused by environmental factors, such as allergen exposure and infection, in genetically predisposed individuals. Respiratory syncytial virus (RSV) infection causes bronchiolitis especially in infants and children, which is an important risk factor for the development of chronic asthma. However, the molecular mechanisms by which RSV infection increases asthma susceptibility still remain unclear. We have discovered that RSV infection induces IL-17E/IL-25, which triggers NKT cell activation and leads to airway hypersensitive reaction (AHR), a cardinal feature of asthma.

Based on CD4/CD8 expression, NKT cells can be divided into two populations, $CD4^+$ and $CD4^-CD8^-$ double negative (DN). These two

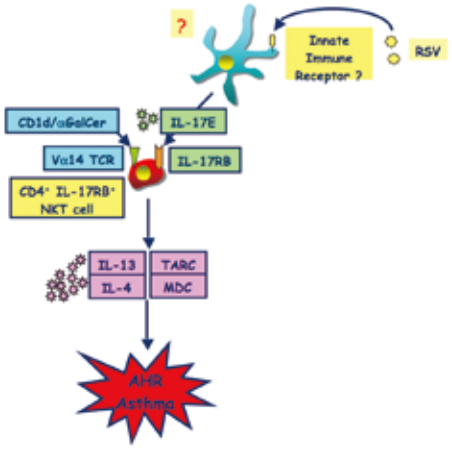

Figure 1 Mechanisms in RSV-induced AHR induction

NKT cell subsets have distinct functions, such as helper and cytotoxic functions, respectively. We have found that IL-17RB, one of the IL-17 receptor family members and a receptor for IL-17E/IL-25, is preferentially expressed on a fraction of NKT cells. None of the other leukocyte populations tested were IL-17RB+ and, among NKT cells, about one third of $CD4^+$ cells, but not the DN cells, expressed the receptor. Moreover, $CD4^+$IL-$17RB^+$ NKT cells proliferate and produce predominantly Th2 cytokines and chemokines (IL-13, IL-4, TARC, MDC, ECF-L) in an IL-17E/IL-25-dependent manner and induce AHR in mice. These results strongly suggest that $CD4^+$IL-$17RB^+$ NKT cells with Th2-like phenotype may have a crucial role, distinct from that of Th2 cells, in the pathogenesis of asthma.

A novel plasmacytoid dendritic cell-specific receptor, PDC-TREM, is responsible for augmented production of type I interferons

Successful host defense against viral pathogens depends largely on inhibition of viral replication during the early stages of infection by rapid and robust production of type I interferons (IFNs). Several viral recognition molecules have been identified, including retinoic-acid-inducible gene I (RIG-I) and melanoma-differentiation-associated gene 5 (MDA5), that mediate type I IFN production in conventional dendritic cells (CDCs). Plasmacytoid dendritic cells (PDCs) are also specialized producers of type I IFNs, however, the mechanism(s) underlying type I IFN production by these cells remain unclear.

Although triggering through toll-like receptors (TLRs) activates PDCs to produce type I IFNs, optimal production does not depend solely on TLRs, but requires TLR-mediated secondary events. Type I IFN-α/β receptor (IFNAR)-deficient PDCs fail to produce type I IFNs in response to TLR agonists, indicating that IFNAR-signaling itself, in a positive feedback loop, is one of these TLR-mediated secondary events. Constitutive expression of IFN regulatory factor (IRF)-7 in PDCs is also thought to augment type I IFN production, however, IRF-7 is detected in various cell types, and the level of IRF-7 expression does not correlate with the amount of type I IFN produced. Thus, other signaling events must be involved in type I IFN production following TLR triggering.

We have identified a novel cell surface molecule, PDC-TREM, a member of the triggering receptor expressed on myeloid cells (TREM) family, which is preferentially expressed on TLR-stimulated PDCs. Surface expression of PDC-TREM requires not only TLR- but also IFNAR-signaling. PDC-TREM forms a molecular complex with another transmembrane protein, Plexin-A1 (PlxnA1). The PlxnA1 ligand Sema6D induces robust production of type I IFNs, and when the association of PDC-TREM with PlxnA1 or the expression of PDC-TREM on the cell surface is prevented, both type I IFN production and PDC-TREM-induced signaling events are inhibited. Therefore, TLR- and IFNAR-signaling are responsible for expression of PDC-TREM, whose signaling cascade further augments production of type I IFNs.

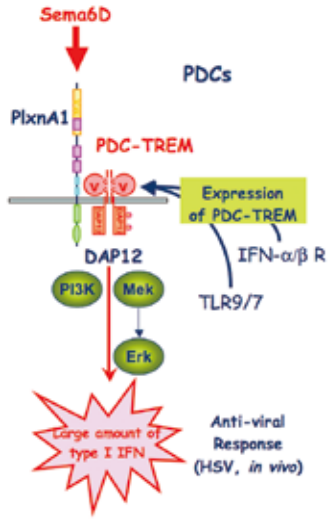

Figure 2 PDC-TREM-dependent typeI IFN production from plasmacytoid dendritic cells

Recent publications

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Laboratory for Immunchaperones

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Unfolded or denatured cellular proteins must be rapidly degraded, otherwise they may cause cell death through apotosis. The destruction machinery for such proteins is the ubiquitin-proteasome pathway. Unfolded or damaged proteins are first recognized by molecular chaperones like hsp90 and hsc70. These chaperones may simultaneously recruit E3 ubiquitin ligase to polyubiquitylate the target proteins, resulting in their delivery to the proteasome. In the context of immunity, this chaperone-assisted quality control system for intracellular proteins is the main source of antigenic peptides presented by MHC class I molecules. In addition to identifying which protein should be degraded, there is another level of regulation that dictates how the protein is degraded. The specific peptide fragments that are produced from the whole protein depend on the structure of the 20S proteasome and its regulatory particles like PA28 and PA700 (19S cap), which in turn determines the peptides presented by MHC class I molecules. The precise regulatory mechanism by which the proteasome produces peptides, however, is not well understood. Studies in our laboratory are focused on defining the mechanisms, especially through PA28 and the molecular chaperone hsp90, that regulate processing of cellular proteins and peptides.

HSPs in Antigen Processing

MHC class I ligands are mainly produced by the proteasome. A significant proportion (~3%) of newly synthesized cellular proteins on ribosomes are misfolded and are termed DRiPs (Defective Ribosomal Products). DRiPs has been implicated as the main source of cellular proteins from which most MHC class I ligands are produced by processing through the ubiquitin-proteasome system. Substrates (possibly DRiPs) to be degraded by the proteasome seem to be associated with the cytosolic chaperone hsp90. Hsp90 (and/or hsc70) binds unfolded proteins and also binds the U-box containing ubiquitin ligase, CHIP (carboxy terminus of hsc70 interacting protein), through its tetratricopeptide repeat (TPR) motif to polyubiquitinate the substrates. As a result, those ubiquitinated proteins are delivered to the 26S proteasome and finally processed into MHC class I ligands. In this case, hsp90 acts as a bridge to link cellular proteins and CHIP, leading toward degradation by the proteasome. It is also thought that hsp90 can

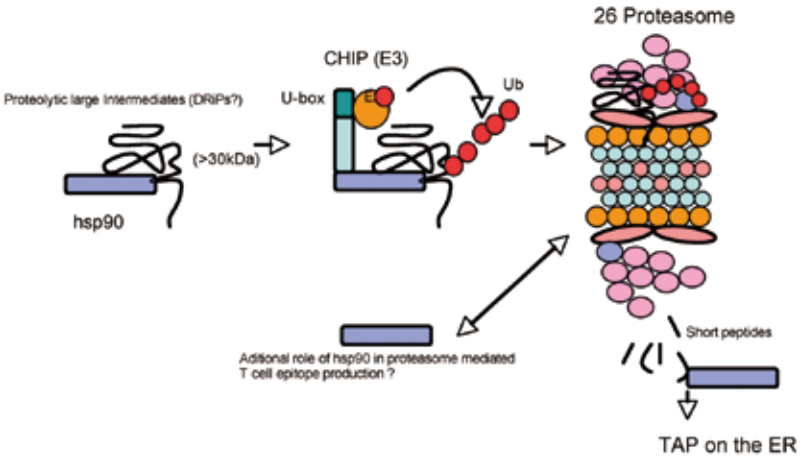

Figure Roles of cytosolic chaperone hsp90 in peptide production by the proteasome

bind to N-terminally extended versions of MHC class I ligands, thus, proteasomal products, and delivers them to TAP (transporter associated with antigen processing) molecules in the ER (endoplasmic reticulum) membrane. Whether hsp90 plays essential or additional role in MHC class I antigen processing remains unknown. We are currently examining the role of hsp90 in the context of peptide production by the proteasome as well as in the biogenesis of the proteasome. We believe that not only hsp90 but also other heat shock proteins such as hsc70/hsp40 participate in T cell epitope production at the level of the proteasome. DCs (dendritic cells) utilize a special pathway, called cross-presentation, to present antigenic peptides in the context of MHC class I even though the proteins are exogenously internalized. Cross-presentation is usually inhibited by proteasome inhibitors and is thus dependent on proteasome activity, implying that this pathway utilizes cytosolic endogenous processing pathways. For this reason, cross-presentation might also involve hsp90 – ubiquitination and delivering antigens to the proteasome and ferrying proteasomal peptides to TAP.

PA28 in Antigen Processing

IFN-γ stimulation increases the formation of football- (homo PA28-20S) and hybrid-shaped (PA28-20S-PA700) proteasomes: The former is a complex where PA28 is attached to both ends of the central 20S proteasome while the latter comprises the 20S proteasome flanked by PA28 on one end and PA700 (alias 19S Cap) on the other, functioning as an ATP-dependent protease, similar to the 26S proteasome, which has PA700 at both ends. The fundamental role of PA28 in the proteasome is still largely unknown. One proposed mechanism by which PA28 stimulates the proteasome is termed the gate-opening theory, where the usually closed α-ring of the 20S proteasome is immediately opened up by association with PA28, enabling translocation of substrates into the core catalytic 20S proteasome, followed by production of antigenic peptides. PA700 is also implicated as a chaperone complex that passes through substrates into the core 20S. Based on that perspective, both PA28 and PA700 may open the α-ring of the 20S to allow substrate loading. It is not known whether gate-opening is the only function of these regulatory particles but, if so, the peptide repertoire produced either by PA28 or PA700 should be the same and depend on the 20S proteasome complex itself. However, we found that the Plasmodium yoelii $CSP_{281-289}$ T cell epitope was processed by the 26S proteasome but not by the PA28-20S proteasome, indicating distinct digestion patterns between these two proteasome complexes. To gain more insight into the mechanism of PA28, we carried LC/MS based peptide digestion assays using many synthetic peptides harboring mouse MHC class I K^b and K^d ligands and the 20S proteasome plus recombinant PA28α and β. We found that many Kd ligands, if not all, were not properly processed by the PA28-20S proteasome, but were processed by the 26S proteasome. We are currently investigating why the PA28-20S proteasome is unable to process K^d ligands.

Roles of the cytosolic chaperone hsp90 in peptide production by the 26S proteasome

Proteolytic large intermediates containing MHC class I ligands (and/or possibly DRiPs) are associated with cytosolic hsp90 to prevent their aggregation. Hsp90 recruits E3 ubiquitin ligase CHIP to polyubiquitinate the bound cellular proteins, thus, hsp90 acts as a bridge between substrates and CHIP, resulting in delivery of them to the 26S proteasome to produce T cell epitopes. Produced epitopes or N-terminal elongated versions of proteasomal products are associated with hsp90 to be transported onto TAP molecules on the ER. We are currently investigating the additional role of hsp90 at the point of epitope production by the proteasome.

Recent publications

Susumu S, Nagata Y, Ito S, Matsuo M, Valmori D, Yui K, Udono H, Kanematsu T. Cross-presentation of NY-ESO-1 cytotoxic T lymphocyte epitope fused to human heat shock cognate protein 70 by dendritic cells. *Cancer Science* Vol.99 107-112. (2007).

Udono H, Wang J, Watanabe T. Antigen presentation to Lymphocytes. Encyclopedia of Life Sciences January (2007), John Wiley & Sons, Ltd.

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Laboratory for Cytokine Signaling

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Assistants : **Ryoko Masuda**
Mizuki Shimura

Despite their increasing prevalence in developed countries, the molecular mechanisms leading to autoimmune diseases and allergies remain poorly understood. The eventual goal of the Cytokine Signaling Research Group is to help elucidate the underlying molecular and immunological mechanisms of autoimmune diseases and allergy from the viewpoint of signal transduction within the immune system. Recently, we have reported 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.

The Role of Zn transporters in immune function.

In a breakthrough discovery, we identified the zinc transporter Zip6 as a target gene of the IL-6 cytokine signaling molecule STAT3 and further demonstrated that Zip6 plays an important role in cell migration during gastrulation in the zebrafish (Yamashita et al, Nature, 2004). We showed that Zip6 is required for nuclear localization of Zn-finger protein Snail, a repressor of E-cadherin. This result led to the prediction that Zn might act as a signaling molecule like $Ca2^{+}$. Zn is a trace element that is essential for the function of many enzymes and transcription factors and thereby Zn is one of the important constitutive components of our body. However, little is known concerning its role as a signaling molecule. Zn deficiency results in defects in innate and acquired immune responses, but little is known how zinc controls immune cell function. In 2006, we found that the Toll-like receptor 4 (TLR4) agonist lipopolysaccharide (LPS) altered the intracellular level of free Zn by changing the expression profile of Zn transporters in dendritic cells (DC). This LPS-induced decrease of intracellular free Zn is one of critical steps for DC maturation; upregulation of surface MHCII and costimulatory molecules required for $CD4^{+}$T cell responses (Kitamura et al, Nature Immunology, 2006). Taken together

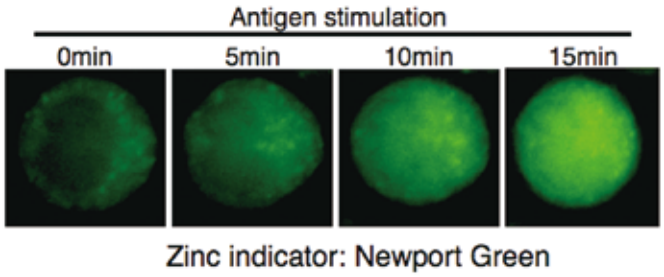

Figure 1 Zinc wave.
Bone marrow derived mast cells were stimulated by FcεRI and intracellular fluorescence to measure Zn ion levels were assessed every 5 minutes.

with the aforementioned fact that FcεR stimulation induces a Zn wave in mast cells, our results showed that Zn is a novel intracellular signaling molecule like Ca^{2+} and that Zn plays crucial roles in immune and allergy responses. To further clarify the *in vivo* roles Zn, we are currently establishing knock out mice deficient in a variety of Zn transporters, including Zip6, Zip7, Zip10 and Zip13.

Mast Cell Biology and molecular mechanisms of mast cell functions.

We are investigating the mechanistic involvement of mast cells in allergy, inflammation, and autoimmune diseases. Recently, we dissected the degranulation process of mast cells. First, FcεRI 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 (Nishida et al., J Cell Biol. 2005). At present, we are especially focused on clarifying the molecular mechanisms of calcium-independent microtubule-dependent granule translocation. Recently, we found that Zn is required for FcεRI-induced granule translocation to the plasma membrane and therefore, Zn chelators could be potential new anti-allergic agents. Zn is also required for cytokine production by mast cells (Kabu et al., J Immunology. 2006). In addition, we showed that Zn chelation in mast cells prevented translocation of protein kinase C (PKC) as well as putative downstream events such as the phosphorylation and nuclear translocation of NF-κB, resulting in the decreased production of IL-6 and TNFα. Based on this finding, we are now searching for Zn-associated molecules that regulate granule translocation and cytokine production in mast cells. Very recently, we found that Zn also functions as a second messenger capable of converting an external signal into internal events (Yamasaki et al., J Cell Biol. 2007). When we generated an external signal in mast cells by cross-linking IgE receptors, we observed an internal wave of free Zn released from the vicinity of the ER within several minutes after stimulation (Fig.1). We named this phenomenon the "Zinc wave". Calcium and activated MAP kinase were necessary for the Zinc wave. Since Zn inhibits tyrosine phosphatases and, in fact, Zn enhanced FcεRI-induced total tyrosine phosphorylation, one possible target of the zinc wave is the tyrosine phosphatases (Fig. 2). The precise origin of the zinc wave and its molecular features remain to be resolved. It is also important to identify authentic targets of the zinc wave. Another important question to be answered is whether the zinc wave is a general phenomenon or is specific to mast cells.

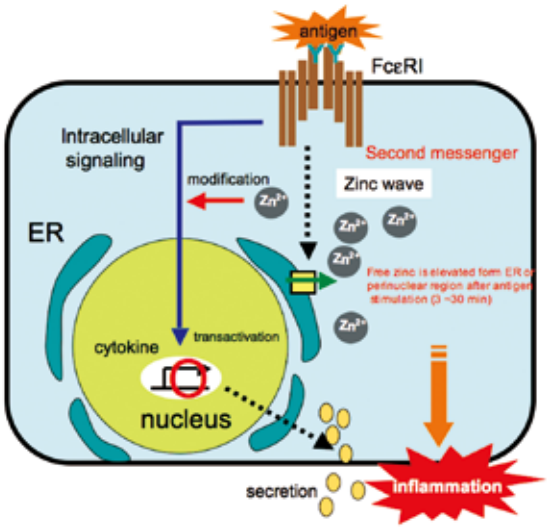

Figure 2 Regulation of the Zinc wave.
Zinc wave was regulated by calcium and activated MAP kinase. Free Zn modulates FcεRI-induced signaling pathway such as total tyrosine phosphorylation, one possible target of the zinc wave is the tyrosine phosphatases

Recent publications

Yamasaki, S., K. Sakata-Sogawa, A. Hasegawa, T. Suzuki, K. Kabu, E. Sato, T. Kurosaki, S. Yamashita, M. Tokunaga, K. Nishida, T. Hirano. Zinc is a novel intracellular second messenger. *J. Cell Biol*. 177, 637-645, (2007)

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Laboratory for Signal Network

Team leader

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Special Postdoctoral Researcher	:	**Shinya Tanaka**
Technical Staff	:	**Yukiko Matsuno** **Yoshie Suz uki** **Maiko Natsume**
Student Trainees	:	**Yasutaka Motomura** (Junior Research Associate) **Hiroko Suyama** **Emi Ohkubo**
Assistant	:	**Michiko Nakamura**

T cells play a central role in the effector and regulatory functions in immunological surveillance, and aberrations of these functions can lead to various immunological disorders. T helper 1 (Th1) cells secrete IL-2, IFN-γ and TNF-α in cellular immune responses against intracellular pathogens and viruses. By contrast, Th2 cells produce IL-4, IL-5, IL-6, IL-10 and IL-13 in humoral immune responses, mainly against extracellular pathogens, and also account for allergic immune responses. Recently, a new subset of Th cells, Th17, has been characterized based on IL-17 production. Th17 cells are associated with many aspects of autoimmune tissue inflammation. Cytokines are critical mediators of the transmission of information from the cytokine receptor to the nucleus as well as in the communication between cells. Thus, the cytokines secreted from effector helper T cells play a critical role in controlling the outcome of immunological surveillance. The overriding goal of our laboratory is to understand the molecular basis of helper T cell differentiation.

Alternative regulation of Th2 cytokines in Th1 cells.

During the last two decades, it has become dogma that theTh1 and Th2 cytokine profiles are strictly conserved and immutable during T cell differentiation. Among the signature Th2 cytokines, IL-4 and IL-13 expression is thought to be controlled by common transcriptional mechanisms. However, we found that chronic antigenic stimulation could preferentially alter the cytokine profile of Th1 cells to elicit significant IL-13, but not IL-4, expression in both mouse and human systems. We designated these IL-13 producing Th1 cells as Th1/13 cells. IL-13 expression is tightly associated with the expression of a putative clock-controlled transcriptional factor, E4BP4, and individual Th1/13 cells from mouse and human consistently co-expressed E4BP4, IFN-γ, and IL-13. The promoter region of the *Il13* locus was constitutively transcriptional accessible, and the binding of the induced E4BP4 to the promoter resulted in IL-13 expression by conventional Th1 cells. Therefore, E4BP4 is a critical regulator for the expression of IL-13 in Th1/13 cells and for activating this normally silent gene in Th1 cells. Th1/13 cells were found in human asthmatic patients who had a less Th2 biased phenotype, and the administration of Th1/13 cells in a mouse asthma model resulted in induction of

eosinophil-mediated asthma responses. These results indicate that E4BP4 controls the induction of a unique T subset, Th1/13, to possibly initiate allergic incidents in otherwise chronic Th1 biased situations. Our findings provide a novel insight into the traditional dogma of Th1 and Th2 balance in allergic diseases.

Identification of the molecular basis of Th2 bias

Genetic background is known to influence the type of immune responses, and the molecular basis of TH2 bias has been a long-standing mystery in the field of immunology. Genetic linkage analyses have been used in a comparison of BALB/c versus B10.D2 strains with respect to their ability to produce IL-4 upon initial TCR stimulation. These studies have identified Determinant of IL-4 commitment (*Dice*) 1.2 on chromosome16 as one trait locus that controls Th2 bias . We found that Myc-induced nuclear antigen with a molecular mass of 53kDa (*mina53*) expression was consistently higher in Th1- prone strains (B10.D2) than Th2-prone strains (BALB/c). The genome sequences of the 5' flanking sequence and the first intron region contained multiple SNPs among different mouse strains, and these tightly associate with the propensity to produce initial IL-4. Interestingly, The SNPs in the promoter region of human *mina53* gene associated with severity of allergic asthma. These results indicated that Mina53 is a useful genetic maker for Th2 bias and allergic predisposition in mouse and human.

Role of memory phenotype CD4 T cells in Th17 mediated immune-regulation

The distal 3' enhancer in the *Il4* locus corresponds to a highly conserved region termed conserved non-coding sequences (CNS)-2. Using a transgenic GFP reporter system, we demonstrated that the CNS-2 enhancer regulated the initial expression of IL-4 in $CD44^{hi}$ memory phenotype (MP) CD4 T cells. These IL-4 producing MP-CD4 cells co-expressed IFN-γ, while GFP^{-} IL-4 non-producing MP cells expressed robust IL-17 and IFN-γ. The IL-17 producing MP (MP-Th17) cells appeared to be distinct population from canonical Th17 cells since MP-Th17 cells developed in the absence of IL-6, which is required for Th17 differentiation. The MP-Th17 cells had an alternative role to induce IL-17 production by dendritic cells (DC) in an IL-17 dependent manner. These results demonstrated that rapid IL-17 production by MP-Th17 cell resulted in the secondary production of this cytokine by DCs to exacerbate inflammatory responses.

Recent publications

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Kano, S., Sato, K., Morishita, Y., Vollstedt, S., Kim, S., Taki, S., Honda, K., Kubo, M., & Taniguchi, T.; Regulation of Th1 vs. Th17 differentiation: Selective contribution of thetranscription factor IRF1 to the IFN-γ-IL-12 axis of signaling networks in $CD4^{+}$ T cells. *Nature Immunology* 9, 34 - 41, 2008

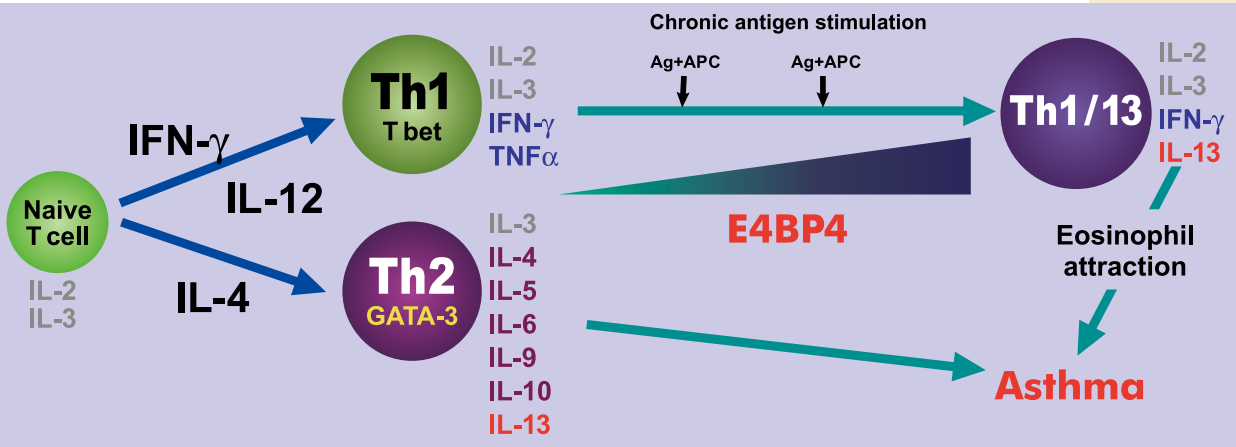

Figure Chronic antigen stimulation induced E4BP4 mediated IL-13 production
Significant IL-13 expression could be induced in mouse Th1 cells following chronic antigenic stimulation. This chronic stimulation initiated expression of E4BP4, leading to IL-13 expression in conventional Th1 cells. The IL-13 producing Th1 cells were found among human T cells from asthmatic patients. IL-13 produced by Th1 cells induced an eosinophil mediated asthmatic response in a mouse asthma model.

Laboratory for Immunogenetics

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Assistant : **Aiko Iyama**

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 on 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 allergic condition 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.

During the first 4 years of this project we have screened 7,600 recessive inheritances from 80 mouse pedigrees. For basic phenotypic screening, we examined the levels of immunoglobulins, cytokines, and autoantibodies, as well as expression of a panel of cell surface markers on peripheral blood cells. Using also examined tissue sections from animals at 16 weeks of age. We have thus clarified the mutant phenotypes by pathological, cytological and molecular biological examinations of affected tissues, lymphoid organs and blood cells.

A phenotype identified in a mutant candidate line is reexamined at a later development stage, and if the same phenotype is confirmed, we begin mating the mice for phenotype inheritance tests. The responsible mutant loci are mapped by backcrossing mutant individuals with the C3H/HeJ strain for gene detection by single-nucleotide polymorphism analysis. After approximate mapping, candidate gene sequences are compared with wild type sequences in public databases by means of the PosMed research system established by RIKEN GSC. Candidate gene mapping has been done in collaboration with the Phenome Informatic Team and Mouse Mutation Resource Exploration Team in GSC, RIKEN

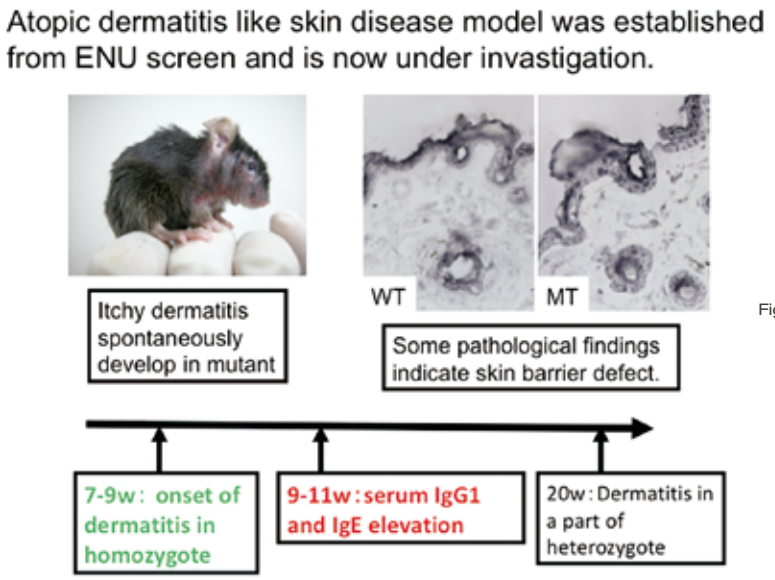

Figure 1 An atopic dermatitis-like mutant mouse was established from the ENU screen. The study of the precise mechanism(s) of the responsible mutation in this disease is now underway.
Above figure: Dermatitis in some of the heterozyogtes.

Allergic disease model mutant mouse model

By ENU recessive mutant screening, 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 and, as the mice aged, they started to scratch the ear skin or face. The serum IgG1 or IgE level of these mice gradually increased. Pathologically, the epidermal layer was hypertrophic and many lymphocytes were found in the dermis of the lesion. These symptoms and findings are compatible with the criteria for human atopic dermatitis (AD). We have mapped the phenotype responsible region to within a few cM and identified a candidate gene mutation. We are now using genetic manipulation to test whether the mutation is responsible for the phenotype .

By ENU recessive mutant screening, we have identified and established more than 140 mutant lines of immune or blood disease models. In keeping with our expectation, more than 50 lines showed phenotypes related to allergic diseases. We are now backcrossing some of the mice to the C3H/HeJ strain in order to map the responsible loci. As of December 2007, we have mapped eight independent mutant-responsible loci to distinct regions, and three of them have been clarified to be point mutations of known genes.

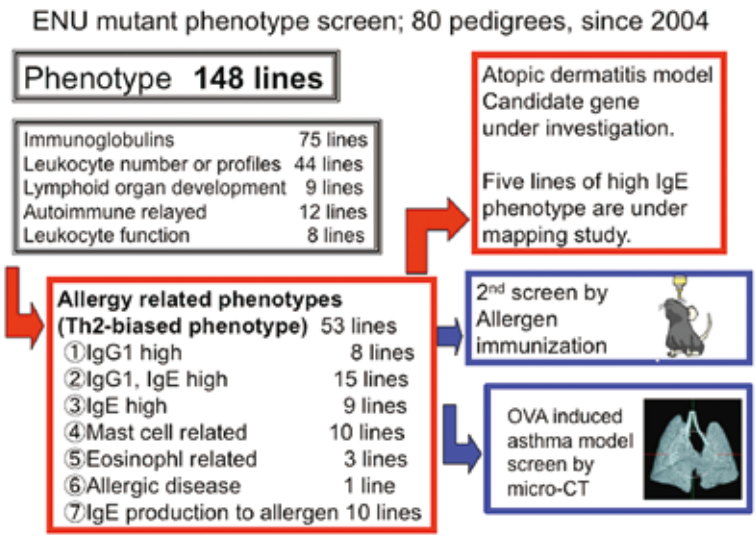

Figure 2 Summary of three years of ENU mutant screening. In total, approximately 7,600 mutant mice from 80 genomes have been screened and 148 phenotypes have been identified in mutant lines.

Recent publications

Hamanaka S, Nabekura T, Otsu M, Yoshida H, Nagata M, Usui J, Takahashi S, Nagasawa T, Nakauchi H, Onodera M.: Stable transgene expression in mice generated from retrovirally transduced embryonic stem cells. *Mol Ther*. 15(3),560-5 (2007)

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Research Unit for Immune Tissue Engineering

Unit leader

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Risa Chihara
Minako Ogawa
Yuri Suzuki

Trainee : **Taku Nishide** (from MBL)

The goal of our project is to develop novel methods and tools to restore, reinforce and/or regenerate the immune surveillance system by regenerating immunological tissues or organs.

Recently, various strategies for immune intervention have been developed to overcome intractable diseases such as severe infection, autoimmune disorders and cancers. Those include antibody therapy, using monoclonal as well as polyclonal antibodies, cytokine therapy, and cell therapy by the administration of hematopoietic stem cells, dendritic cells or antigen- specific immune effector cells. These have sometimes proven to be effective strategies to combat the above diseases. However, it is known that immune tissues and organs are often destroyed or severely damaged by disease-causing agents such as pathogens or tumor cells, and by medical treatment itself, which can result in irreparable damage leading to death. It is also known that the function of the immune system is severely reduced by aging.

One approach to overcome a severely impaired immune surveillance system would be to newly construct or regenerate immune tissues or organs in order to reinforce and restore the function of primary or secondary lymphoid tissues. Up to now, only a few reports including ours have been published concerning generation of artificial lymphoid tissues with efficient immune function.

Generation of artificially constructed lymph node-like tissues (aLNs)

We previously demonstrated that artificially constructed lymph node-like tissues (aLNs), which were generated by transplantation of a stromal cell-embedded biocompatible scaffold (collagen sponge) into the renal subcapsular space in mice, possess a well organized tissue structure similar to secondary lymphoid organs. The aLNs contain compartmentalized B cell and T cell clusters, high endothelial venule (HEV)-like vessels, germinal centers and follicular dendritic cell networks. Furthermore, the aLNs were transplantable to naive normal as well as to severe combined immunodeficient (SCID) mice. Antigen-specific IgG isotype antibody formation could be induced in the artificially constructed antigen-primed lymphoid tissues soon after intravenous administration of the same antigen to the secondary recipients.

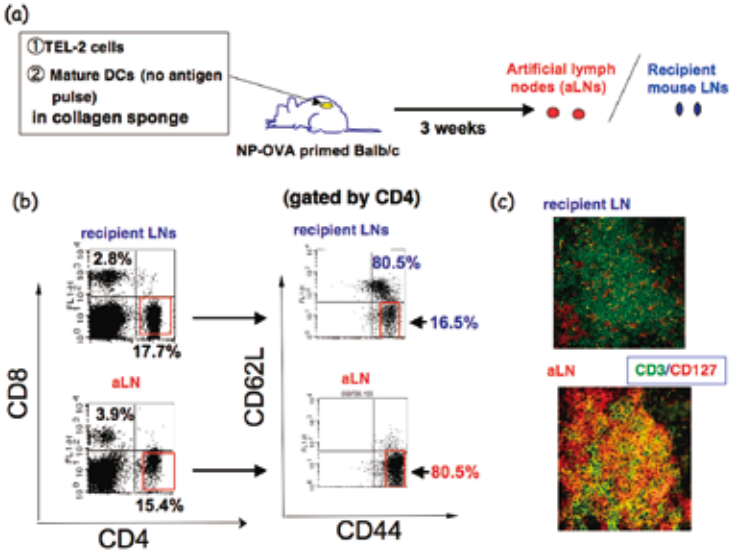

Figure
Enrichment of CD44^{hi}CD62L^{lo}CD127(IL-7Rα)$^{+}$ memory type T cells in artificial lymph nodes (aLNs). Collagen sponges containing stromal cells and mature dendritic cells were transplanted into the renal subcapsular spaces of antigen-preimmnunzed Balb/c mice. Two to three weeks later, aLNs were formed. Profiles of T cells from aLNs and lymph nodes of recipient mice were analyzed. (a) schema for the generation of aLNs in Balb/c mice. (b) The ratio of CD4 and CD8 positive T cells in aLNs are equivalent to that of normal recipient lymph nodes. In CD4 positive T cells, CD44^{hi}CD62L^{lo} T cells were highly enriched in aLNs. (c) Many of CD127 (IL-7Rα) positive T cells are found in T cell area of aLNs.

Enrichment of memory type lymphocytes in aLNs

Memory type CD4^{+} T cells (CD44^{hi}, CD62L^{lo}) were highly enriched in the aLNs as well as in spleens of SCID mice bearing the aLNs. We then attempted to clarify the nature of the T cells enriched in aLNs. Besides memory type CD4^{+} T cells, another major population of CD4^{+} helper T cells enriched in aLNs was the antigen specific follicular helper T cell (TFH). The TFH are a distinct subpopulation of helper T cells that promote activation of antigen-specific B cell responses, plasma cell development, class-switching, and acceleration of memory B cell development. Enrichment of this particular subclass of helper T cells in aLNs may drive the potent antigen-specific secondary responses that we have observed. The helper T cells enriched in aLNs strongly express ICOS which supports secondary memory and effecter T cells responses by influencing their cell survival. The helper T cells enriched in aLNs also produce large amounts of IL-21, a cytokine, known to promote CD8^{+} memory T cell accumulation, suggesting that aLNs may also provide the appropriate environment for generation of T cell-mediated immune responses. Not only memory type helper T cells but also memory type B cells accumulated in aLNs and spleen in aLN-transplanted SCID mice. We are currently defining the molecular signature(s) of the memory T and B cells in aLNs.

Anti-tumor activity of aLNs

The aLNs efficiently suppressed tumor growth in tumor-bearing hosts. The suppressive activity was much stronger than that induced by simple administration of antigen primed dendritic cells. Two weeks after transplantation of tumor cells into naïve mice, the original tumors were removed. At that point, a collagen sponge containing stromal cells, TEL-2 cells, and tumor-antigen primed BM-derived mature dendritic cells was transplanted into renal subcapsular space of the mice and aLNs were formed 2-3 weeks after. Recurrent tumor growth was effectively suppressed by the formation of aLNs. The aLNs were then removed and transplanted into tumor-bearing SCID mice and tumor growth was significantly suppressed . These preliminary data suggest the possibility of using aLNs for cancer treatment.

Determination of molecular signature(s) of lymph node organizers (stromal cells)

In the last decade, remarkable progress has been made in understanding the cellular and molecular mechanisms involved in the organization of secondary lymphoid tissues. However, the origins as well as molecular features of stromal cells (organizers) involved in the secondary lymphoid tissue organization are not well understood. This year, we have begun to establish monoclonal antibodies (mAb) which specifically recognize mouse mesenchymal stromal cells in the lymph node anlagen of E15.5 mouse embryos. So far, several mAb have been isolated. One recognizes the embryonic lymph node (but not spleen) anlage mesenchymal cells as well as a new subpopulation of follicular dendritic cells (FDC) in adult lymph nodes and Payer's patches. Another mAb has a broader reactivity, recognizing mesenchymal cells in lymph nodes as well as spleen of embryonic and adult mice. We plan to use these mAb to isolate mesenchymal stromal cells (organizers) from lymph node anlagen of mouse and to determine molecular signature(s) for lymph node organizer cells by the analysis of their gene expression profile.

Recent publications

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Research Unit for Human Disease Model

Unit leader

Fumihiko Ishikawa

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Takehiko Doi

Technical scientist : **Hidetoshi Ozawa**

Technical staff : **Nahoko Suzuki**
Akiko Sone
Mariko Tomizawa

Assistant : **Mizuka Kimura** (**Nao Akimori**)

The development of *in vivo* animal models of human hematopoietic and immune systems, normal as well as pathological, has been a long sought goal of investigators interested in human studies. Several different immucompromised mouse strains have been constructed in the past with varying degrees of success. However, incomplete immunodeficiency, the short life spans and age-dependent leakiness of murine humoral immunity have presented obstacles for achieving high levels of long term human cell engraftment. To overcome these problems, we have created a novel immunodeficient mouse strain, NOD/SCID/IL2rγ^{null}. Transplantation of purified human HSCs into these mice during the neonatal period results in a significant humanization of their hematopoietic system. This strain, with a life expectancy of >90 weeks, is more robust than previous models, allowing the assessment of reconstitution and immune system development of human HSCs and progenitor cells. Using NOD/SCID/IL2rγ^{null} newborns, we have created humanized mice with a full representation of human hematopoietic and immune systems. Engrafted human T cells and B cells undergo a physiological maturation process in primary and secondary lymphoid organs of the recipients. These findings suggest that the NOD/SCID/IL2rγ^{null} newborn transplant model is a powerful model to study the human immunohematopoietic system *in vivo*. Currently we are investigating the biology of human leukemic stem cells and creating mouse models of human diseases such as leukemia and primary immunodeficiency by transplanting human disease stem cells into the neonatal NOD/SCID/IL2rγ^{null} mice.

Studying normal human hematopoietic stem & progenitors in the NOD/SCID/IL2rγ^{null} mice

To directly examine human immunity and hematopoiesis, we have been attempting to reconstitute human immune subsets in mice. In the past, full development of the human immunohematopoietic system *in vivo* has been hindered by the lack of a suitable mouse model. For instance, NOD/SCID mice support human B cell differentiation, but not T cells or dendritic cells, from transplanted human hematopoietic stem cells (HSCs). Moreover, the engrafted human B cells undergo maturation arrest so that it is not possible to analyze the functions of mature B cells and their progeny, such as human Ig production. Using the novel newborn NOD/SCID/IL2rγ^{null} HSC transplantation model, purified human cord blood $CD34^{+}CD38^{-}$ cells

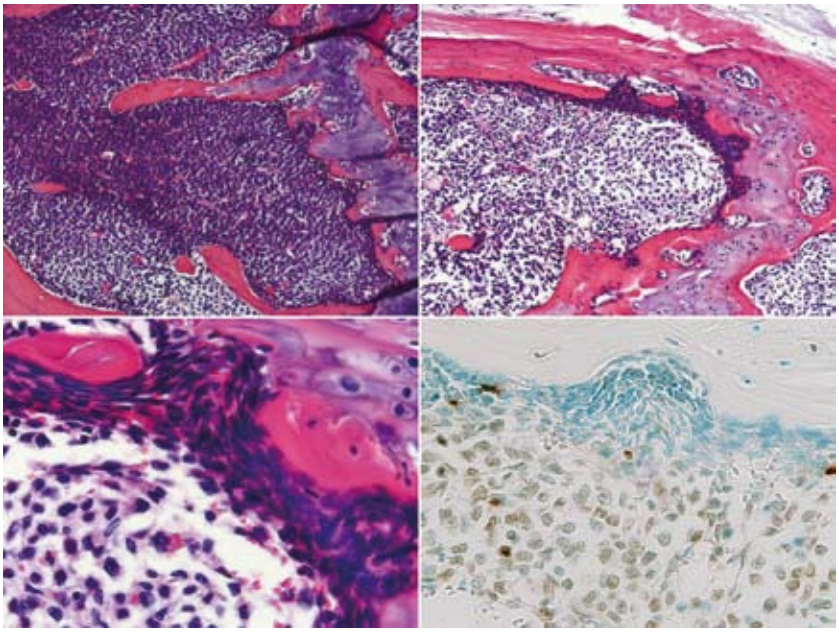

Figure Femoral bone from untreated and Ara-C-treated AML-engrafted mice. In the untreated femur, the BM space is packed with AML blasts (upper left; HE). At day 3 after chemotherapy, there is a significant decrease in cellularity in the BM space except at the endosteal surface of the bone (upper right and lower left; HE). TUNEL staining confirms the massive apoptosis of cells in the central BM cavity while the cells at the endosteal surface are relatively spared (lower right).

consistently give rise to high levels of human hematopoietic chimerism for over six months. In this model, human HSCs produced mature differentiated progeny including myelomonocytes, T and B lymphocytes, dendritic cells, erythroid cells, and platelets. Functionally, human B cells underwent normal class switching, and engrafted human T cells exhibited HLA-dependent cytotoxic activity. Using this system, we have recently been investigating the differentiation capacity of myeloid and lymphoid progenitors. When purified human myeloid progenitors and lymphoid progenitors were intravenously transplanted into NOD/scid/IL2rγ^{null} newborn mice, both progenitors displayed significant expansion in the xenogeneic host. Human conventional and plasmacytoid DC progeny were found in recipients transplanted with myeloid progenitors and those transplanted with lymphoid progenitors, respectively. Thus, successful short-term engraftment by lymphoid and myeloid progenitors in the newborn NOD/SCID/IL2rγ^{null} mouse model enabled us to identify the origins of human conventional DCs and plasmacytoid DCs. The NOD/SCID/IL2rγ^{null} newborn transplant model is thus an excellent system to study the properties of hematopoietic stem and progenitor cells as well as to study the human immune system *in vivo*.

Examination of human primary leukemia stem cell biology by the development of primary human acute myelogenous leukemia model

While advances in chemotherapeutic agents and stem cell transplantation have improved survival of patients with acute myelogenous leukemia (AML) over the last 30 years, chemoresistant disease, recurrence after transplantation, and inability to undergo transplantation due to lack of suitable HLA-matched donors continue to adversely affect clinical outcomes. Development of more effective therapeutic agents is needed, but the testing of such agents has so far relied on data obtained from mouse models or *in vitro* cell culture using human primary cells or cell lines. The ability to test therapeutic agents on human leukemic cells *in vivo* would be important since it would allow for the evaluation of novel therapeutics in a more physiologically relevant setting, as well as the assessment of therapeutic efficacy for individual patients.

As a proof-of-principle, we have treated primary human AML-engrafted NOD/SCID/IL2rγ^{null} mice with the chemotherapeutic agent cytarabine (AraC). By using this approach, we were able to show that the number of human AML cells in the peripheral blood decreased after a single pulse treatment with AraC, however this was followed by eventual relapse originating from surviving leukemia stem cells (LSCs). Importantly, the LSCs are resistant to conventional chemotherapy, while the majority non-stem leukemic cells are induced to undergo apoptotic cell death. The surviving LSCs efficiently develop AML in secondary and tertiary recipients, suggesting that the chemoresistance of LSCs is a major cause of AML relapse. Thus we need to consider novel ways to eradicate LSCs to minimize AML relapse. We are currently investigating the mechanism underlying the chemoresistance of LSCs.

Recent publications

Ishikawa F, Yoshida S, Saito Y, Hijikata A, Kitamura H, Tanaka S, Nakamura R, Tanaka T, Tomiyama H, Saito N, Fukata M, Miyamoto T, Lyons B, Ohshima K, Uchida N, Taniguchi S, Ohara O, Akashi K, Harada M, Shultz L D. Chemotherapy-resistant human AML stem cells home to and engraft within the bone marrow endsteal region. *Nature Biotechnology*, 25(11):1315-21, 2007.

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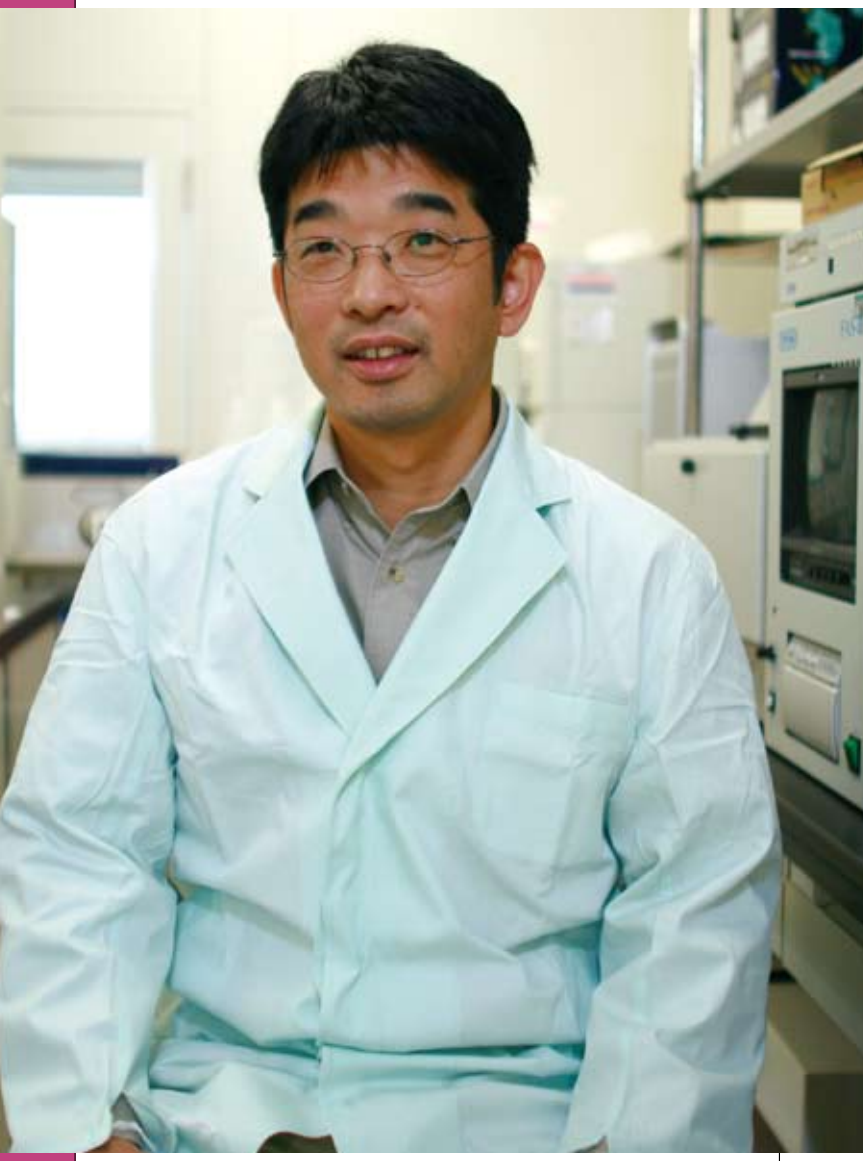
Research Unit for Cellular Immunotherapy



Unit leader

Shin-ichiro Fujii

Technical staff : **Yohei Shozaki**

Assistant : **Kaori Aki**

We have studied the full maturation of dendritic cell (DC) *in situ* for tumor immunity by focusing on the link between innate and adaptive immunity, especially through the interaction between DC maturation and NKT cells. The goal of our laboratory is to establish an antitumor therapeutic strategy by elucidating the function of effector cells in tumor bearing models, leading to the launch of clinical studies, in collaboration with Dr. Shimizu, Therapeutic Model Research Unit.

We also have been developing a joint clinical study with Chiba University using α-galactosylceramide (α-GalCer)-pulsed autologous DC therapy to evaluate the immunological and clinical responses to NKT cell therapy in advanced non-small cell lung cancer patients. In the phase I study we detected an increase in the number of NKT cells in the high dose DC administration group, and then used that cell number in a subsequent Phase IIa trial. Based on our initial analyses, we have encouraging immunological and clinical results in the phase I/IIa trials (A collaboration with RCAI Director Dr. Taniguchi and Drs. Nakayama and Motohashi in Chiba University).

Induction of Adjuvant Effects of NKT Cells Leading to Adaptive Immunity in Cancer Therapy (A collaboration with Dr. Shimizu, Therapeutic model research unit)

Initially, we studied the different types of APCs for loading α-GalCer to induce stronger innate tumor immunity. We have pursed the capacity of different CD11c- leukocytes to present α-GalCer by *in vitro* loading, and found that as long as different types of CD1d expressing leukocytes were able to capture glycolipid, they could stimulate NKT cells. When we used α-GalCer-loaded tumor cells instead of DC/Gal, we found more augmenting NK cell activity as “adjunct effects”. In addition, we recently found that a low dose of B16 tumor cells loaded with α-GalCer and injected intravenously could induce a protective T cell immune response to subsequent challenge with B16 melanoma subcutaneously. To analyze the events taking place in this system *in vivo*, we tracked labeled α-GalCer-loaded tumor cells by confocal microscopy and found that α-GalCer did indeed activate NK and NKT cells to kill tumor cells. The tumor debris were captured by DCs in the spleen, which matured to present the tumor antigen peptide to $CD4^+$ and $CD8^+$ T-cells,

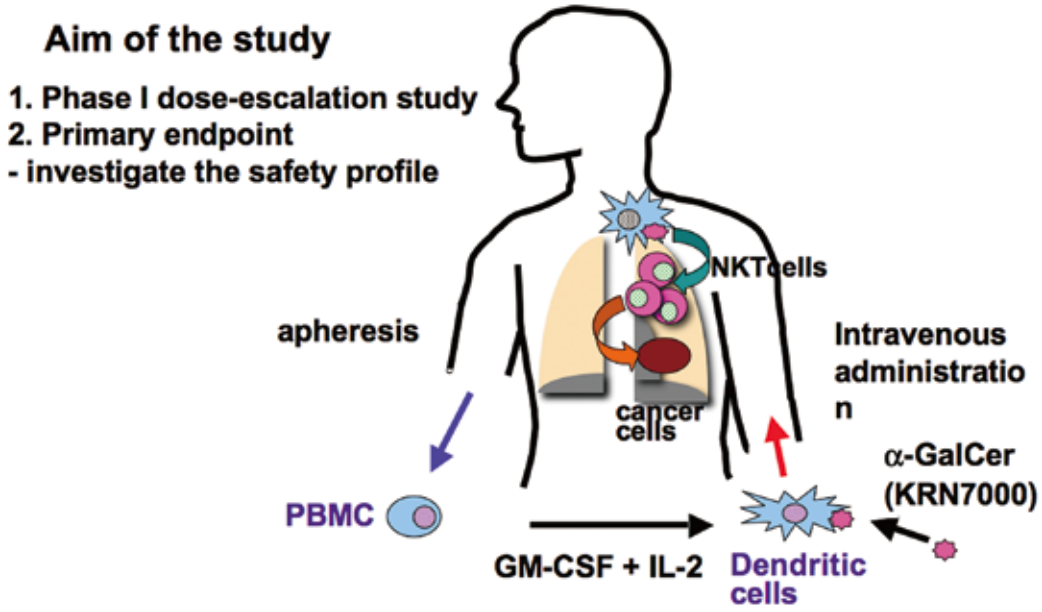


Figure 1 Strategy for α-GalCer (KRN700)-pulsed DC immunotherapy for advanced lung cancer patients Peripheral blood mononuclear cells (PBMCs) were collected and separated by density gradient centrifugation. To generate DCs, the cells were cultured with GM-CSF (800 U/mL) and IL-2 (100U/mL) and loaded with α-GalCer (100 ng/mL) for 24 h before administration to patients. The α-GalCer/DCs were administered to patients intravenously.

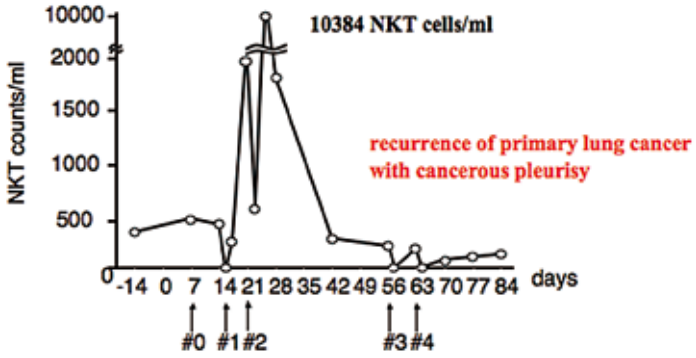


Figure 2 The numbers of Vα24+ NKT cells increased in patients treated with α-GalCer-pulsed DCs Results from a patient sensitive to DC/Gal therapy are shown. After administration of high doses of DC/Gal (1x10^9/m^2) at the indicated time points, the Vα24+NKT cells in PBMCs were highly elevated. Severe adverse events were not observed in any of the treated patients.

promoting an adaptive immune response against that particular tumor (cross-presentation 1). Also the tumor debris-capturing DCs were able to present the α-GalCer ligand to NKT cells again (cross-presentation 2). This immune response persisted for at least six months.

Phase I/IIa immunotherapy for advanced non-small cell lung cancer (NSCL) patients refractory to standard treatment as a post second line therapy (A collaboration with Chiba University and RCAI Director Dr. Taniguchi)

In the phase I dose escalation study using α-GalCer (KRN7000)-pulsed DCs, 11 patients were enrolled and no adverse events were observed. Patients treated with a high dose of DC/Gal (1x10^9/m^2) responded better than those receiving a medium (2.5x10^8/m^2) or low dose of DC/Gal (5x10^7/m^2). In the high dose group, a dramatic increase of NKT cells in peripheral blood was observed. Furthermore, 2 patients remained clinically stable for more than a year. Then, we decided to use the high dose and have been developing a Phase I/IIa clinical study of the application of NKT cell therapy for advanced lung cancer patients (stage IIIB, IV or recurrence).

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Shimizu K, Hidaka M, Kadowaki N, Makita N, Konishi N, Fujimoto K, Uchiyama T, Kawano F, Taniguchi M, Fujii S. Evaluation of the function of human invariant NKT cells from cancer patients using α-alactosylceramide-loaded murine dendritic cells. *J Immunol.* 177, 3484-92 (2006).

Fujii S, Shimizu K, Hemmi H, Fukui M, Bonito AJ, Chen G, Franck, RW, Tsuji M, Steinman RM. Glycolipid α-C-galactosylceramide is a distinct inducer of dendritic cell function during innate and adaptive immune responses of mice. *Proc. Natl. Acad.Sci. USA.* 103, 11252-11257 (2006).

Laboratory for Vaccine Design

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Haruka Katagiri
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Several antigens have been used for antigen-specific immunotherapy for allergic diseases, however, the risk of anaphylaxis after the administration of these antigens has not been completely excluded. Furthermore, despite the fact that allergic immunotherapy has been in clinical practice for decades, the mechanisms by which such therapy may attenuate allergic symptoms remain unclear. Since the allergic reaction in mice is quite different from that of humans, other suitable animal models have been sought. Dogs have an allergic reaction similar to humans and manifest clinical signs of allergy. Therefore, in terms of clinical evaluation, the dog is an appropriate model for investigation of therapeutic efficacy prior to application of any therapy to humans.

Appropriate antigens for sublingual immunotherapy (SLIT) for Japanese cedar pollinosis may be the native cedar pollen antigens (e.g., proteins such as Cry j1 and Cry j2). However, the risk of anaphylaxis induced by these antigens cannot be excluded even with a mucosal therapy like SLIT. Allergic reactions occur following binding of the allergen to IgE antibodies occupying Fcε receptors on the surface of mast cells, resulting in mast cell degranulation and release of potent immunological mediators. In cases where high levels of allergen specific IgE are bound to mast cells in vital organs, systemic anaphylaxis can ensue on exposure to even minute amounts of allergen, resulting in life threatening bronchconstriction and hypotension. It is known that most IgE antibodies recognize conformational epitopes of the antigens, therefore one could predict that denatured antigens would show low or no binding to IgE antibodies specific to the native form of the same antigen. With that in mind, we prepared a recombinant fusion protein joining the Cry j1 and Cry j2 proteins in a non-native state for use as a possible allergy vaccine.

The aims of this project are: to develop safe and efficient antigens that can be utilized for SLIT and to investigate the therapeutic effects of these reagents on the allergic reaction and its clinical symptoms in an allergy-prone dog colony with high basal IgE levels.

Study of the therapeutic efficacy of sub-lingual immunotherapy (SLIT) for Japanese cedar pollinosis

Structural genes of mature region encoding Japanese cedar major antigens, the Cry j1 and Cry j2 proteins, were fused, and the resultant

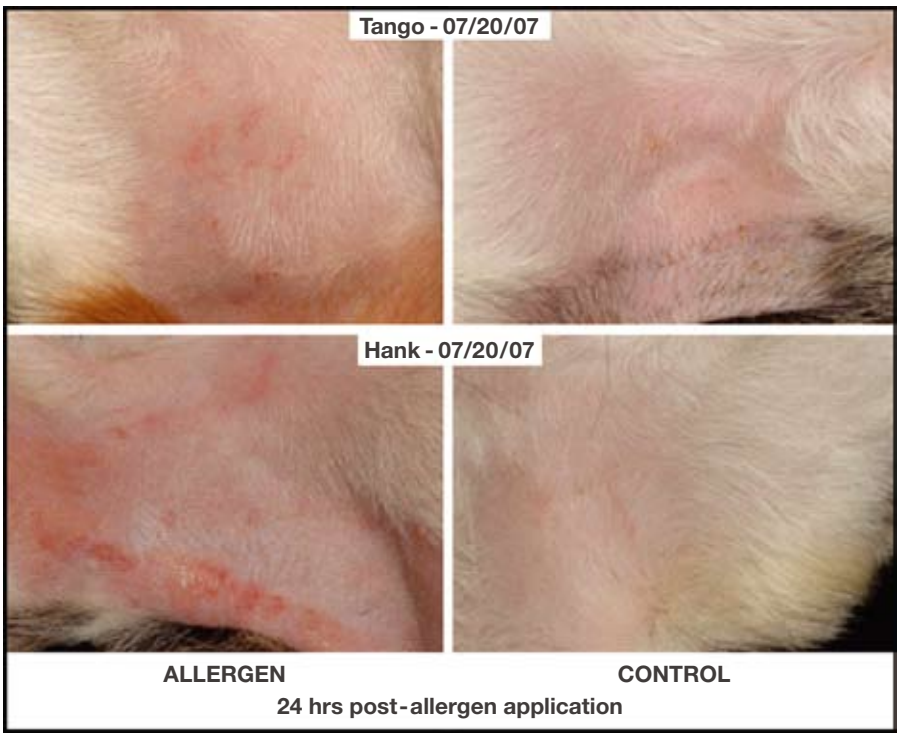

Figure Evaluation of skin lesions induced by Cry j 1 slurry application to Japanese cedar pollen-sensitized Maltese beagle allergy (MBA) dogs

recombinant fusion protein (hereinafter referred to as the "recombinant Cry j 1/2 fusion protein") was expressed in *E. coli* or yeast cells. Since the recombinant Cry j 1/2 fusion protein was denatured and solubilized, it could not bind IgE antibodies derived from the pollinosis patients. However the fusion protein still contains all the T cell epitopes from both the Cry j1 and the Cry j2 proteins. Therefore, the fusion protein has distinct advantages in that it can be the safely administered without causing anaphylaxis, yet it may still induce immunity (e.g., cellular immunity and humoral immunity such as IgG) specific for the cedar pollen antigen. Being a protein rather than a peptide antigen, all of the T cell epitopes appropriate for patients with cedar pollen disease, no matter what their MHC haplotype, should be present in the fusion protein.

Study of the therapeutic efficacy of α-GalCer-liposome-recCry j1/2 fusion protein for Japanese cedar pollinosis

In our previous studies, we found that NKT cell-derived IL-10 appears to play a critical role for the development of antigen-specific Treg cells and immune tolerance. Also, splenic DCs acquire the properties of regulatory DCs in an IL-10-dependent fashion after the injection of liposome formulations of α-galactosylceramide (α-GalCer) in mouse models. To evaluate the efficacy of the liposomes in clinical studies, Maltese-Beagle Allergy (MBA) dogs were sensitized with native Cry j1 and Cry j2 antigens. Based on the results of intradermal testing, ten dogs that show positive reaction to Cry j 1/2 were enrolled into the study. The dogs will be divided into two groups based on the serum concentration of Cry j 1- specific IgE. The treatment group will receive the liposome formulation of α-GalCer encapsulated recombinant Cry j1/2 fusion protein.

A total of twenty eight MBA dogs were assigned to the present study. They received two intraperitoneal injections of the purified Cry j1 and Cry j2 proteins and additional repeated epicutaneous sensitization at two-week intervals with the crude antigens extracted from the Japanese cedar pollen. To evaluate the cutaneous allergic reactions of the sensitized MBA dogs, a slurry of the purified Cry j 1 protein was applied to to the skin of right pinna and diluent saline was applied to left pinna. The skin lesions resulting from the allergen application were evaluated grossly and histologically during the phase of skin application. "Tango" and "Hank" are representative sensitized MBA dogs (Figure).

Recent publications

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Laboratory for Immunogenomics

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Temporary employee : **Yuki Kobayashi**, **Tetsuhiro Moriya**, **Masako Mori**, **Seiko Watanabe**, **Nobutake Suzuki**, **Satomi Takahashi**, **Kayoko Nagata**, **Aoi Ozawa**, **Noriko Sawai**, **Tatsufumi Nagase**, **Yukiko Sakaguchi**

Assistant : **Kazuyo Nomura**

An important and basic mission of our research group is to function as a "Gateway" to genomics for immunologists. To achieve this goal, our research group has taken a three-pronged approach: (1) central support activities; (2) strategic and collaborative research activities; and (3) exploratory research activities aimed at new technology development. In 2007, we have made a significant effort to enhance strategic and collaborative research activities, particularly for the human primary immunodeficiency (PID) network project. We have also strengthened our collaborative relationships with other research groups at RCAI even more than before. For example, our collaboration with the Research Unit for Human Disease Model (unit leader, Dr. Ishikawa) has given us many opportunities to learn how to make genomic approaches more fruitful in immunology. One of our current interests that originated from this collaboration is how to analyze mRNA and protein profiles of very limited numbers of immune cells. This pursuit was originally motivated by the need to solve practical problems frequently encountered in the central support activities as well as in collaborative research activities. However, if we can make a breakthrough in genomic analysis at the single cell level, we believe that this path will lead us to substantial breakthroughs in analysis of complicated immune systems from a systems biology viewpoint. We anticipate that the interactions among our three-pronged research activities, which are driven by collaboration with immunologists, will play a crucial role in development of Immunogenomics at RCAI. Below we describe two of our research activities in 2007.

Enhancement of an open-access Immunogenomics database, RefDIC

As explained in the 2006 annual report, we launched an open-access Immunogenomics reference database of immune cells, abbreviated as RefDIC, and have described this database in a recent publication (Bioinformatics 2007). However, it was obvious from the outset that the dataset in RefDIC had to keep growing in size as well as in quality. To achieve this, we have taken advantage of our central support activities for DNA chip-based mRNA profiling: Because we analyzed all the samples for mRNA profiling in the same way as those for the dataset of RefDIC, it was quite straightforward to incorporate the data from either the central

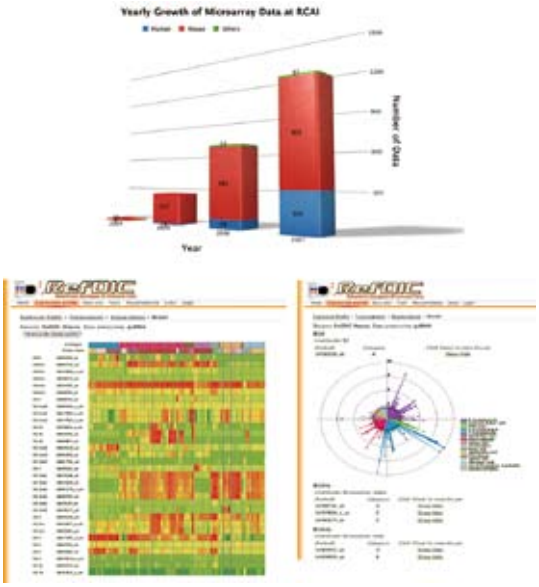

Figure 1 **Enhanced mRNA profile viewer in RefDIC**

support activities or the strategic/collaborative research activities after obtaining approval from clients/collaborators for deposition of the data into RefDIC. We are confident that this approach will allow for sustainable growth of RefDIC. In fact, the number of the microarray datasets was only 159 when RefDIC was opened to the public last year, but now the total number of datasets currently accumulated exceeds 1200 at the end of 2007 (Figure1 upper panel). We also had to revise the informational platform of RefDIC to accommodate the increased number of the datasets. Figure 1 shows an updated version of the RefDIC mRNA profile viewer as an example. RefDIC has been accessed by a large number of immunologists world-wide and will keep growing as well as becoming a more integrated Immunogenomics database through links to other bioinformatics databases and incorporation of new primary data from our own experiments.

The Primary Immunodeficiency Disease Project

The aim of this project is to analyze the immunological features and genetic causes of primary immunodeficiency diseases (PID) in a collaborative framework with clinical immunologists and was initiated in late 2006. Towards this end, various platforms are needed. The first is an informational platform to fill the gaps among patients, clinicians, basic immunologists, and genomic scientists. The second is a platform for analysis of PID samples from immunological and genetic perspectives. The third is an integrated database which stores various threads of information regarding PID and will enable us to make a diagnosis more accurately and rapidly than before. The informational and genomic technology will certainly play a crucial role in construction of such multidisciplinary platforms. In practice, we set up a collaboration framework with clinical immunologists in 13 Japanese universities/colleges, the Kazusa DNA Research Institute (KDRI;Chiba, Japan), and the Institute of Bioinformatics (IOB;Bangalore, India) for this project. In this regard, the PID project might be viewed as a pioneering venture for RCAI in an immune disease-oriented multidisciplinary collaboration. In RCAI, the Immunological Memory Research Group, the Immunogenomics Research Group, the Research Unit for Human Disease Model and the Immunoinformatics Research Unit are all involved in this project (See Collaborative Networks). Our research group (including the RCAI IT-support group) has worked particularly for construction of the clinical information repository of PID patients and for construction of the DNA diagnosis framework in collaboration with clinical immunologists and KDRI. Construction of the open-access PID database is also being conducted by our group, in collaboration with the Immunoinformatics Research Unit, KDRI and IOB. Consequently, the clinical information repository, designated PIDJ, is now open to the public and more than 40 patient samples were subjected to DNA analytical diagnosis in 2007. We will make every effort to mature these platforms to support patients and clinicians who are fighting PID.

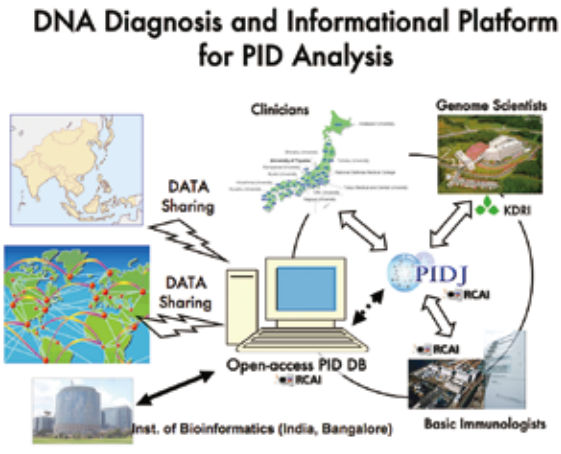

Figure 2 **The framework of the primary immunodeficiency disease project**

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Ishikawa F, Yoshida S, Saito Y, Hijikata A, Kitamura H, Tanaka S, Nakamura R, Tanaka T, Tomiyama H, Saito N, Fukata M, Miyamoto T, Lyons B, Ohshima K, Ucida N, Taniguchi S, Ohara O, Akashi K, Harada M, Shultz DL Chemo-therapy-resistant human AML stem cells home to and engraft within the bone marrow endosteal region. *Nature Biotechnology* 25, 1315-1321 (2007)

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Research Unit for Immunoinformatics

Unit leader

Sujatha Mohan

Research Associates : **Shivakumar Keerthikumar**
Rajesh Raju

An important mission of our research unit is to develop and maintain an open resource bioinformatics platform and data resources in order to gain insights into primary immunodeficiency diseases (PID) through the accumulation and analysis of genomic, transcriptomic, and proteomic data. 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.

Generation of Primary Immunodeficiency Disease Database from Asia

Primary immunodeficiency diseases are rare and genetically heterogeneous group of disorders affecting various components of the innate and adaptive immune systems. Despite rapid developments in the science of PID, early diagnosis and effective treatment of PID have posed new challenges to physicians all over the world. In this milieu, the development of a freely accessible dynamic web-based integrated tool on PID has become a paramount need of different target groups such as clinical immunologists, researchers, patients and family members.

We hope that this initiative will assist clinical immunologists and physicians in the identification of susceptible populations followed by cost effective regular screening procedures in order to minimize clinical deterioration and provide researchers with essential clues to analyze candidate PID genes. We also hope to create awareness among PID patients to help them lead a near normal quality of life and to support affected family members with relevant PID information.

The strength of this project is that it makes access to biological information simple for those without any bioinformatics knowledge and integrates it with experimental data from a diverse set of biological platforms including FACS and DNA genotyping to clinical phenotypes and signaling pathways. Our first focus is on the analysis of primary immunodeficiency diseases because RCAI investigators have been working intensively on PID in collaboration with outside researchers and have accumulated a wide array of clinical and experimental data..

To achieve our goals, we propose to carry out strategic approaches such as: i) curation of PID literature information along with publicly available clinical information into a web accessible knowledge base, ii) development of PID

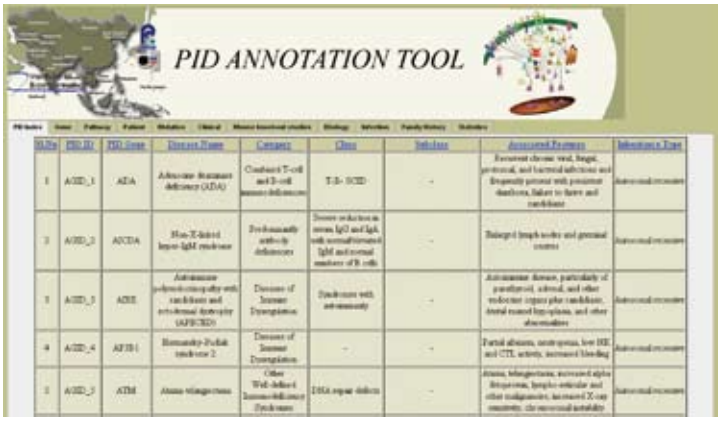

Figure 1 PID annotation tool web interface.

annotation tools, iii) accumulation of cell specific immune and other signaling pathway information and depiction of signaling cascades with various biological processes using a visualization tool, iv) analysis of known PID gene mutations and, where applicable, the mechanism of defective regulation in signaling pathways, v) identification of candidate genes by an in silico approach, vi) three dimensional structural prediction of mutant PID proteins relative to their normal counterparts.

Overall architecture of the PID database

The PID database will be constructed as a web-based tool that is built on a traditional three-tier architecture consisting of a client application as the first tier, Python and DTML code running on the Zope server engine as the second tier, and a backend database running MySQL as the third tier. This architecture will facilitate annotation of PID information, storage, retrieval, review, and update and generate a standard output format.

Future plans

We propose to develop a web-based integrated dynamic database for PID using a simple graphical user interface (Figure 1). We are also in the process of standardizing the entire work flow of a prediction program in order to automate and streamline the process to identify the most probable candidate PID genes for further experimental validation (Figure 2). Given the rare occurrence of PID, we plan to encourage direct submission of patient data to this resource by physicians, thus ensuring the continuous flow of relevant information as well as sufficient numbers of updated records.

We sincerely believe that this repository will serve as a prototype for other immunological diseases and will be of immense value to physicians in clinical decision making and diagnosis. This effort will combine the skills of clinicians as well as scientists from molecular biology, immunology, genomics, proteomics and bioinformatics fields from other countries especially from Asian regions.

The PID project has been initiated in collaboration with the Institute of Bioinformatics (IOB, Bangalore, India), the Immunogenomics research group at RIKEN RCAI, Japan, and the Kazusa DNA Research Institute (KDRI), Japan.

This research unit is 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).

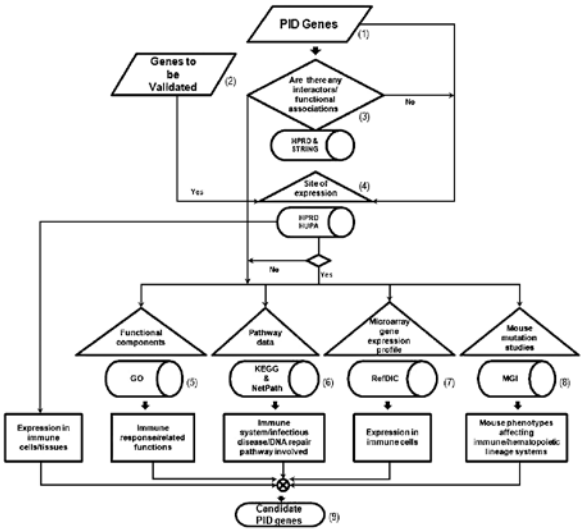

Figure 2 Flow chart outlining our current approach to the proposed PID prediction algorithm.
A list of known PID genes (1) or any genes (2) is given as an input data and passed through various gateways such as Human Protein Reference Database, HPRD - http://www.hprd.org/, STRING - http://string.embl.de/ (3), HuPA - http://humanproteinpedia.org/ (4), GO - http://www.geneontology.org/(5), KEGG -http://www.genome.jp/kegg/ and NetPath - http://netpath.org/ (6), RefDIC - http://refdic.rcai.riken.jp/welcome.cgi (7), MGI - http://www.informatics.jax.org/ (8).
Each gene is scored and ranked based on the number of successful gateways that are being passed for identifying the most probable candidate PID genes (9).

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Laboratory for Lymphocyte Development

Unit leader

Willem van Ewijk

Technical Staff : **Eric Vroegindewey**

Student trainees : **Stijn Crobach**
Janneke Rood
Nienke Grotenhuis

Thymic microenvironments critically support the development of T lymphocytes. The stromal cell types composing these microenvironments are epithelial in origin, and form as non-polarized cells a three-dimensional (3-D) organized network (Fig.1).

This type of organization is unique for epithelial cells in the thymus, because in other organs epithelial cells are polarized and placed on a basal lamina forming sheets of cells. The 3-D organization of thymic epithelial cells (TECs) forms the basis of microenvironments, allowing both for migration of thymocytes through the epithelial network, as well as for lympho-stromal interaction. In this way, TECs control various steps in T cell development, like clonal expansion, and also positive and negative selection. Reciprocally, the integrity of thymic microenvironments depends on the physical presence of thymocytes within the epithelial network. We have previously shown that removal of thymocytes from the thymic environment, either by genetic manipulation or by biochemical methods leads to a dramatic shift in the phenotype and organization of TECs, inducing the formation of epithelial cell types which are normally found in the gastro-intestinal tract and the respiratory tract (Fig.2). These experiments have uncovered a mutual interdependency between TECs and thymocytes, a phenomenon designated as “thymic crosstalk” (Fig.3). Thus, thymic crosstalk regulates the integrity and maintenance of the thymic stroma, and in this respect, thymocytes continuously promote their own development (see references 1-4).

Cellular and molecular analysis of thymic crosstalk.

The actual mechanisms of thymic crosstalk have remained obscure for many years. Using a “gain of function” approach we have started to analyze the role of the Notch signaling pathway in TEC development. Using fetal thymic organ culture (FTOC), we first showed that thymic crosstalk is a T cell specific phenomenon. Although progenitor B-lymphocytes may reside and develop in between TEC’s, they are only marginally able to influence the organization of thymic microenvironments. We then showed that B cells infected with a DLL-1 expressing retrovirus induce proper development of the thymic epithelial reticulum. These experiments particularly reveal that Notch activation by the DLL expressing B lymphocytes induces the 3-D non polarized phenotype in TECs (5).

Our FTOC experiments have also shown that complete loss of crosstalk leads to a dramatic shift in the development of TECs. Within a period

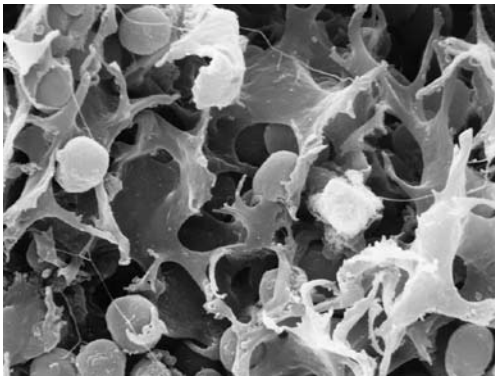

Figure 1 The 3-D organized epithelial network supports migration and differentiation of T lymphocytes.

Figure 2 Absence of crosstalk signaling induces generation of epithelial cells normally found in the intestinal and respiratory tracts.

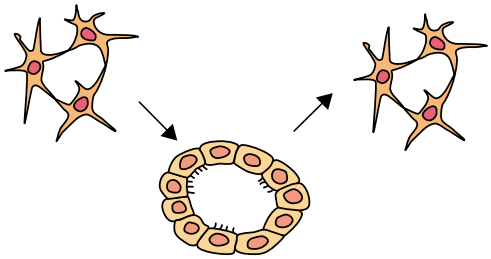

Figure 3 Principle of "thymic crosstalk".

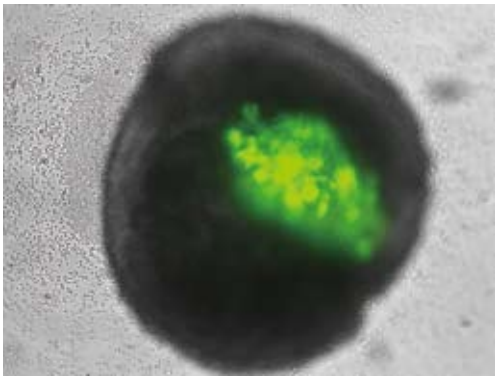

Figure 4 Introduction of GFP expressing intestinal epithelial cells in the embryonic thymus induces a thymus specific phenotype in these cells.

of 6 days, TEC's lose their long cytoplasmic processes and re-associate in cysts.

Apparently, in absence of thymic crosstalk signals, TECs start to follow a "default" developmental program, leading to the formation of 2-D polarized TECs associating on a basal lamina. It has been speculated by others that thymic cysts have an extra-thymic origin. However, by inducing cysts in FoxN1Cre; lox GFP mice we have shown that cyst lining cells do have an intra-thymic origin, since FoxN1 (a thymus specific transcription factor only present in TECs) was found to be expressed in cyst-lining epithelial cells (6).

The artificial thymus.

Based on technology previously developed by Watanabe et al. (J Clin Invest. 2007 Apr;117 (4):997-1007) we are currently attempting to construct an artificial thymus, where stromal cell types of other organs cultured on a scaffold are used to promote T cell development *in vitro*. Such an artificial thymus may allow the generation of T lymphocytes outside the body, which will be beneficiary for patients with T cell immunodeficiencies.

In collaboration with Dr. W. Germeraad, (University of Limburg, the Netherlands) we are investigating the role of human keratinocytes to promote T cell development *in vitro*. Likewise, we have found that the DLL expressing cell line TsT4 supports development of mature T lymphocytes, when transplanted under the kidney capsule of nude mice. An important issue of these experiments is to elucidate factors which promote professional TEC development from other stromal cell types. To gain insight in such TEC inducing factors, we have developed a novel experimental system where we introduce epithelial cells from other organs in the thymic environment (Fig.4).

We have found that epithelial cells derived from other epithelial organs, like the intestine, adopt a "thymus phenotype" upon introduction into the thymic environment. Normally, epithelial cells isolated from duodenal crypts, differentiate into a 2-D organized intestinal epithelium, but in striking contrast, intra-thymic injection of these cells induces the thymus specific 3-D phenotype. Apparently, the thymic environment "overrules" the developmental program of the injected gut epithelial cells.

The experiments mentioned above will provide insight in the development of thymic microenvironments, which is essential knowledge required for further development of an artificial thymus. In particular, our cell transfer studies will enable future analysis of genes, expressed during conversion of other epithelial cells into professional TEC's.

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Research Unit for Immunoepigenetics

Unit leader

Miguel Vidal

Technical staff : **Asako Shibano**
Noriko Ishikawa

The products of the Polycomb group (PcG) of genes encode a set of evolutionary conserved proteins that are typically considered regulators of developmental processes since they were first identified in *Drosophila melanogaster*. Recent evidence, however, shows their involvement in a wide range of processes that include genomic imprinting, maintenance of stem cell self renewal and also in cancer development. The PcG proteins associate in a variety of multiprotein complexes that act as epigenetic transcriptional repressors, through still largely unknown molecular mechanisms that involve, at least, two histone modifying activities. A histone H3 methyltransferase encoded by the Ezh2 gene, a subunit of Polycomb repressive complex (PRC) 2, and a histone H2A E3 monoubiquitin ligase activity, encoded by the paralogs Ring1A and Ring1B,which belongs to PRC1. Both, PRC2 and PRC1 are the best known Polycomb complexes, although PRC1 subunits are found also as components of a heterogeneous set of complexes. We are using the hematopoietic compartment to address the role of the Polycomb system in an ongoing differentiative process. To this end we are using loss of function mouse models of Ring1A/ Ring1B to investigate their function during hematopoiesis.

Ring1B function in hematopoietic homeostasis

Ring1B is essential for embryonic development. Therefore, in collaboration with H. Koseki (Laboratory for Developmental Genetics), we generated a conditionally mutant mouse line in which Ring1B inactivation occurs upon Cre-mediated deletion of coding sequences flanked by loxP sites. We used an inducible (interferon-responsive) Cre transgenic line to inactivate Ring1B in the bone marrow. Analysis in my lab in Madrid showed that mutant mice developed a hypocellular bone marrow that paradoxically contained an enlarged, hyperproliferating compartment of immature (myeloid) cells, with an intact differentiation potential. These alterations were associated with differential upregulation of cyclin D2, which occurred in all mutant bone marrow cells, and of p16Ink4a, observed only in the differentiated compartment. Concurrent inactivation of Ink4a rescued the defective proliferation of maturing cells but

did not affect the hyperproliferative activity of progenitors and resulted in a shortening of the onset of lymphomas induced by Ink4a inactivation. We conclude that Ring1B restricts the progenitors' proliferation and promotes the proliferation of their maturing progeny by selectively altering the expression pattern of positive and negative cell cycle regulators along hematopoietic differentiation.

To investigate these events in more detail we have initiated a characterization of targets affected by the deletion of Ring1B, analyzing gene expression patterns (H.Kitamura, Laboratory for Immunogenomics) in isolated progenitor cells. We are also studying binding of Ring1B to genomic regions of selected targets, with an emphasis on loci encoding known components of the cell cycle machinery. Finally, using a 4-hydroxitamoxifen inducible Cre sytem we study, in colaboration with Dr. Kawamoto (Laboratory for Lymphocyte develoment) the behaviour of adult and fetal hematopoietic mutant cells under a variety of *in vitro* differentiation conditions.

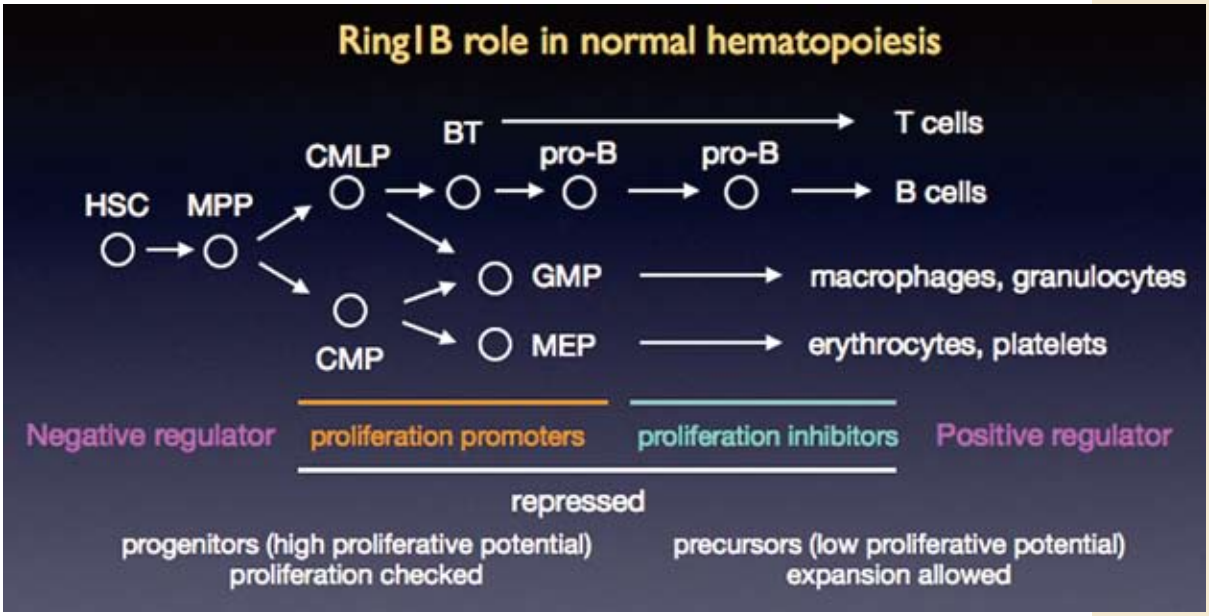

Figure Ring1B role in normal hematopoiesis

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Central Facilities

Central Facilities in RCAI provides all researchers in the Center with access to the most advanced equipment and technologies. The 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.

Monoclonal Antibody Lab

Collaborative Researcher : Shinji Seki (MBL)
Technical Staff : Tomomi Aoyama and Hajime Inamochi

In March 2008, operation of the Monoclonal Antibody Lab was tentatively terminated after five years of productive activities.

The Monoclonal Antibody (mAb) Lab was established in RCAI in collaboration with Medical & Biological Laboratories Co., Ltd. (MBL). Since its establishment in 2004, the lab received 92 requests and produced 73 mAbs. These mAbs were used at RCAI for FACS, immunohistochemistry, and ELISA analyses and, in some cases as receptor antagonists, and MBL merchandised the products. In 2007, the Lab produced 18 mAbs to various antigens, recombinant proteins, synthetic peptides, cells, native protein or even an unknown tissue target (table) upon requests by 11 teams. However, because of the completion of the collaborative agreement with MBL, the mAb Lab ceased operation in March 2008. The laboratoy may be reorganized in a different way.

Table Successful production of monoclonal antibodies classified by immunogen character (2007)

	Success ratio	Application of mAb
Recombinant (Ig-fusion)	5/6 (83%)	FACS (3) Immunohistochemistry (2)
Recombinant (silkworm)	2/2 (100%)	FACS (1) Antagonist (1)
Recombinant (E. coli)	2/3 (67%)	Immunocytochemistry (1) FACS (1)
Peptide-KLH	3/3 (100%)	WB (3)
Cell (stable transfectant)	3/4 (75%)	FACS (2) Antagonist (1)
Native protein	2/4 (50%)	ELISA (1) FACS (1)
Tissue (unknown targets)	1/1 (100%)	Immunohistochemistry (1)

FACS Lab

Technical Scientist :
Hanae Fujimoto
Techinical Staff :
Yukiko Hachiman

Photo Cell analyzer (FACS Calibur)

The FACS Lab provides a range of support for flow cytometry and cell sorting, procedures that are essential for nearly all immunological experiments. The FACS Lab contains several Becton Dickinson instruments: 4 FACS Vantages, 2 FACS Aria, 6 FACS Caliburs (photo) and 1 LSR. The laboratory's activities are divided into three main parts: technical support and training for FACS users, a cell sorting operation service, and management/maintenace of the FACS machines.

1. Technical support and training

In 2007, the facility offered 18 technical training courses (13 for cell sorting and 5 for cell analysis). Courses were held at seven different levels, LSR, Calibur basic, Calibur option, Vantage basic, Vantage option, Aria basic and Aria option. A total 48 researches in RCAI took the courses in 2007.

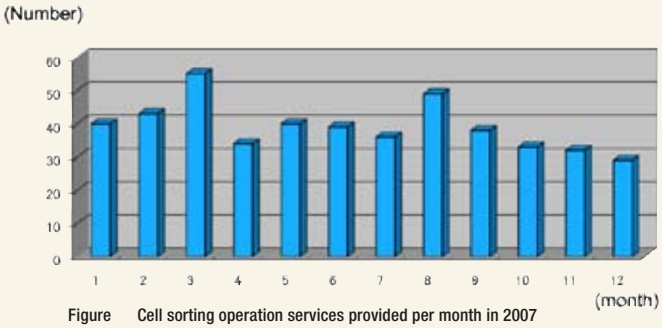


Figure Cell sorting operation services provided per month in 2007

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 2007, 468 cell sorting operation services were provided by the Lab (Figure).

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 staff, engineers from Becton Dickinson visit once a week to provide maintenance and technical support.

Confocal Lab.

Collaborative Researcher : Akiko Furuno (Leica Co. Ltd.)

The Confocal Lab provides imaging equipment and technical support. The Confocal Lab is managed in collaboration with Leica Microsystems. There are 5 confocal microscope systems: 1. Inverted system with visual and multiphoton (MP) lasers which is suitable for time-lapse imaging of living cells and organs. 2. Inverted system with a 405 nm laser which is suitable for a time-lapse imaging of living cells in a controlled environment (CO2, temperature, and humidity). 3. Inverted system with visual and UV lasers

which can be used for calcium detection. 4. Upright system with visual and UV lasers which is suitable for standard fixed specimen observation. 5. Intravital upright system with visual laser and MP laser which can be used for in vivo imaging of various tissues such as lymph nodes observations. During 2007, the total running time of the microscopes was over 2500 hours. In addition to the management of the equipment, Dr. Furuno from Leica provided 18 training courses for 35 people in 2007.

Genomics Lab

Senior research scientist : Hiroshi Kitamura
Research scientist : Yayoi Kimura
Technical staff : Ryo Yokoyama, Tomoko Yuasa, Satomi Takahashi, Tatsufumi Nagase, Noriko Sawai, Yukiko Sakaguchi, Nobutake Suzuki, Kayoko Nagata, Yuki Kobayashi, and Masako Mori

The Genomics Lab provides various services to the members of the Center; cDNA/Genomic clone distribution, DNA sequencing, DNA microarray experiments, RNA preparation from tiny amounts of samples, protein identification by mass spectrometry, and two-dimensional gel electrophoretic analysis (including differential image analysis).

1. Clone distribution

The lab provides cDNA and genomic clones in FANTOM (Fuctional Annotation of the Mouse) projects, lead by the RIKEN Genomics Science Center. In 2007, 88 clones were provided to 9 RCAI research groups.

2. DNA sequencing

During 2007, the lab sequenced 30,712 DNA samples (19,000 samples by 36cm capillary sequencers and 11,712 samples by 50 cm capillary sequencers) from 23 research groups.

Photo Mass spectrometry analysis

3. DNA Microarray analysis

Microarray analysis is conducted using the GeneChip system (Affymetrix) . In 2007, 772 arrays (386 human, 384 mouse and 2 chicken) were conducted.

4. Proteome analysis

Mass spectrometry analysis (302 samples), 2-D gel electrophoresis (40 samples) and in vitro translation (7 samples) were carried out in 2007. The samples were mainly from humans, mice, and bacteria (E. coli and bifidobacteria), and the requests were from 8 RCAI research groups.

Animal Facility

Senior technical scientist : Takanori Hasegawa
Technical scientists : Shinobu Mochizuki
Technical staff : Masashi Matsuda, Tomoyuki Ishikura, Naomi Ootsuka, Isamu Hisanaga, Momoko Ogoshi, Natsumi Saito
Administrative staff : Hiroko Iwamoto

The Animal Facility has been maintaining over 52,500 mice and 10 rats in the SPF area and 500 mice in an isolated area. The facility provides the following services for the users in RIKEN Yokohama Institute.

1. Supports for the ENU mutant mice project

The Animal Facility supports the ENU mutant mice project (a collaborative project between RCAI and GSC) by producing third generation (G3) offspring for phenotype-driven screening of the ENU-induced mutants. During 2007, seven lines of G3 (376 mice) were generated by in vitro fertilization.

2. Generation of SPF mice and cryopreservation of fertilized embryos

In order to maintain its SPF level, the Animal Facility performs in vitro fertilization to sanitize mice obtained from outside institutes. In 2007, 3,054 mice of 119 lines were obtained by this procedure. Cryopreservation of fertilized embryos reached 42,835 embryos of 276 lines.

3. Generation of transgenic mice

The Animal Facility generates transgenic mice requested by laboratories of the RIKEN Yokohama Institute. In 2007, 27 transgenic mouse lines were generated using 39 vectors provided by six laboratories.

Photo Vinyl isolator

4. Generation of knockout mice

1) Injection method
387 male chimeras were generated using 26 vectors from 9 laboratories.
2) Aggregation method
1,477 male chimeras were obtained using 59 vectors from 11 laboratories

5. Sperm cryopreservation

Two lines were cryopreserved in 2007.

RIKEN Special Postdoctoral Researcher (SPDR) and Junior Research Associate (JRA)

RIKEN Special Postdoctoral Researchers (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, three postdocs,

Dr. Tomoyuki Suzuki
(Lab. for Cytokine Signaling)(01)
Dr. Shinji Fukuda
(Lab. for Epithelial Immunobiology) (02)
Dr. Shinya Tanaka
(Lab. for Signal Network) (03)
conducted their research at RCAI through the SPDR program.

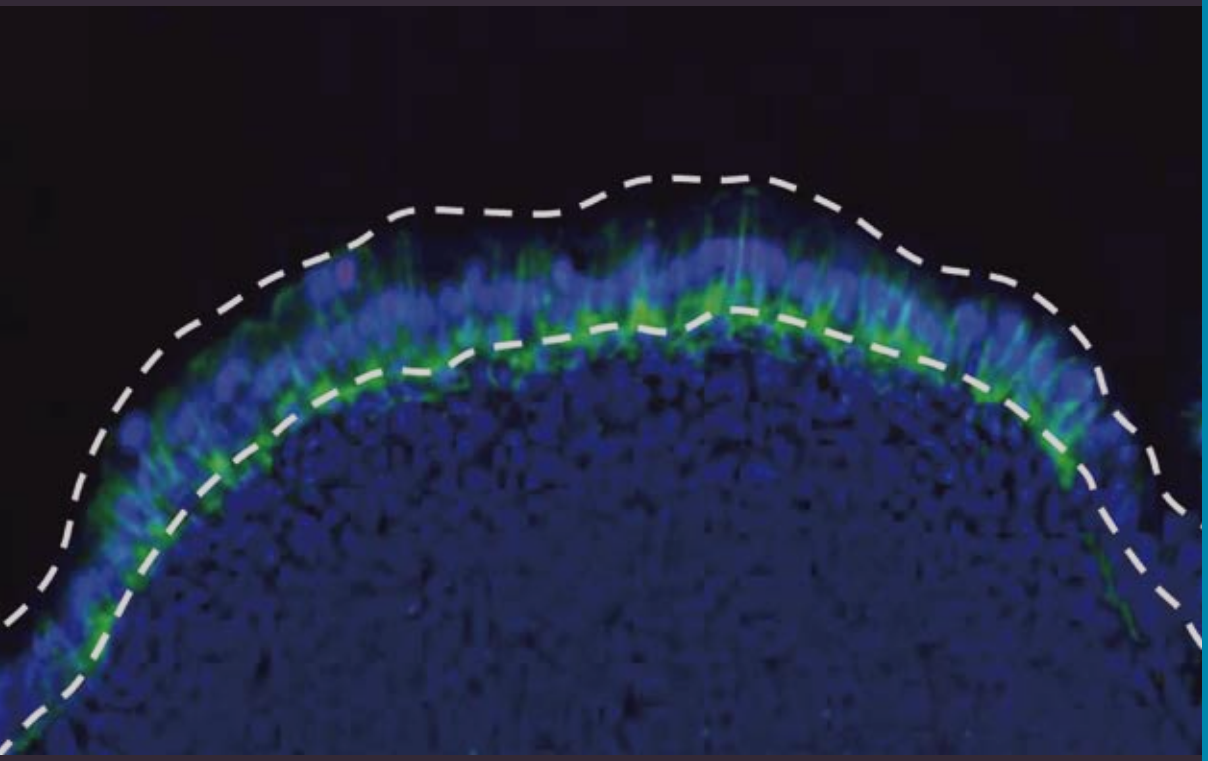
RIKEN's Junior Research Associate (JRA) Program

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, veteran scientists with years of experience. This program provides part-time positions at RIKEN for young researchers enrolled in university PhD 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, ten JRA students studied in RCAI.

Shintaro Hojo	(Lab. for Cytokine Signaling) (04)
Masayo Harada	(Lab. for Developmental Genetics) (05)
Takahiro Sugiyama	(Lab. for Host Defense) (06)
Yasutaka Motomura	(Lab. for Signal Network) (07)
Masashi Tachibana	(Lab. for Transcriptional Regulation) (08)
Shigeharu Fujita	(Lab. for Dendritic Cell Immunobiology) (09)
Noriko Komatsu	(Unit for Immune Homeostasis) (10)
Gaku Nakato	(Lab. for Epithelial Immunobiology) (11)
Asuka Terashima	(Lab. for Immune Regulation) (12)
Naomi Hongo	(Lab. for Immune Regulation) (13)

2007 Collaborative Networks



Opening of the RIKEN Jeffrey Modell Diagnostic and Research Center for Primary Immunodeficiencies

Photo From left, back row: Masaru Taniguchi (Director, RCAI), Peter Turner (President, CSL Behring LLC), Shigeaki Nonoyama (Professor, National Defense Medical College), and Michio Oishi (Director, KDRI); front row: Fred Modell (Founder, JMF), Ryoji Noyori (President, RIKEN),, and Vicki Modell (Founder, JMF).

RCAI established the RIKEN-Jeffrey Modell Diagnostic and Research Center for Primary Immunodeficiencies (Director, Dr. Toshitada Takemori), in collaboration with the Kazusa DNA Research Institute (KDRI) and the clinical research team for "Investigative Research on Primary Immunodeficiency Disease Syndrome". This team is sponsored by the Ministry of Health, Labor and Welfare and consists of 13 domestic universities/colleges. This center was partly supported by the Jeffrey Modell Foundation (JMF), a United States non-profit organization whose goal is to improve the quality of life of PID patients and to promote basic and clinical research in PID. In commemoration of the opening of the center, RIKEN held a ceremony on January 15, with participation by KDRI, a leader of the clinical research team, JMF founders Mr. Fred and Mrs. Vicki Modell, the Tsubasa no Kai (a Japanese advocacy group for PID patients and their families), and guests from the Ministry of Science, Technology and Sports and the Ministry of Health, Welfare and Labor. The RIKEN-Jeffrey Modell Diagnostic and Research Center will focus the collective efforts of domestic organizations specializing in the diagnosis and treatment of PID and biomedical scientists. The long-term goal of this strategy is to establish a database linking clinical, genetic, and immunological information to enable the prompt and accurate diagnosis of PID, allowing early decisions concerning appropriate treatment options, thereby helping to improve the quality life of PID patients.

Primary Immunodeficiency (PID) syndromes cause disorders in immune functions due to congenital abnormalities, making these patients very susceptible to infection by pathogens such as bacteria, viruses, and fungi. These are extremely serious disorders, which at times are accompanied by malignant tumors at a young age, autoimmune diseases, and allergies. It is estimated that about 10,000 people in Japan suffer from these syndromes. At present more than 150 causative PID genes have been identified, but there are many forms of PID for which the genetic defect has not yet been determined. In cases where the causative gene has been identified, treatments such as haematopoietic stem cell transplants and gene-repair therapy may be administered, resulting in the potential to cure some of these disorders. However, because there is often a delay in diagnosis, there are many cases where patients become afflicted with serious infectious diseases and die as a result. Particularly in cases where the causative gene has not been identified, diagnosis and adequate treatment methods have not been established. In Japan, cases of PID have been dispersed and no central database, which could amass clinical information or results of genetic analyses, has existed, making it difficult to identify causative genes.

In an effort to elucidate the pathogenesis of PIDs, establish methods of early diagnosis and find effective treatments, RCAI formed a collaboration in 2006 with 13 universities/colleges in Japan which belong to the research team for Investigative Research on Primary Immunodeficiency Disease Syndrome*, ("Ministry of Health, Labour and Welfare Survey Research Team", Research Leader: Toshiro Hara, Professor and Chairman of the Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University). RCAI and this team, in cooperation with KDRI, have been promoting research on the immunological analyses and identification of causative genes in anonymized PID patients. Through this collaboration, RCAI accepts the DNA of patients suffering from PID, performing genetic analyses and building a database containing the analytical results.

The work of the RCAI to date has been highly regarded by the Jeffrey Modell Foundation (JMF), an American NPO established in 1987 to prevent PID and to promote its diagnosis and treatment throughout the world, and a decision was made by RIKEN and JMF to jointly engage in a project to build a diagnostic research and clinical data platform. The establishment of the RIKEN Jeffrey Modell Research Center for Primary Immunodeficiencies with the cooperation of the RCAI and the KDRI as well as 13 universities/colleges across Japan will not only contribute to the study of basic human immunology, but is also expected to play a major role in speeding up the diagnosis of PID and the selection of more appropriate treatment approaches.

In order to meet these goals, it is extremely important to have an information network that allows access to accurate data by hospital clinicians, basic researchers, clinical PID experts, and PID patients. The RIKEN Jeffrey Modell Immunodeficiency Research Center, located within RCAI's research building in Tsurumi, Yokohama, began its work to build a PID clinical archive with the support of RCAI's IT- team, clinicians, and KDRI. Thanks to their devoted effort, the database, designated PIDJ (Primary Immunodeficiencies Database Japan) was successfully launched in November 2007. This general diagnostic and therapeutic database (http://pidj.rcai.riken.jp/medical.html) not only contains clinical data collected from all over Japan, but also integrates the basic analytical data including immunological and DNA / RNA analyses performed by the RIKEN Jeffrey Modell Immunodeficiency Research Center. This information platform is expected to provide an accurate PID diagnosis framework and promote research, including the development of new diagnostic and therapeutic methods, which can then contribute to PID patients, their families and physicians.

Currently, RCAI has also succeeded in developing a model mouse where human PID has been reproduced using humanized mouse technology. This new approach is also expected to promote basic research into the pathogenesis of PID and the development of new treatment technologies.

The RIKEN Jeffrey Modell Immunodeficiency Research Center will continue to strengthen the cooperation among clinicians, researchers and other Jeffrey Modell Diagnostic Centers worldwide, and to promote basic research into the treatment of PID, as well as increasing our understanding of the development and function of the human immune system.

***Investigative research team for Investigative Research on Primary Immunodeficiency Disease Syndrome, a research program of the Ministry of Health, Labour and Welfare for overcoming intractable disease:** At present, the following 13 universities/colleges are participating as members of the research investigation group: University of Miyazaki, Gifu University, Nagoya University, Kyushu University, Hiroshima University, Kanazawa University, University of Toyama, Tokyo Medical & Dental University, Tohoku University, Hokkaido University, Kyoto University, Shinshu University, National Defense Medical College. Dr. Toshiro Hara, Professor and Chairman of the Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University is the team leader.

Generation of the Asian Primary Immunodeficiency Disease Database

To build an integrated information platform for Primary Immunodeficiency Diseases (PID) and also as a part of our actions to initiate a network of PID research in Asia, RCAI started a collaborative research project with the Institute of Bioinformatics (IOB, Bangalore, India) and the Kazusa DNA Research Institute (KDRI), 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).

Photo from left, Mr. Rajesh Raju, Dr. Sujatha Mohan and Mr. Shivakumar Keerthikumar

Primary Immunodeficiency diseases are rare and genetically heterogeneous group of disorders affecting various components of the innate and adaptive immune systems. Despite rapid developments in the science of PID, early diagnosis and effective treatment pose new challenges to physicians all over the world. In this milieu, the development of a freely accessible dynamic web-based integrated database for PID has become a paramount necessity for different target groups such as clinical immunologists, researchers, patients and family members. The main objective of this database is to provide detailed information about genes and proteins that are involved in PID along with other pertinent information about molecular alterations, signaling pathways and protein-protein interaction networks.

A new research unit, Research Unit for Immunoinformatics, was established in RCAI, and Dr. Sujatha Mohan moved from IOB to start the project activity at RCAI as its unit leader. The unit also accepted two Ph.D. students, Shivakumar Keerthikumar and Rajesh Raju (photo). "We sincerely believe that this repository will serve as a prototype for other immunological diseases and will be of immense value to physicians in clinical decision making and diagnosis. This will combine the efforts of clinicians as well as scientists from molecular biology, immunology, genomics, proteomics and bioinformatics fields from other countries especially from Asian regions," said Dr. Mohan.

Collaboration with Foreign Institutes

RCAI has established collaborations with the Max Planck Institute of Immunobiology, the Max Planck Institute for Infection Biology, and the German Rheumatism Research Center (DRFZ) in Germany, Inserm Pasteur Institute in France, A*STAR in Singapore, and the La Jolla Institute of Allergy and Immunology in San Diego, USA. During FY2007, the first RIKEN-Max Planck joint workshop, a RIKEN-A*STAR joint symposium, and a RIKEN-Inserm joint symposium took place.

RIKEN RCAI and the Max Planck Society / German Rheumatism Research Center Joint Meeting on Activation and Regulation of the Immune System

The first Max Planck Society (MPG) - RIKEN joint workshop "Activation and Regulation of the Immune System" was held April 16th and 17th in Berlin. Participating scientists were from the RIKEN Research Center for Allergy and Immunology (RCAI, Yokohama), the Max Planck Institute for Infection Biology (MPIIB), the Max Planck Institute for Immunobiology (MPI), and the German Rheumatism Research Center (DRFZ). The sessions were held in a modern research facility that houses both the MPIIB and DRFZ located on the historic Charité medical campus in the heart of Berlin. This venue was particularly fitting for a joint German/Japanese immunology meeting because it was here that German and Japanese bacteriologists Emil Behring and Kitasato Shibasaburo collaborated in groundbreaking studies in the 1880's. They were the first to identify substances in blood of immunized animals that could be used to passively protect against tetanus toxin, substances subsequently termed antibodies.

The goal of the meeting, which featured more than 20 talks by researchers from the three participating institutes, was to promote future research collaborations between the Max Planck immunology institutes and RCAI. The meeting covered the spectrum of current immunologic research, providing an opportunity for scientists from both countries to learn the latest developments in their respective fields and to establish personal relationships that should foster future collaborations.

The meeting featured talks by senior scientists including Thomas Boehm (MPI) described his efforts to trace the evolutionary origin of the MHC-peptide system used for antigen presentation in contemporary vertebrates, with the hypothesis that the MHC prototype may have been a soluble peptide carrier. He presented data that olfactory neurons can discriminate and respond to different MHC peptides and is cloning the peptide receptor. Sergei Nedospasov (DRFZ) described ongoing efforts to create mice humanized for the cytokine tumor necrosis factor (TNF) and TNF receptors as a model to allow study of the in vivo effects of TNF blockers. These are currently in use clinically to treat human diseases such as rheumatoid arthritis and the goal is to use these mice to develop new and more effective therapeutics. Michael Reth (MPIB) described new transgenic mice in which Cre recombinase is driven by mb-1 regulatory elements, resulting in very robust Cre expression at all stages of B cell development. Using this system he could study a wave of B cell development in previously B cell deficient mice and found, surprisingly, that ligation of the B cell receptor, which should mimic encounter with self antigen and delete these newly formed B cells, had no effect. Toshitada Takemori (RCAI) issued a challenge to the textbook immunology view that memory B cells are generated during the germinal center reaction. Using a variety of cell sorting and gene expression array strategies, he has concluded that germinal center B cell and memory B cells develop along separate pathways. Masaru Taniguchi (RCAI) and colleagues have solved a long standing puzzle of how BCG immunization suppresses IgE production and have identified the central role of the NK T cell, specifically the production of IL21 by these cells. The clinical implications of these studies in terms of allergy abatement are currently being tested by BCG immunization in humans. Importantly for future collaborations, many younger researchers were also invited to speak. In addition to the speakers, the audience included scientists and students from the Berlin area.

The initial memorandum of understanding to establish formal collaborations between RIKEN and MPG was signed in 1984. The participants at this joint meeting all agreed that it was a successful first edition that would stimulate collaborations and scientific exchanges and that there should be more such meetings in the future.

RIKEN and A*STAR Joint Immunology and Developmental Biology Meeting

The Biomedical Research Council of the Agency for Science, Technology and Research (A*STAR) of Singapore and RIKEN co-organized "Joint Symposium 07", held on May 16 and 17 at Biopolis in Singapore. Biopolis is a biomedical research hub currently consisting of seven contemporary buildings with two new ones, Immunos and Neuros, set to open soon as immunology and neurobiology research centers. Participating groups from A*STAR were the Singapore Immunology Network (SIgN), the Institute of Molecular and Cell Biology (IMCB), the Institute of Medical Biology (IMB), and the Genome Institute of Singapore (GIS) and from RIKEN were the Research Center for Allergy and Immunology (RCAI) and the Center for Developmental Biology (CDB). Immunology was the focus of first day's meeting and Developmental Biology the second. These seemingly disparate scientific disciplines have much in common. Studies of blood cell and lymphocyte development have been very informative models for our understanding of other developmental systems. Moreover, similar genetic programs, e.g. the involvement of hierarchies of transcription factors, regulate development of immune as well as other cell types.

The Symposium, organized by Drs. Kong Peng Lam and Mike Jones of A*STAR and Drs. Shin-ichi Nishikawa and Takashi Saito of RIKEN, was broad in scope with twenty speakers in fields such as cell signaling, tumor immunology, lymphoid organogenesis, humanized mice, myogenesis, embryogenesis, and evolution. Dr. Nishikawa (CDB) described a new model of Peyer's patch (PP) organogenesis involving chemokine receptor-mediated sequential relocation of PP inducer cells resulting in an influx and segregation of the various cellular constituents that make up the mature PP. Along a similar vein, Dr. Takeshi Watanabe (RCAI) described the generation of artificial lymph nodes (LN) that recapitulate conventional LN development but with intriguing differences including a predominance of memory cells. He showed that these structures could be primed to suppress tumor cell growth and ultimately hopes to move these studies into humans. Dr Jean Pierre Abastado (SIgN) has studied a spontaneous mouse tumor model and finds that tumor cells can attract and polarize immune cells, particularly macrophages, so that they support tumor cell growth and inhibit immune mediated tumor rejection. Interference in this complex ecology may have therapeutic benefit. Dr. Phil Ingham (IMCB) is studying myogenesis in zebrafish and has made a myoblast EST library, the largest yet generated from this model organism. He has isolated several genes required for normal muscle development and this library is publically available to other zebrafish researchers, an important resource.

The goal of this Symposium was to allow senior and junior investigators from both countries to present data in areas of common interest in order to initiate and foster long term collaborations. The participants all agreed that this was a very good beginning.

RIKEN RCAI and Inserm Pasteur Joint Immunology Symposium

The first Inserm/Pasteur Institute/RIKEN RCAI Joint Immunology Symposium was held March 3 – 5, 2008 at the La Ferme des Vallees (The Farm of the Valleys) in Auffargis, France. Participating scientists were from the RIKEN Research Center for Allergy and Immunology (RIKEN RCAI, Yokohama), the RIKEN SNP Research Center, the Pasteur Institute, and various INSERM-affiliated research institutes.

The meeting opened on Monday afternoon with comments by Mr. Stéphane Roy from the Inserm Department of International Relations. He emphasized that the aim of the Symposium was to initiate an immunology network between France and Japan.

The first session dealt with TCR signaling and the immune synapse. Philippe Bousso (Inserm, Pasteur) used two photon microscopy to examine intratumoral cytotoxic T cell (CTL) activity. A novel FRET-based sensor whose function is dependent on ZAP-70 activity was used by Alain Trautmann (Institut Cochin, CNRS-Inerm) to demonstrate the existence of the "antisynapse", a clustering of signaling molecules on the pole of the cell opposite from the immunological synapse. The power of modern imaging was further emphasized in studies by Takashi Saito (RIKEN RCAI) who has used total internal reflection fluorescence microscopy to identify TCR signaling microclusters.

In the T-cell function and homeostasis session, Benedita Rocha (Inserm, Necker, Paris) asked whether all CD8 T cells responding to a given antigen have identical gene expression profiles. Agnés Lehuen (Inserm, Cochin, Paris) described her studies of invariant NKT cells in autoimmune diseases and viral infection. Talks on regulatory T cells by Shohei Hori (RIKEN RCAI) and Lucienne Chatenoud (Inserm, Necker) illustrated the potency of these cells in regulating autoimmune diseases.

The Tuesday session featured talks on NK and dendritic cell (DC) biology. James DiSanto (Inserm, Insitut Pasteur) described an important role for the transcription factor GATA3 in development of both T and NK cells. Tsuneyyasu Kaisho (RIKEN RCAI) identified differential roles for various signaling pathways in the production of type I interferons by DC. Marc Dalod (Inserm, CIML, Marseilles) used genome wide expression analysis to determine the relationship among DC subsets.

The session on BCR recombination and B cell development began with a talk by Jean-Pierre de Villartay on the role of the DNA repair system in immunoglobulin gene and class switch recombination. Nadel Bertrand (Inserm, CIML) described the early steps of follicular lymphoma genesis. Ana Cumano (Inserm, Pasteur) described her studies of cytokine requirements of early lymphoid precursors in fetal and adult mice.

The afternoon began with a session on Innate immunity. Yves Delneste (Inserm, Angers) described studies to harness the capacity of antigen cross presentation by dendritic cells to combat tumors. Marc Bonneville (Inserm, Nantes) observed that γδ T cells recognize a mitochondrial protein found on the surface of some tumor cells.

In the Mucosal immunity and Lymphoid organ/Lymphocyte development session, Sidonia Fagarasan (RIKEN RCAI) described her studies on the function and formation of intestinal isolated lymphoid follicles. Hiroshi Ohno (RIKEN RCAI) studies the M cells that overlay Peyer's patches and has identified a molecule that binds bacteria and mediates their transcytosis, the initial step in the local immune response.

On the final day, the session Clinical immunology and immunotherapy began with Laurence Zitvogel (Inserm, IGR, Villejuif), who described studies indicating that chemo- and radiotherapy, in addition to affecting tumor cells, can activate the innate immune system. Danila Valmori (Inserm, Nantes) described the outcome of a large clinical trial organized by the Cancer Vaccine Collaborative, an international collaboration whose objective is to translate laboratory discoveries into cancer therapy. Kazuhiko Yamamoto (RIKEN SRC) concluded the session and the symposium discussing the results of genome-wide association analysis as a means to identify rheumatoid arthritis susceptibility genes.

RCAI Annual Scientific Review Meetings 2007

RCAI Advisory Council conducts “Annual Reviews” every year and “Term Reviews” every 2-3 years. This year, Annual Reviews took place at RCAI in RIKEN Yokohama Institute. The major goal of Annual Reviews is to provide the scientists with external support and guidance and to identify potential problems early on. The reviews were divided to eight blocks consisted of 3-5 research teams and 2-3 AC members who have expertise in the field.

RCAI Advisory Council members


Dr. Max. D. Cooper, Chair


Dr. Rudolf Aebersold


Dr. Antonio Coutinho


Dr. Alain Fischer


Dr. Paul W. Kincade


Dr. Bernard Malissen


Dr. Diane Mathis


Dr. Ruslan Medzhitov


Dr. Masayuki Miyasaka


Dr. Nobuyuki Miyasaka


Dr. Hiromitsu Nakauchi


Dr. William E. Paul


Dr. Klaus Rajewsky


Dr. Takehiko Sasazuki


Dr. Ralph M. Steinman


Dr. Kazuo Sugamura


Dr. Kiyoshi Takatsu


Dr. Dale Umetsu


Dr. Arthur Weiss

RCAI Advisory Council

Throughout the Annual Scientific Review Meetings 2007 (Table 1), the Advisory Council remained impressed with the progress of the institute and the research quality of the individual research laboratories. They commented that many young scientists at an early stage in their career presented well and were in good shape. However, they also pointed out that, as the institute approaches its first 5-year milestone in 2009, some considerations on how to sharpen the research focus of the Center and how to refresh research staff are now in order.

Table 1 : RCAI Annual Scientific Review Meetings 2007

Date	Block	Organizer	Laboratory	AC Members
Nov. 27-28	Development and Differentiation	Koseki	Developmental Genetics [Koseki]	Paul W. Kincade* Hiromitusu Nakauchi
			Lymphocyte Cloning [Rybouchkin]	
			Lymphocyte Development [Kawamoto]	
			Transcriptional Regulation [Taniuchi]	
			Human Disease Model [Ishikawa]	
Oct 1-2	Molecular Events in Cellular Signaling	Saito	Cell Signaling [Saito-T]	Arthur Weiss* Kazuo Sugamura Bernard Malissen*
			Lymphocyte Differentiation [Kurosaki]	
			Single Molecule Imaging [Sakata-Sogawa]	
Oct 22-23	Mucosal Immunity and Lymphoid Organ	Ohno	Epithelial Immunobiology [Ohno]	Max D. Cooper* Masayuki Miyasaka Klaus Rajewsky *
			Mucosal Immunity [Fagarasan]	
			Immunological Memory [Takemori]	
			Antigen Receptor Diversity [O-Wang]	
Jul 6-7	Immune Regulation 1	Kaisho	Host Defense [Kaisho]	Ralph M. Steinman* Ruslan Medzhitov
			Infectious Immunity [Ishido]	
			Dendritic Cell Immunobiology [Sato]	
			Innate Cellular Immunity [Tanaka]	
			Therapeutic Model [Shimizu]	
Jun 7-8	Immune Regulation 2	Kanagawa	Autoimmune Regulation [Kanagawa]	Antonio Coutinho* Takehiko Sasazuki Diane Mathis *
			Immune Homeostasis [Hori]	
			Immunochaperones [Udono]	
			Immune Regulation [Taniguchi]	
Oct 18-19	Allergy Research	Hirano	Cytokine Signaling [Hirano]	William E. Paul* Kiyoshi Takatsu
			Signal Network [Kubo]	
			Immunogenetics [Yoshida]	
Jan 18-19	Human Immunology	Watanabe	Immune Surveillance [Watanabe]	Alain Fisher* Nobuyuki Miyasaka* Dale Umetsu
			Cellular Immunotherapy [Fujii]	
			Vaccine Design [Ishii]	
			Immunodeficiency Network [Takemori]	
Oct 4-5	Central Facilities & Systems Biology	Ohara	Genomics [Ohara]	Bernard Malissen* Rudolf Aebersold
			Animal Facilities [Koseki]	
			ENU Mutagenesis [Yoshida]	
			Immunoinformatics [Mohan]	
			Molecular Systems Immunology [Tokunaga]	

* Core Advisory Member

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 15 million JPY/year to each collaborative research project for up to three years.

Since the program started in 2004, 12 projects have been funded (table 2) and in FY2007, Drs. Vidal and Koseki's collaborative project on Ring1 genes resulted in three successful publications (Development (in press), Mol Cell Biol, 2008 and Nat Cell Biol, 2007). Six collaborative projects were in place during FY2007, and contributed not only to scientific discovery but also to the international exchange of scientists and international visibility.

Table 2: RCAI International Collaboration Award Program Awardees

Year	Investigator	Title of Research	Host Laboratory
2004-2006	**Michael Dustin** New York University School of Medicine	Analysis of dynamism and function of limmunological synapse using planar membrane and knock-in T cells	**Takashi Saito** Lab for Cell Signaling
2004-2006	**Willem van Ewijk** Leiden University Medical Center	Regulatory role of lymphoid progenitors during development of thymic microenvironments	**Hiroshi Kawamoto** Lab for Lymphocyte Development
2004-2006	**Miguel Vidal** Centro de Investigaciones Biologicas, CSIC	Genomic and functional analysis of the role of the Polycomb Ring1 genes in B-cell development	**Haruhiko Koseki** Lab for Developmental Genetics
2004-2005	**Steven F. Ziegler** Benaroya Research Institute at Virginia Mason Medical Center	Role of NKT cells in TSLP-Mediated Aallergic linflammation	**Masaru Taniguchi** Lab for Immune Regulation
2004-2006	**Peter D. Burrows** University of Alabama at Birmingham	Expression and function of FcRY-a novel Fc receptor-related gene expressed in B cells	**Jiyang O-Wang** Lab for Antigen Receptor Diversity
2005-2007	**Wilfried Ellemeier** Institute of Immunology, Medical University Vienna Hilde Cheroute La Jolla Institute for Allergy and Immunology	Study of T cell differentiation mediated by regulated expression of CD8 genes	**Ichiro Taniuchi** Lab for Transcriptional Regulation
2005-2007	**Mark Bix** University of Washington, Seattle, Washington	Understanding genetic regulation of interleukin 4 production by a CD4(+) T cell-intrinsic mechanism.	**Masato Kubo** Lab for Signal Network
2005-2006	**Yun-Cai Liu** La Jolla Institute for Allergy and Immunology	Gene-array analysis and proteomics of Th2 tolerance	**Yasuyuki Ishii** Lab for Vaccine Design
2005-2007	**Kenneth M. Murphy** Howard Hughes Medical Institute Washington University School of Medicine	Visualization of STAT protein in the cytokine mediated signaling at a single molecular level.	**Osami Kanagawa** Lab for Autoimmune Regulation
2005-2007	**Facundo Damian Batista** Cancer Research UK London	Role of signaling molecules in B cell synapse formation and its maintenance	**Tomohiro Kurosaki** Lab for Lymphocyte Differentiation
2006-	**Sunhwa Kim and Michael Karin** Department of Pharmacology, Univ. of California, San Diego	Identification of Novel Necrotic Molecules from Necrotic Hepatocytes and Examination of Its Effect on the Inflammatory Response	**Masato Tanaka** Lab for Innate Cellular Immunity
2007	**Andrea Brendolan** Cornell University Medical Center, Department of Cell and Developmental Biology	A study on the spleen and lymph nodes mesenchymal cells that participate in the assembly of artificial secondary lymphoid organs	**Takeshi Watanabe** Research Unit for Immune Surveilance

International Progams

Short-term Lectureship Program

The Center's Short-term Lectureship Program invites internationally recognized investigators to give lectures and also to stay for one week at RCAI to discuss ongoing research with young investigators. In FY2007, the program invited nine distinguished researchers from six countries for seminars and discussions (table 3).

Table 3 : Short-term Lectureship Program 2007

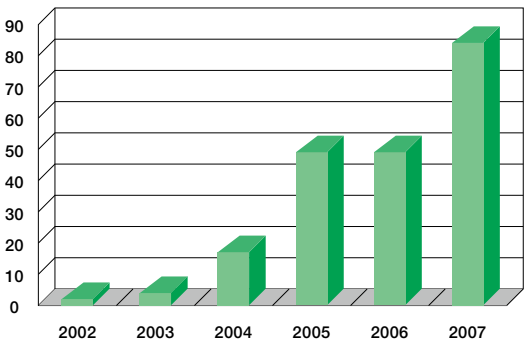
Month	Lecturer	Affiliation	Seminar Title
October, 2007	**Kong Peng LAM**	Institute of Molecular and Cell Biology, Center for Molecular Medicine, Lab of Molecular and Cellular Immunology/ Singapore Immunology Network, Singapore	Modulation of thymocyte apoptosis by FAIM
October, 2007	**Hans-Christian Reinecker**	Harvard Medical School, Medicine/ Massachusetts General Hospital, Gastrointestinal Unit, USA	Mucosal dendritic cell systems in control of tolerance and mucosal defenses
October, 2007	**José A. Villadangos**	The Walter and Eliza Hall Institute of Medical Research, Immunology Division, Australia	Regulation of antigen presentation in the dendritic cell network *in vivo*
November, 2007	**Hua Gu**	Columbia University/Microbiology, USA	The Cbl family of proteins at the crossroads of immune system development, autoimmunity, and anti-tumor immunity
November, 2007	**Edward Palmer**	Basel University, Dept. Research, LabTransplantation Immunology and Nephrology, Switzerland	Central tolerance: How much affinity can you tolerate?
December, 2007	**Tim Sparwasser**	Technical University of Munich, Medicine, Medical Microbiology& Immunology, Germany	Novel approaches for vaccine design: Bypassing Treg activity enhances T cell-mediated immunity
February, 2008	**Tom Cupedo**	Erasmus University Medical Center, Erasmus University Rotterdam, Department of Hematology, The Netherlands	Inducers and organizers in human fetal lymph node
March, 2008	**Steven L. Reiner**	University of Pennsylvania, Medicine/Infectious Diseases, USA	Specifying diverse T cell fates for immunity
March, 2008	**Mark Shlomchik**	Yale University, Laboratory Medicine and Immunobiology,USA	(1)Toll-like receptors dictate autoantibody specificity (2)B cell response and dynamics

Support for Patent Applications

RCAI regards the development of new technology in immunological studies as one of its missions. In order to support such innovations, RCAI has developed the following unique support system for patent applications.

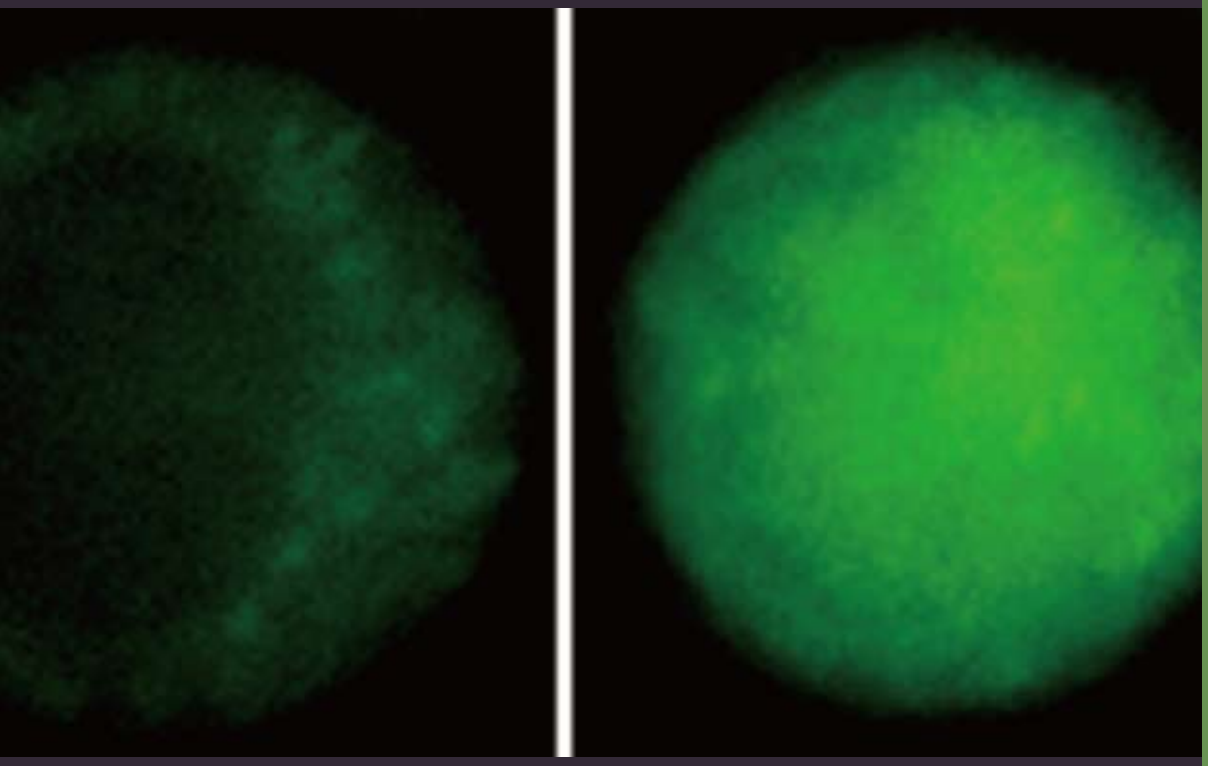
A Japanese patent law firm visits RCAI twice a year to conduct hearings on the candidate inventions for patent applications. This law firm prepares the necessary documents on the inventions that are considered suitable for application as patents. Scientists submit the reports to the RIKEN Center for Intellectual Property Strategies.

The number of patent applications increased dramatically after this system was introduced in 2004. The number of application in FY2007 reached 82, including 24 domestic applications and 58 overseas applications.



Graph: Number of patent applications

2007 **Events**



RCAI International Summer Program

(RISP 2007)

The second RCAI International Summer Program (RISP 2007) was held in Yokohama July 20-27. Forty-three graduate students and postdoctoral fellows from sixteen countries participated in RISP 2007. During the first part of the program, held for four days at RCAI, there were poster and oral presentations by the participants and a series of lectures by invited speakers from RCAI, Japanese universities and abroad. The second part of the program was the joint RCAI/JSI meeting "Development and Maintenance of the Immune System" held at Pacifco Yokohama. Fifteen of the participants stayed on at RCAI for an additional week long laboratory internship from July 30 - August 3.

The research interests of the participants were quite varied, although T cell development and regulatory T cells were a major focus. Everyone who attended the sessions was very impressed by the high quality of the oral presentations and by the insightful questions asked during these presentations and during the invited lectures. These lectures provided an overview of the immune system ranging from molecular analysis of antigen receptor signaling to whole animal studies. Each lecturer incorporated introductory material pertinent to their topic as well as recent highlights from his or her own research.

Several of the invited lecturers also gave talks at the RCAI/JSI meeting, providing nice continuity with the material covered earlier at RCAI. There were also many new topics, thus further increasing the breadth of the immunology coverage accessible to the RISP participants. Awards for best RISP posters were presented at a reception on the first evening of the RCAI/JSI meeting. Toward the end of the reception, all the participants gathered as a group and their spokesman, Omar Duramad, a graduate student from the University of Texas, MD Anderson Cancer Center expressed their deep appreciation to the organizers for inviting them to RISP 2007, an exceptional experience. The success of this unique program is due to the efforts of the Organizing Committee, chaired by Dr. Tomohiro Kurosaki, the RISP Secretariat who made sure everything went smoothly, and in particular to the efforts of the outstanding participants. Planning is already underway for the next RISP, tentatively scheduled for June 2008.



Lecturer	Title
Prof. Kayo Inaba, Kyoto University	C-type Lectins in Dendritic Cells and Macrophages
Prof. Yoshihiro Kawaoka, Institute of Medical Science, University of Tokyo	Why Influenza Kills and will Kill Again
Dr. Takashi Saito, RIKEN, RCAI	Immunological synapse and T cell activation
Prof. Willem van Ewijk, Leiden University Medical Center	Thymic microenvironments, come and go….
Prof. Cornelis Murre, University of California, San Diego	Regulation and function of helix-loop-helix proteins in lymphocyte evelopment
Dr. Fumihiko Ishikawa, RIKEN, RCAI	Studying human normal and malignant stem cells in NOD/SCID/IL2rγnull mice
Dr. Sidonia Fagarasan, RIKEN, RCAI	Dynamic interactions between bacteria and gut associated lymphoid tissues
Dr. Tsuneyasu Kaisho, RIKEN, RCAI	How dendritic cells sense and respond to immune adjuvants
Prof. Alexander Y. Rudensky, Washington University	Regulatory T cell
Prof. Kenneth M. Murphy, Washington University	Development of Effector T cell Subsets
Prof. Marc K. Jenkins, University of Minnesota Medical School	Enumeration of Polyclonal Antigen-Specific $CD4^+$ T Cells in Naïve and Immunized Mice
Prof. Diane Mathis, Joslin Diabetes Center	Aire control of immunological tolerance
Prof. Kathryn Calame, Columbia University	Blimp-1 Regulates Lymphocyte Differentiation and Function

Participants (Country)			
Minna Anthoni (Finland)	Yung-Chi Chang (Taiwan)	Nicolas Chevrier (France)	Jin Wook Choi (Korea)
Maria Ciofani (Canada)	Nichole Danzl (USA)	Ella Day (Ausutralia)	Ivana Djuretic (Serbia/Montenegro)
Michael Drennan (Germany)	Omar Duramad (USA)	Klaus Gossens (Germany)	Marie Gotter (France)
Yaron Gruper (Israel)	Edwin Hawkins (New Zealand)	Sook Yuin Jessica Ho (Singapore)	Marisa Juntilla (USA)
Taras Kreslavsky (Russia)	Supeecha Kumkate (Thai)	Anne Lai (USA)	Queenie Lam (Canada)
Michelle Linterman (New Zealand)	Jared Lopes (Canada)	Dalam Ly (Canada)	Ana Miletic (USA)
Nadia Ouaked (Canada)	Vikram Palanivel (USA)	Amiya Patra (India)	Ivan Poon (Australia/China)
Anatoly Rubtsov (Russia)	Aurore Saudemont (France)	Petra Schnorrer (Germany)	Barbara Schraml (Austria)
John Sedy (USA)	Till Strowing (Germany)	Melanie Stumpf (Germany)	Andy Tan (Singapore)
Michele Teng (Malaysia)	Datsen Wei (USA)	Robert Welner (USA)	Luke Williams (USA)
Tim Worbs (Germany)	Hsin-Jung Wu (Taiwan)	Santiago Zelenay (Argentina)	

RCAI-JSI International Symposium 2007

Development and Maintenance of the Immune System

A joint RCAI/JSI meeting "Development and Maintenance of the Immune System" was held July 26th and 27th at the Pacifco Yokohama Conference Center. Speakers were invited from Japan, Canada, Germany, the UK, and the US. Among the participants in this meeting were the students from the RCAI International Summer Program and some of the lecturers from that program also spoke here.

The first day's talks concerned the development of the immune system and mechanisms of lymphocyte selection. Dr. Toshio Suda (Keio University) focused on a very early developmental stage, the hematopoietic stem cell (HSC). He described the importance of the osteoblast, a cell normally thought of in terms of its role in bone formation, in the stem cell niche, a specialized microenvironment where the HSC reside in a quiescent state. Dr. Juan Carlos Zúñiga-Pflücker (University of Toronto, Canada) demonstrated an important role for Notch signaling in early T cell development. A Notch signal is required for expansion of αβT cells and their survival at the single positive stage and PI3 Kinase and Akt are downstream effectors. By contrast, the γδ T cells can survive without Notch once they express a TCR. Dr. Al Singer (NIH, USA) described an interesting mouse model in which the TCR co-receptors CD4 and CD8 are deleted and signaling to the developing thymocytes occurs strictly through the TCR. Remarkably, if the major histocompatibility complex (MHC), which is normally what thymic T cells are selected on, is also inactivated, T cell development still takes place but the T cells in the periphery are not MHC restricted. Dr. Dianne Mathis (Harvard Medical School, USA) has been examining obesity and the role of low grade inflammation in adipose tissue in the development of type-2 diabetes. She found that regulatory T cells (Tregs) accumulate to an extraordinarily high fraction of CD4 T cells in the abdominal adipose tissue of mice as they age and that the cells have an unusual phenotype in comparison with Tregs in other tissues. Understanding the physiological role of these cells may provide new prophylactic treatment options for preventing diabetes.

Differentiation of lymphocyte subsets and the maintenance of a functional immune system were the focus of the second day's talks. Dr. Shinsuke Taki (Shinshu University) discussed basophils as a potential initial source of IL-4 for polarization of helper T cells toward the Th2 pathway. Analysis of gene targeted mice allowed him to define important regulatory molecules in IL-3 induced IL-4 production by basophils. Dr. Brigitta Stockinger (National Institute for Medical Research, UK) described physiological inducers for the development of Th17 cells, a recently described subset of helper T cells that produce IL-17. Zymosan, a yeast cell wall component and *Mycobacterium tuberculosis* were found to induce Th17 cells. She also noted that reported differences between human and mouse Th17 cells may not be real, but rather are an artifact of not starting with sufficiently pure naïve T cells when inducing human Th17 cell development in vitro. Dr. Marc Jenkins (University of Minnesota Medical School, USA) discussed strategies to enumerate polyclonal antigen-specific CD4 T cells in unimmunized mice in order to avoid the use of transferred TCR transgenic T cells to study the expansion and half life of these cells during an immune response. The frequency of peptide specific T cells is remarkably low and varies among different peptide epitopes, e.g. 220 versus 20 cells per mouse. The memory T cells generated after immunization decline slowly over time, with a half life of ~ 50 days for the one specificity studied in detail, and this may account for the loss of T cell immunity. Dr. Masato Tanaka (RCAI) has developed a system for tolerance induction in experimental autoimmune encephalomyelitis (EAE), a mouse model for human multiple sclerosis. Apoptotic cells expressing EAE self antigens are injected intravenously and protect the mouse against subsequent induction of EAE. Conditional knockout mice were used to identify the marginal zone macrophages in the spleen as important mediators of tolerance induction.

The next RCAI-JSI International Symposium on Immunology is tentatively scheduled for June of 2008.

Session I : Development of immune system	
Toshio Suda, Keio University, Japan	Quiescent Stem Cells Regulated by Niche Cells
Thomas Boehm, Max-Planck Institute, Germany	Evolutionary history of the thymus
Hiroshi Kawamoto, RCAI, Japan	Thymic T cell progenitors that have shut off B cell potential retain potential to generate macrophages
Juan Carlos Zúñiga-Pflücker, University of Toronto, Canada	Early T cell development, differential dependency on Notch for $\alpha\beta$ vs $\gamma\delta$ lineage choices
Cornelis Murre, University of California, San Diego, USA	3D-Architecture of the Immunoglobulin Heavy Chain Locus
Session II : Mechanism of lymphocyte selection	
Alfred Singer, National Institutes of Health, USA	Basis for Thymic Selection of an MHC-Restricted $\alpha\beta$ T-cell Repertoire and How it can be Circumvented
Ichiro Taniuchi, RCAI, Japan	Transcriptional Factors Network in CD4/CD8 Lineage Choice
Diane Mathis, Joslin Diabetes Center, Harvard Medical School, USA	Fat Tregs
Mitsuru Matsumoto, University of Tokushima, Japan	Thymic stromal factors that regulate establishment and maintenance of self-tolerance
Jun-ichiro Inoue, University of Tokyo, Japan	Identification of TRAF6 as a critical intracellular signal transducer for controlling autoimmunity
Shiv Pillai, Harvard Medical School, USA	BCR signal strength and a cell surface acetylation/deacetylation paradigm for peripheral B lymphoid lineage commitment and tolerance
Session III : Functional differentiation of lymphocyte subsets	
Shinsuke Taki, Shinshu University, Japan	Positive and negative regulation of IL-3 signals in murine basophils, important for controlling Th2 development
Brigitta Stockinger, MRC National Institute, UK	Differentiation and function of the Th17 T cell subset
Kathryn Calame, Columbia University College, USA	Blimp-1 Regulates Lymphocyte Differentiation and Function
Takeshi Watanabe, RCAI, Japan	Generation of artificial lymph nodes (aLNs) and their immunological function
Session IV : Maintenance of functional immune system	
Marc K. Jenkins, University of Minnesota, USA	Enumeration of Polyclonal Antigen-Specific $CD4^+$ T Cells in Naïve and Immunized Mice
Jonathan S. Bromberg, Mount Sinai School of Medicine, USA	Trafficking of T Cells and Antigen Presenting Cells for Transplantation Tolerance
Masayuki Miyasaka, Osaka University, Japan	Dynamics of regulatory T cells, dendritic cells, and eosinophils in the small intestinal lamina propria
Jason G. Cyster, University of California, San Francisco, USA	Cell Migration Dynamics During the B Cell Immune Response
Masato Tanaka, RCAI, Japan	Tolerance induction by apoptotic cell clearance
Hedda Wardemann, Max-Planck-Institute, Germany	Autoantibodies as part of the antigen-experienced B cell repertoire in healthy donors and patients with Systemic Lupus Erythematosus
Chandra Mohan, UT Southwestern Medical Center, USA	Lupus susceptibility loci at the interface of tolerance and autoimmunity

2nd JSI-RCAI Workshop

Lessons learned from PID: From bedside to bench and from bench to bedside

2nd JSI-RCAI Workshop was held on May 25, 2007 at RCAI, RIKEN Yokohama Institute. The workshop was entitled "Lessons learned from PID: From bedside to bench and from bench to bedside". Ninety-six people including both clinicians and basic researchers gathered and freely discussed various aspects regarding PID.

The history of immunology began with the discovery of primary immunodeficiency diseases (PID). The pathogenesis of PID discloses the function of the human immune system, and Dr. R. A. Good called PID an "experiment of nature". In the 1950-60s, PID including X-linked agammaglobulinemia, DiGeorge syndrome, and severe combined immunodeficiency were identified, and the understanding of these diseases indicated that the immune system was divided into cellular and antibody immunities. Surprisingly, these findings were observed 10 years before the recognition of T cells and B cells. PID includes not only a defect in T and B cells, but also a defect in NK cells, neutrophils, phagocytes, and complements. Interestingly, a defect in innate immunity as well as adaptive immunity is also associated with PID. Modern immunology primarily focuses on the study of immunology in mice using gene-engineered mice. The function of the same gene between mice and humans could differ, and the gene-engineered mice are also not considered to be true models for human diseases. PID is a rare disease, however, a better understanding of PID could help to improve our overall understanding of the immune system in humans. Thereby, through the discussions between clinicians and basic immunologists, people could learn much regarding the immune system in humans.

Morning Session	
Hirokazu Kanegane (University of Toyama)	Lessons learned from XLA-history of primary immunodeficiency diseases
Fumihiko Ishikawa (RCAI)	Studying human primary immunodeficiency diseases using a novel xenotransplant model
Satoru Kumaki (Tohoku University)	X-SCID gene therapy: reality and future direction
Yousuke Takahama (University of Tokushima)	Lessons learned from ENU-mutagenized athymic mutants
Selected oral presentation (from the poster presentation)	
Chikako Tono (Aomori Rosai Hospital)	Correction of immunodeficiency associated with NEMO mutation by unrelated cord blood transplantation using a reduced-intensity conditioning regimen
Hiroyuki Nunoi (University of Miyazaki)	Analysis of gp91 phox^{+} variant Chronic Granulomatous Disease in Japan
Poster session 1	
Afternoon Session	
Tsuneyasu Kaisho (RCAI)	Immunological phenotype of pattern recognition receptor defects: from knockout mice to human immune disorders
Yoshiyuki Minegishi (Tokyo Medical and Dental University)	Molecular genetics and funnctional characterization of human hyper-IgE syndrome
Masamichi Muramatsu (Kyoto University)	AID; its function and deficiency
Kohsuke Imai (National Defense Medical College)	Defect of immunoglobulin class switch recombination in human
Poster session 2	

3rd JSI-RCAI Workshop

Regulation of the Mucosal Immune Response: Crosstalk between Basic and Clinical Research

The 3rd JSI-RCAI Workshop "Regulation of the Mucosal Immune Response: Crosstalk between Basic and Clinical Research" took place on March 18, 2008 at RCAI, RIKEN Yokohama Institute. This workshop provided a good opportunity for mucosal immunity researchers in Japan from both basic and clinical fields to get together and discuss the regulation of mucosal immune responses and generate new insights in this field.

Inflammatory bowel disease (IBD), including ulcerative colitis and Crohn's disease, features chronic inflammation and ulcers in the small and large intestine. Many reports have shown that excessive responses by intestinal immune cells, which normally control innate and adoptive immunity to external antigens such as food antigens and luminal bacteria, occur in IBD. These conditions may be caused by genetic abnormalities. In animal models, it has been reported since the early 1990s that mice deficient in genes such as IL-2, IL-10, or TCRα, as well as SCID mice with colitis induced by the transfer of $CD45RB^{high}CD4^{+}$T cells, suffer from mucosal inflammation only in the presence of the intestinal commensal bacteria. Taken together, the immune responses to external antigens should be tightly regulated in the intestine. The intestinal immune system plays a major defensive role in protection against pathogens and in the induction of tolerance to many non-pathogenic factors, for example food antigens. This regulatory system for external antigens, however, appears to be impaired in IBD. Recently, a new treatment for IBD using an anti-TNFα antibody, which was generated from a 'bench to bedside' effort, has been successfully launched.

One-hundred and ten researchers from basic and clinical fields gathered and discussed the latest findings in IBD and intestinal immunity.

Session 1 Chariperson: Hiroshi Ohno (RCAI)	
Jun Kunisawa (The University of Tokyo)	Functional roles of sphingosine 1-phosphate in the immunosurveillance and immunological homeostasis in gut
Tadakazu Hisamatsu (Keio University)	Intestinal macrophages for gut homeostasis and inflammation
Koji Hase (RCAI)	Function and differentiation of intestinal follicular M cells
Toshiaki Ohteki (Akita University)	Mechanisms of IgA production in the mucosa-associated lymphoid tissue
Masaru Yoshida (Kobe University)	The role of Fc receptors for mucosal immunity
Poster discussion	
Session 2 Chariperson: Sidonia Fagarasan (RCAI)	
Kenya Honda (Osaka University)	Commensal bacteria drive lamina propria T_H17 cells
Kanai Takanori (Tokyo Medical and Dental University)	The life span of colitogenic $CD4^{+}$ T cells
Tomohiro Watanabe (Kyoto University) (selected from poster presentations)	Muramyl dipeptide activation of nucleotide binding oligomerization domain 2 protects mice from experimental colitis
Keiichiro Suzuki (RCAI) (selected from poster presentations)	Activation of follicles in Peyer's patches is dependent on TLR-MyD88 signaling stimulated by commensal flora
Session3 Chariperson: Masaru Yoshida (Kobe University)	
Taeko Dohi (Research Institute International Medical Center of Japan)	Searching targets for the treatment of chronic intestinal inflammation and mucosal injury
Tsutomu Chiba (Kyoto University)	A novel mechanism for mutagenesis in inflammation-associated carcinogenesis: Roles of activation-induced cytidine deaminase (AID) as a genome mutator

Guidance session for adjunct graduate school programs

May 12, 2007

RCAI accepts graduate students through the mechanism of adjunct professorships with various universities. In April 2007, RCAI and the Graduate School of Frontier Biosciences, Osaka University, agreed to collaborate for research education. Dr. Tomohiro Kurosaki (Group Director, Laboratory for Lymphocyte Differentiation) and Dr. Ichiro Taniuchi (Team Leader, Laboratory for Transcriptional Regulation) were newly appointed as visiting professors, and Dr. Keigo Nishida (Senior Researcher, Laboratory for Cytokine Signaling) became a visiting associate professor of the graduate school. The total number of adjunct professors/associate professorships in RCAI is now 16, and the Center collaborates with 5 university graduate schools. (Table)

On May 12, RCAI held a guidance session on adjunct graduate school programs. Twenty-nine participants gathered from all over Japan including Kumamoto, Niigata, Gunma, Tottori, Gifu and Tokyo. After Dr. Saito, Center Deputy Director, briefly summarized the adjunct graduate school programs, representatives from eight laboratories presented their research activities. In addition to those oral presentations, each laboratory introduced its projects during a poster session.

"We decided to hold this event on Saturday afternoon this year so that attending from remote areas would be easier. As a result, attendance was more than twice last year's," said Dr. Takemori, the organizer. The session provided an opportunity for students to directly visit and talk with lab leaders, and to consider their future directions. More than 17 laboratories received student visits on the day after the session. In the end, 15 participants indicated that they were interested in entering an adjunct graduate school to study at RCAI.

Table

Graduate School of Frontier Bioscience, Osaka University	Tomohiro Kurosaki (visiting professor)
	Ichiro Taniuchi (visiting professor)
	Keigo Nishida (visiting associate professor)
Graduate School of Medicine, Osaka University	Osami Kanagawa (visiting professor)
	Tsuneyasu Kaisho (visiting professor)
	Toshiyuki Fukada (visiting associate professor)
Department of Immunology, School of Medicine, Chiba University	Takashi Saito (visiting professor)
	Haruhiko Koseki (visiting professor)
	Hiroshi Ohno (visiting professor)
	Shinichiro Fujii (visiting associate professor)
	Yasuyuki Ishii (visiting associate professor)
	Fumihiko Ishikawa (visiting associate professor)
School of Biomedical Science, Tokyo Medical and Dental University	Takashi Saito (visiting professor)
	Tomohiro Kurosaki (visiting professor)
	Sidonia Fagarasan (visiting associate professor)
International Graduate School of Arts and Sciences,Yokohama City University	Hiroshi Ohno (visiting professor)

RIKEN Yokohama Open Campus

June 23, 2007

The RIKEN Yokohama Institute Open Campus took place on June 23, 2007. The fine weather brought a record high number of visitors (1,820) including 468 children, more than 10 % increase from last year. At RCAI, 14 research laboratories exhibited posters, and researchers explained their projects to the visitors. Among the displays, a new handmade model of immune receptors was especially popular with the children. While enjoying the 3-D puzzle representing immune receptors and ligands (photo), the children learned why immune cells only react to specific antigens, a sometimes difficult concept to convey. RCAI also provided two other hands-on learning opportunities. The Laboratory for Immunogenetics prepared stained samples of fetal mice. The visitors, ranging in age from kindergarten to retirees, observed the samples through microscopes and asked questions. The Laboratory for Lymphocyte Development arranged a sophisticated cell-sorting experiment for those in high school or above. After hearing a lecture on lymphocyte development and the concept of the fluorescent activated cell sorter, participants were divided into groups and isolated $CD4^{+}CD8^{+}$ cells from mice, a remarkable experience. In addition to these lectures and hands-on activities, two original RCAI videos "Ushering in a New Era for Medical Care; Therapies Using Immunologically Humanized Mice" and "Unlocking the Mysteries of the Immune System; RIKEN Research Center for Allergy and Immunology" were shown in the conference room.

Super Science High School

August 21

"Super Science High Schools" are schools designated by the Ministry of Education, Culture, Sports, Science and Technology to focus specifically on scientific education. There are currently 101 Super Science High Schools in Japan. Because more young Japanese are moving away from the sciences, the ministry aims to strengthen and improve scientific education especially at these schools. RCAI holds a special workshop every year in Kanagawa prefecture for students from these Super Science High Schools. This year, 31 students and 7 teachers from Hakuyoh, Seisho, Yokosuka, Zushi and Kanagawa Sogo Sangyo High Schools were invited. After lectures on "AIDS Virus and Immunity" and "Immunological Organs" by Drs. Tomohiro Kurosaki and Hisahiro Yoshida, the

students were divided into groups for hands-on practice of the dissection and histological staining of immunological organs. “I was surprised to see the organs of a mouse. They were so much alike with humans,” one student said. “I realized that biology was a beautiful academic world.”

In addition to this workshop, RCAI accepted student visits throughout the year. Students from Jisyuukann Junior High School enjoyed Dr. Kawamoto’s basic immunology lecture and a lab tour on July 18, Takasaki High School students listened to Dr. Ishii’s lecture on allergy therapies on July 25, students from Sendai Daiichi High School had a brief facilities tour with Dr. Iwano on Sep. 26, and Yokohama Suifuu High School students had a discussion talk with Dr. Kitamura and Mr. Ishii on Nov. 1.

RCAI Retreat

Sep 3-4, 2007

The aim of the annual RCAI retreat is to stimulate communication and collaborative activities. This is facilitated by gathering all the members in one venue in the countryside. This year, three publishing editors of well regarded scientific journals, *Nature Immunology*, *Nature Medicine* and *Immunity*, visited the meeting at the Nihon Aerobics Center in Chiba Prefecture together with 160 RCAI members.

On the first day, RCAI held two sessions of poster presentation. There were 86 posters in total, and both new unpublished data and on-going projects were introduced to the attendants. As people participated in extensive discussions, each laboratory head visited all the posters and voted for the Excellent Poster Awards. During the evening banquet, the13 awardees were identified (see *Excellent Poster Awards*). On the next day, the winners of the Excellent Poster Awards were given the opportunity to introduce his/her projects in a 10-minute English oral presentation. “This year, we tried to focus especially on unpublished new results,” Dr. Udono said.

The dedicated efforts of organizers contributed to the success of the retreat. Dr. Udono and Kaisho arranged the general organization of the meeting. Drs. Takemori, Kanagawa and Kurosaki held special training sessions for poster presentations. Ms. Imaizumi’s coordination of travel, food, and accommodation ensured that the program went very smoothly.

RIKEN Science Lecture: Immunology Creates the Future

Feb 2, 2008

RIKEN RCAI held a public lecture “Immunology Creates the Future” on Saturday, Feb. 2 at the Marunouchi Building. Holding the lecture on Saturday in central Tokyo was one of the keys for its success; it gathered nearly 400 people from all over Japan, even from Fukuoka, Nagasaki, Tokushima and Okayama prefectures. Following opening remarks by the RIKEN President, Ryoji Noyori, three RCAI researchers and an invited speaker gave talks: Dr. Fumihiko Ishikawa’s presentation “Humanized mice: the foundation for medical revolution” attracted people’s interest because of the tremendous possibility for the applications of the mice. Dr. Saito introduced his research “Monitoring single molecules to elucidate the immune mechanisms” and explained the contribution of the single molecule microscope. Dr. Taniguchi’s talk, “Can we overcome allergy?,” effortlessly explained the etiology and the current status of allergy vaccine development. The last speaker, Dr. Kishimoto, introduced his research on IL-6 and the development of antibody drugs that saved a boy from an immunodeficiency disease.

“I flew from Kyusyu with my son because he wanted to listen to Dr. Ishikawa’s talk. His research was very interesting and inspiring,” a participant noted.

In Memory of Konomi Nakamura

Satoshi Ishido, Leader, Lab. for Infectious Immunity

Ms. Konomi Nakamura, the assistant in the Laboratory for Infectious Immunity, passed away on Dec. 25, 2007 because of gall bladder cancer.

Photo: Ms. Nakamura (*center*) with the laboratory staff, in the summer of 2007.

Ms. Nakamura was a great source of support from the very beginning of the lab. Our laboratory started in April 2004 when, along with RCAI, we launched our actual activity in Yokohama. Most people in the Center had moved from outside RIKEN, but she already had a career in RIKEN as the assistant of Dr. Sakaki, Director of the Genomic Science Center. With her considerable institutional knowledge, she supported not only our lab but also the other RCAI assistants.

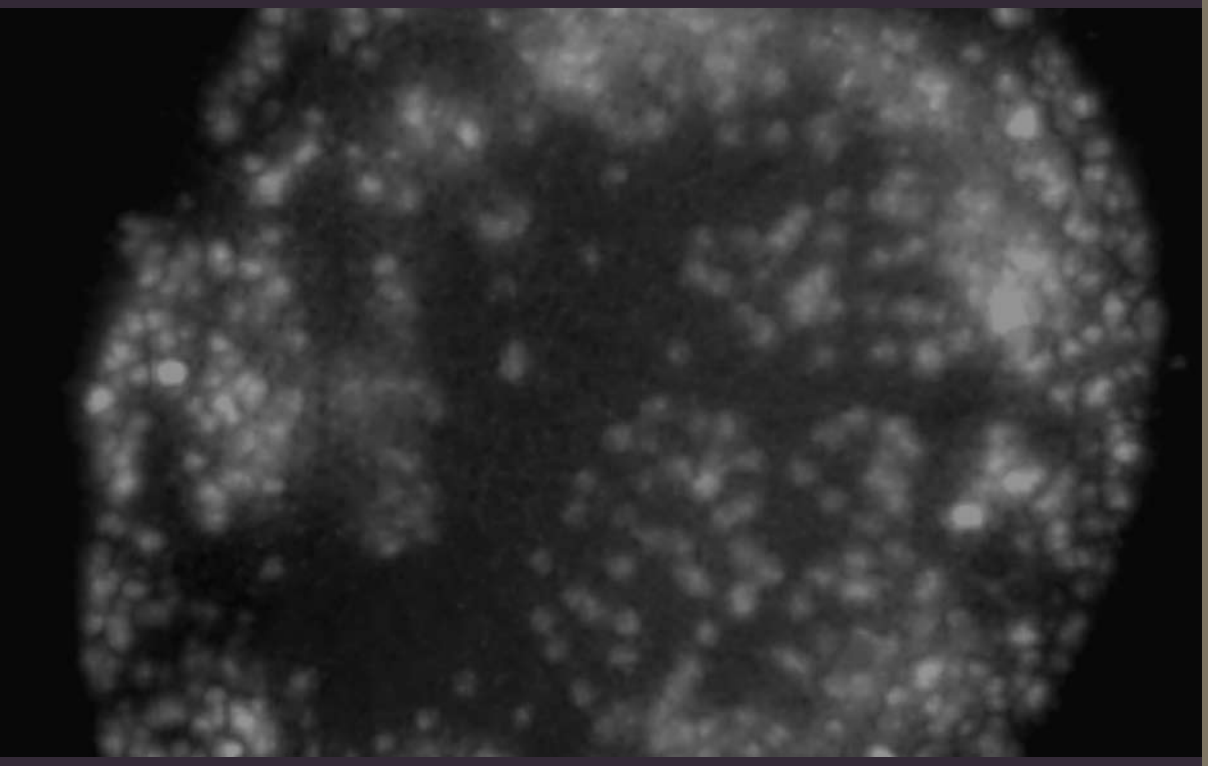
I can think of two major contributions she made to RCAI. First, she started the annual briefing session in the Adjunct Graduate School Program for students interested in studying at RCAI. Second, she organized RCAI's retreat meeting in 2006. In addition to these accomplishments, in 2005 she lead the organizational effort for the Symposium on Infection and Immunity, which was supported by the Special Study Group on Membrane Trafficking, Ministry of Education, Culture, Sports, Science and Technology. She showed great leadership in organizing all of these events, and her contributions were essential for their success.

Her biggest dream was to be a pianist. I heard that during her days in Musashino Academia Musicae, she had stayed in Europe alone to carry out her musical activities and complete her thesis. However, in her career at RIKEN, I think she had another goal, because I became keenly aware of her interest in science. During our weekly progress meeting, she asked pointed questions based on her wider social view. Her insights may have stemmed in part from her own experiences, because she suffered from severe atopic dermatitis. Her opinions from the patients' viewpoint often made me think more deeply about the implications of our work.

Thus, Ms. Nakamura did many great and unselfish things for our lab and for RCAI. We hope to extend the system she established, the various systems for research communication, and promise her to promote life science research.

Thank you Nakamura-san. May your soul rest in peace.

2007 Data & Statistics



Publications FY2007 Apr. 2007-Mar. 2008

Publication Table (Apr. 2007-Mar. 2008)

Journal Title	IF (2006)	FY2007
Science	30.0	1
Cell	29.2	1
Nature Reviews Immunology	28.7	1
Nature Medicine	28.6	1
Nature Immunology	27.6	5
Nature	26.7	1
Nature Genetics	24.2	1
Nature Biotechnology	22.7	2
Nature Cell Biology	18.5	1
Immunity	18.3	3
Journal of Clinical Investigation	15.8	5
Genes & Development	15.1	1
Nature Methods	15.0	1
Journal of Experimental Medicine	14.5	5
Immunol Rev	10.8	1
Blood	10.4	7
Journal of Cell Biology	10.2	2
PNAS	9.6	2
J Allergy Clin Immun	8.8	1
Stem Cells	7.9	2
Arthritis Rheum	7.8	2
Cell Death Differ	7.5	2
Am J Transplant	6.8	1
Molecular and Cellular Biology	6.8	7
Journal of Immunology	6.3	9
Molecular Therapy	5.8	1
Journal of Biological Chemistry	5.8	5
Curr Med Chem	5.2	1
International Immunology	4.0	3
Other Journals		44
Total		119

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Invited Presentations

Meeting	Title	Month - Year	Speaker
RIKEN-MPG “Activation and Regulation of the Immune System”, Berlin, Germany	Role of Runx complexes in CD4/CD8 lineage choice.	Apr - 07	Taniuchi, I.
RIKEN-MPG “Activation and Regulation of the Immune System”, Berlin, Germany	Type I interferon induction mechanism in dendritic cells.	Apr - 07	Kaisho, T.
RIKEN-MPG “Activation and Regulation of the Immune System”, Berlin, Germany	Mechanisms of tolerance induction to cell-associated antigens.	Apr - 07	Tanaka, M.
RIKEN-MPG “Activation and Regulation of the Immune System”, Berlin, Germany	Generation of artificial transplantable lymph node tissues and their efficient immunological function.	Apr - 07	Watanabe, T.
RIKEN-MPG “Activation and Regulation of the Immune System”, Berlin, Germany	Mechanisms of Treg-mediated immune regulation: lessons learned from naturally occurring foxp3 gene mutations.	Apr - 07	Hori, S.
RIKEN-MPG “Activation and Regulation of the Immune System”, Berlin, Germany	Myeloid potential retained by early T cell progenitors: Towards a unified model of hematopoiesis.	Apr - 07	Kawamoto, H.
RIKEN-MPG “Activation and Regulation of the Immune System”, Berlin, Germany	Memory B cell development and maintenance	Apr - 07	Takemori, T. Kaji, T.
The Second International Symposium on Immune Surveillance, Tokyo, Japan	Induction of potent secondary immune responses by artificial lymph nodes in naïve and immunodeficient mice.	Apr - 07	Watanabe, T.
35th Ehime rheumatism workshop, Matsuyama, Japan	Visualization of molecular dynamics and single molecule imaging in immune cells.	Apr - 07	Tokunaga, M. Sakata-Sogawa, K.
The 27th General Assembly of the Japan Medical Congress, Osaka, Japan	Cytokine signaling and autoimmune diseases.	Apr - 07	Hirano, T.
University, Stanford CA, USA	Crosstalk in the mouse thymus.	Apr - 07	Ewijk, W.
The 2nd RCAI-JSI Workshop. Yokohama, Kanagawa	Immunological phenotype of pattern recognition receptor defects --- from knockout mice to human immune disorders.	May - 07	Kaisho, T.
The 2nd RCAI-JSI Workshop. Yokohama, Kanagawa	Development of human immune system in the NOD/SCID/IL2rγ null mice	May -07	Ishikawa, F.
RIKEN-A*STAR Joint Symposium, Singapore	Generation of artificial transplantable lymph node tissues and their efficient immunological function.	May -07	Watanabe, T.
RIKEN-A*STAR Joint Symposium, Singapore	Development of human immuno-hematopoietic system in the NOD/SCID/IL2rγ null mice	May -07	Ishikawa, F.
RIKEN-A*STAR Joint Symposium, Singapore	Roles of BCAP and BANK in B Lymphocyte Development and Activation.	May -07	Kurosaki, T.
EMBO Conference Series on “Signaling in the immune system” Lymphocyte antigen receptor and coreceptor signaling, Siena, Italy	Roles of BCAP and BANK in B Lymphocyte Development and Activation.	May -07	Kurosaki, T.
EMBO Conference Series on “Signaling in the immune system” Lymphocyte antigen receptor and coreceptor signaling, Siena, Italy	TCR microclusters and co-stimulation for T cell activation.	May -07	Saito, T.
AIST symposium, Tsukuba, Japan	A new activation mechanism of innate immunity through CARD9.	May-07	Saito, T.
The 7th Annual Meeting of Protein Science Society of Japan, Sendai, Japan	Spatiotemporal regulation of T cell activation by TCR-CD28-microclusters.	May-07	Yokosuka, T.
JSI Meneki-Fushigi-Mirai 2007 exhibition, Mirai-kan, Odaiba, Tokyo, Japan	How are immune cells generated?	May-07	Kawamoto, H.
Joint Workshop of Nanyang Technical University with Riken, Singapore	Lineage restriction pathway in early hematopoiesis: Towards a unified model, From Gemomics Towards Genomic Medicine.	May-07	Kawamoto, H.
Maastricht University, Maastricht, USA	Lineage restriction pathway in early hematopoiesis.	May-07	Kawamoto, H.
University of Rostock, Germany	Lymphostromal interaction in the mouse thymus.	May-07	Ewijk, W.
Special Seminar in Immunology Department at University of Washington, Seattle, USA	Transcription factors network in CD4/CD8 Lineage Choice.	June - 07	Taniuchi, I.
LIAI seminar, San Diego, USA	Transcription factors network in CD4/CD8 Lineage Choice.	June - 07	Taniuchi, I.
4th International Leukocyte Signal Transduction Workshop, Rhodes, Greece	Regulation of type 2 helper T cell responses by Notch signaling.	June - 07	Kubo, M.
4th International Leukocyte Signal Transduction Workshop: Clinical implications of signaling pathways, Rhodes, Greece	Initiation and sustenance of T cell activation by TCR microclusters.	June - 07	Saito, T.
FASEB Summer Research Conference: Signal Transduction in the Immune System, Snowmass Village, CO, USA	Roles of BCAP and BANK in B Lymphocyte Development and Activation.	June - 07	Kurosaki, T.
FASEB Summer Research Conference: Signal Transduction in the Immune System, Snowmass Village, CO, USA	Differential regulation of pre-TCR- and αβTCR-mediated signaling.	June - 07	Yamazaki, S.
The 19th Spring Meeting of Japanese Society of Allergology, Yokohama, Japan	Zinc and Immunity/Allergy.	June - 07	Hirano, T.
The 24th Meeting of Japan Trace Nutrients Research Society, Kyoto, Japan	Does zinc act as a signaling molecule? : Zinc is a novel second messenger.	June - 07	Hirano, T.
The Skirball Institute, New York University School of Medicine, New York, USA	Spatio-temporal regulation of T cell activation by TCR microclusters.	June - 07	Saito, T.
The Japanese Society for Epigenetics, Osaka, Japan	The role of mammalian Polycomb group proteins to maintain pluripotency of ES cells.	June - 07	Koseki, H.
RCAI International Summer Program 2007. Yokohama, Japan	How dendritic cells sense and respond to immune adjuvants.	Jul - 07	Kaisho, T.
RCAI International Summer Program 2007. Yokohama, Japan	Studying human immunity in mice	Jul - 07	Ishikawa, F.
RCAI International Summer Program 2007. Yokohama, Japan	Thymic Microenvironments: “come and go”.	Jul - 07	Ewijk, W.
RCAI-JSI International Symposium on Immunology “Development and Maintenance of Immune System”, Yokohama, Japan	Transcription factors network in CD4/CD8 Lineage Choice.	Jul - 07	Taniuchi, I.

RCAI-JSI International Symposium on Immunology “Development and Maintenance of Immune System”, Yokohama, Japan	Tolerance induction by apoptotic cell clearance.	Jul - 07	Tanaka, M.
RCAI-JSI International Symposium on Immunology “Development and Maintenance of Immune System”, Yokohama, Japan	Generation of artificial transplantable lymph node tissues and their efficient immunological function.	Jul - 07	Watanabe, T.
5th Meeting on: “Dendritic Cell Vaccination and other Strategies to tip the Balance of the Immune System.” Bamberg, Germany	Potent T-cell mediated antitumor immunity through fully mature, antigen-capturing DCs following Vaccination with ligand-loaded CD1d-experiencing tumor cells.	Jul - 07	Shimizu, K., Steinman RM., Fujii, S.
Aso symposium, Aso, Japan	Human Immunology Research	Jul - 07	Ishikawa, F.
Cytometry, Chiba, Japan	In vivo study of human stem cells	Jul - 07	Ishikawa, F.
13th International Congress of Mucosal Immunity 2007. Tokyo, Japan	Functional roles of NKT cells in the protective immune responses.	Jul - 07	Taniguchi, M.
Seminar of frontier field for disease and medicine, Komaba Eminence, Tokyo, Japan	Visualization of molecular dynamics in immune cells and single molecule imaging and quantification.	Jul - 07	Tokunaga, M.
ICMI2007 13th International Congress of Mucosal Immunology, Tokyo, Japan	Role of zinc and zinc signaling in immunity: Zn is an intracellular second messenger.	Jul - 07	Hirano, T.
FASEB Summer Research Conference: Lymphocytes and the Immune System, Tucson, AZ, USA	Action mechanisms of adaptor molecules in B lymphocytes.	Jul - 07	Kurosaki, T.
The 3rd Annual Meeting for Young Scientist in Hematology, Tokyo, Japan	Actin cloud induced by LFA-1 stimulation is critical for T cell activation.	Jul - 07	Suzuki, J-I
Disease Biology Excellent Lecture Series (DBELS) Workshop, Nagasaki, Japan	Molecular basis for polycomb regulation.	Jul - 07	Koseki, H.
University of Edinburgh, Scotland	Crosstalk in Thymic Microenvironments.	Jul - 07	Ewijk, W.
Runx Meeting 2007, Singapore	Roles of Runx proteins during thymocytes development.	Aug - 07	Taniuchi, I.
12th LAGID (Latin American Group for Immunodeficiency) and 1st BRAGID (Brazilian Group for Immunodeficiency) Meeting (Satellite Symposium of the 13th International Congress of Immunology). São Paulo, Brazil	The crucial role of regulatory T cells in natural tolerance: lessons learned from naturally occurring foxp3 gene mutations.	Aug - 07	Hori, S.
13th International Congress of Immunology. Rio de Janeiro, Brazil	Regulatory mechanisms of iNKT cells in the immune system.	Aug - 07	Taniguchi, M.
13th International Congress of Immunology. Rio de Janeiro, Brazil	Regulatory mechanisms of iNKT cells in the immune system.	Aug - 07	Taniguchi, M.
13th International Congress of Immunology. Rio de Janeiro, Brazil	Dynamic regulation of T cell activation through TCR miclocluster s.	Aug - 07	Saito, T.
13th International Congress of Immunology. Rio de Janeiro, Brazil	Spatiotemporal regulation of T cell activation by TCR-CD28-microclusters.	Aug - 07	Yokosuka, T.
Bulk Institute for Age Research, California, USA	Mechanisms of A:T mutations and its role in increasing antibody affinity.	Aug - 07	O-wang, J.
St. Jude Children’s Research Hospital Special Immunology Seminar, Memphis TN, USA	Genetical Regulation of the Th2 Cytokines.	Aug - 07	Kubo, M.
Tokyo Tech Special Symposium on Genes and Development: Toward Understanding of Complex Biological Systems, Kanagawa, Japan	Polycomb impacts on ES cells.	Aug - 07	Koseki, H.
3rd Network of Hematology and Immunology in Kanazawa, Kanazawa, Japan	Role of epithelial cells in the intestinal immune system: FAE and M cells.	Sep - 07	Ohno, H.
Nagoya University Immunology Seminar, Nagoya, Japan	Generation of tissue-engineered lymph node-like organoid with immunological function.	Sep - 07	Watanabe, T.
International Society of Hematology Meeting, Beijing, China	Studying Biology of Human Hematopoietic and Leukemic Stem Cells using a Novel Xenograft System	Sep - 07	Ishikawa, F.
1st Bio-nano Research Conference, Higashi-Azuma, Japan	Single molecule measurements and MD simulation by Molecular force microscopy.	Sep - 07	Tokunaga, M.
Japan Society of Hematology Meeting, Yokohama, Japan	Studying human stem cells using a novel xenograft model	Oct - 07	Ishikawa, F.
CBR seminar, Boston, USA	Transcription factors network in CD4/CD8 Lineage Choice.	Oct - 07	Taniuchi, I.
The 20th Naito Conference “Innate Immunity in Medicine and Biology [III]”, Kanagawa, Japan	IκB kinase-α as a critical regulator for dendritic cell function.	Oct - 07	Kaisho, T.
The 11th Symposium of the Japanese Association for Animal Cell Technology, Tokyo, Japan	Trials to construct the artificial immune tissues and organs of mouse and human.	Oct - 07	Watanabe, T.
35th Annual Meeting of the Japan Society for Clinical Immunology	Antigen-specific immunotherapy	Oct - 07	Ishii, Y.
10th Anniversary of Kazusa ARC International Symposium on Advanced Functional Genomics, Chiba, Japan	Kazusa Mammalian Genome Resources: From contents to platform	Oct - 07	Ohara, O.
7th Annual ORFeome meeting, Boston, USA	Kazusa ORFeome Project	Oct - 07	Ohara, O.
5th International Antigen Processing and Presentation Workshop, Dunk Island, Australia	A novel family of membrane-bound E3 ubiquitin ligases.	Oct - 07	Ishido, S.
Institute Gulbenkian de Ciencia,Lisbon,Portugal	Imaging and other new technologies at RIKEN.	Oct - 07	Kanagawa, O.
The 20th Naito Conference—Innate Immunity in Medicine and Biology [III], Hayama, Japan	CARD 9-mediated innate signaling in myeloid cells.	Oct - 07	Saito, T.
Liaison Laboratory Research Meeting, Kumamoto University, Kumamoto, Japan	Developmental pathway from hematopoitic stem cells to T cell progenitors.	Oct - 07	Kawamoto, H.
NYU immunology club seminar series, New York, USA	Transcription factors network in CD4/CD8 Lineage Choice.	Nov - 07	Taniuchi, I.
The 37th Annual Meeting of the Japanese Society for Immunology Shinagawa, Japan	Mechanisms of tolerance induction to cell-associated antigens.	Nov - 07	Tanaka, M.
The 37th Annual Meeting of the Japanese Society for Immunology Shinagawa, Japan	Identification of molecules expressed in FAE and M cells to be involved in mucosal immunity.	Nov - 07	Kawano K., Hase, K., Ohno H.

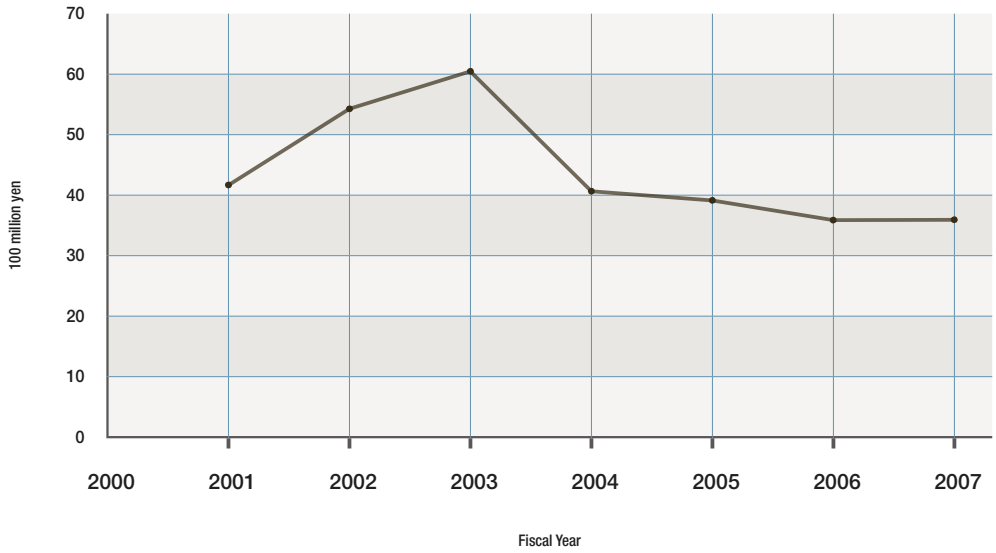
The 37th Annual Meeting of the Japanese Society for Immunology Shinagawa, Japan	M-Sec regulates tunneling nanotubule (TNT) formation in intestinal M cells.	Nov - 07	Hase, K., Takatsu H., Kawano K., Ohno H.
The 37th Annual Meeting of the Japanese Society for Immunology Shinagawa, Japan	Dynamic interactions between bacteria and immune cells leading to intestinal IgA synthesis.	Nov - 07	Fagarasan S.
The 37th Annual Meeting of the Japanese Society for Immunology Shinagawa, Japan	The mechanism of germinal center activation in gut immune system.	Nov - 07	Suzuki, K.
The 37th Annual Meeting of the Japanese Society for Immunology Shinagawa, Japan	Helper T cell subsets and cytokine regulation.	Nov - 07	Kubo, M.
The 37th Annual Meeting of the Japanese Society for Immunology Shinagawa, Japan	T cell recognition and activation through TCR microclusters.	Nov - 07	Saito, T.
Kawashima Nephrology Conference 2007, Kagamihara, Japan	M cells, a specialist for antigen uptake in the intestinal immune system.	Nov - 07	Ohno, H.
International Symposium on Membrane Traffic, Awajishima, Japan	Identification of molecules expressed in M cells to be involved in mucosal immunity.	Nov - 07	Ohno H., Kawano K., Hase, K.
Microbiology Seminar Series at University of Alabama at Birmingham, Birmingham, Alabama, USA	Generation of transplantable artificial lymphoid tissues with immune function.	Nov - 07	Watanabe, T.
57th Annual Meeting of Japanese Society of Allergology	New approach to treatment of allergic diseases	Nov - 07	Ishii, Y.
13th Blood Science Seminar. Tokyo, Japan	Cellular Immunotherapy by NKT cells.	Nov - 07	Fujii, S.
Developmental Technology and Disease Models, Tokyo, Japan	Humanized mouse research	Nov - 07	Ishikawa, F.
Japan Society of Immunology Meeting, Yokohama, Japan	Creating human immunity and recapitulating human diseases in the NOD/SCID/IL2rγ null mice	Nov - 07	Ishikawa, F.
Tokyo University, Tokyo, Japan	Ontogeny of thymus and T cells.	Nov - 07	Kawamoto, H.
Leiden University. Netherlands	Development of Thymic Microenvironments.	Nov - 07	Ewijk, W.
Singapore immunology network seminar	Transcription factors network in CD4/CD8 Lineage Choice.	Dec - 07	Taniuchi, I.
The 256th Chiba Bio-science seminar, Chiba, Japan	What is a terminal point of T cell differentiation?; IL-13 expression in Th1 cells.	Dec - 07	Kubo, M.
Ministry of Health, Labor and Welfare Japan Acute Leukemia Research Group, Nagoya, Japan	Investigating human leukemia stem cells' kinetics	Dec - 07	Ishikawa, F.
Japan Society of Pediatric Hematology / Oncology Meeting, Sendai, Japan	Development of normal and disease human immune system	Dec - 07	Ishikawa, F.
TWMU (International Research and Educational Institute for Integrated Medical Sciences IREIIMS) Tokyo,Japan	T cellmediated autoimmunity.	Dec - 07	Kanagawa, O.
The 30th Annual Meeting of the Molecular Biology Society of Japan, The 80th Annual Meeting of the Japanese Biochemical Society, Yokohama, Japan	Single molecule imaging in cells of molecular dynamics and interactions: five-dimensional imaging and quantification.	Dec - 07	Tokunaga, M. Shinkura, K. Sakata-Sogawa, K.
The 30th Annual Meeting of the Molecular Biology Society of Japan, The 80th Annual Meeting of the Japanese Biochemical Society, Yokohama, Japan	T cell receptor microclusters as signaling unit for antigen-recognition and cell activation of T lymphocytes.	Dec - 07	Saito, T.
The 30th Annual Meeting of the Molecular Biology Society of Japan, The 80th Annual Meeting of the Japanese Biochemical Society, Yokohama, Japan	Polycomb impacts on ES cells.	Dec - 07	Koseki, H.
International Symposium on Metallomics 2007, Nagoya, Japan	Zinc is an intracellular signaling molecule.	Dec - 07	Hirano, T.
MIVAC International Conference and MUVAPRED Annual Meeting. Gothenburg, Swedwn	"Gut barrier function and the role of IgA". Dynamic interactions between bacteria and B cells in GALT.	Jan - 08	Fagarasan S.
Hamamatsu, Japan	Cancer immunotherapy by NKT cell-mediated mature dendritic cells (DCs).	Jan - 08	Fujii, S.
Research Institute for Microbial Diseases,Osaka University , Osaka, Japan	Imaging and other new technologies at RIKEN.	Jan - 08	Kanagawa, O.
Kyoto University medical school,Kyoto,Japan	Imaging and other new technologies at RIKEN.	Jan - 08	Kanagawa, O.
Keystone Symposia,Keystone,USA	Migration of naïve CD4 T cells for the interaction with self-antigens.	Jan - 08	Tomura, M.
Keystone Symposium on Lymphocyte Trafficking, Keystone, USA	CRTAM regulate retention of T cells within lymph node and deliver to effector sites.	Jan - 08	Saito, T.
Hepatic Inflammation and Immunity 2008 (2008 Joint Hepatitis Panel Meeting, U.S. Japan Cooperative Medical Sciences Program), Galveston, USA	Differential cellular adaptors mediating innate and adaptive immunity.	Jan - 08	Saito, T.
The Kick-off Symposium on the MEXT Priority Research for "Immunological Self –Recognition and its Disorder". Kyoto, Japan	Regulatory T cells in the control of autoimmunity.	Feb - 08	Hori, S.
Immunology Program at UCSF, San Francisco, CA, USA	Signaling pathways in B lymphocyte development and activation.	Feb - 08	Kurosaki, T.
Keystone Symposium: Lymphocyte Activation and Signaling, Snowbird, Utah, USA	Role of Ras/Erk pathway in B lymphocyte development and activation.	Feb - 08	Kurosaki, T.
The 11th Membrane Research Forum, Kyoto, Japan	Spatiotemporal regulation of T cell activation and co-stimulation by microclusters.	Feb - 08	Saito, T.
RIKEN-INSERM/Institut Pasteur Joint Symposium, Paris, France	Dendritic cell function regulated by nucleic acid adjuvants.	Mar-08	Kaisho, T.
RIKEN-INSERM/Institut Pasteur Joint Symposium, Paris, France	Dissecting the Foxp3-dependent program of regulatory T cell differentiation and function: lessons learned from naturally occurring foxp3 gene mutations.	Mar - 08	Hori, S.
IFReC Kick-off Symposium International Symposium on Immunology and Imaging, Osaka, Japan	Imaging of initiation of T cell activation.	Mar - 08	Saito, T.

Date	Title	Lecturer	Affiliation
2-Apr-2007	Development of a Non-Inflammatory Relationship between a Human Gut Symbiont and its Host	**Dr. Daniel A. Peterson**	Patholory & Immunology,Washington University School of Medicine, St.Louis, USA
18-Apr-2007	Receptor function and specificity of a natural structural variant of the mouse TCR b-chain	**Dr. Janet L. Maryanski**	INSERM Unit576 (National Institute for Health and Medical Research),Nice, France
13-Jun-2007	Thymoproteasomes regulate development of CD8+ T cell in the thymus	**Dr. Shigeo Murata**	Tokyo Metropolitan Institute of Medical Science, Japan
17-Jul-2007	Molecular Aspects on the Development of Mammalian Spleen	**Dr. Andrea Blendolan**	Cornell University Medical Center, N.Y., U.S.A San Raffeale Scientific Institute, Milan, Italy
18-Jul-2007	Prevention and remission of encephalomyelitis by regulatory T cells	**Dr. Santiago Zelenay**	Gulbenkian Institute for Science, Oeiras, Portugal
6-Aug-2007	Conformational Regulation of Leukocyte Integrins: From Crystal Structures to Drug Delivery	**Dr. Motomu Shimaoka**	CBR Institute for Biomedical Research & Harvard Medical School
7-Aug-2007	The gene regulation by microRNAs	**Dr. Nikolaus Rajewsky**	Max-Delbruck-Center / Charite Berliln-Buch, Germany
13-Sep-2007	Regulation of XY body chromatin	**Dr. Willy Baarends**	Department of Reproduction and Development, Erasmus MC, Rotterdam, The Netherlands
27-Sep-2007	Regulation of IgA production by TNF/iNOS-producing dendritic cells	**Dr. Toshiaki Ohteki**	Department of Immunology, Akita University School of Medicine, Japan
2-Oct-2007	Deconstructing the complexity of the dendritic cell network found in the skin	**Dr. Bernard Malissen**	Centre d'Immunologie de Marseille-Luminy, France
18-Oct-2007	Structural mechanisms of West Nile virus neutralization	**Dr. Daved Fremont**	Pathology&Immunology,Biochemistry&Molecular Physics, Washington University Medical school, USA
23-Oct-2007	MicroRNA control in lymphocytes	**Dr. Klaus Rajewsky**	Harvard Medical School Immune Disease Institute, USA
24-Oct-2007	Toll-like receptor, heat shock protein gp96 and the myth in between	**Dr. Zihai Li**	Center for Immunotherapy of Cancer and Infectious Diseases, Department of Immunology, University of Connecticut Health Center (UCHC), USA
24-Oct-2007	Modulation of Thymocyte Apoptosis by FAIM	**Dr. Kong-Peng Lam**	A*STAR, Singapore
29-Oct-2007	Mucosal Dendritic Cell Systems in Control of Tolerance and Mucosal Defenses	**Dr. Hans-Christian Reinecker**	Massachusetts General Hospital and Harvard Medical School, USA
30-Oct-2007	Regulation of antigen presentation in the dendritic cell network *in vivo*	**Dr. Jose A. Villadangos**	The Walter and Eliza Hall Institute of Medical Research, Australia
31-Oct-2007	Mechanisms of Allergic Inflammation	**Dr. Cezmi A. Akdis**	Swiss Institute of Allergy and Asthma Research (SIAF), Switzerland
1-Nov-2007	The Cbl family of proteins at the crossroads of immune system development, autoimmunity, and anti-tumor immunity	**Dr. Hua Gu**	Department of Microbiology Columbia University College of Physicians and Surgeons, USA
7-Nov-2007	Epigenetic mechanisms in mammalian genomic imprinting	**Dr. Robert Feil**	CNRS Institute of Molecular Genetics, Montpellier, France
8-Nov-2007	Real-time imaging of the B cell immune response by 2-photon microscopy	**Dr. Takaharu Okada**	Associate Professor, Department of Synthetic Chemistry and Biological Chemistry Graduate School of Engineering Kyoto University, Japan
9-Nov-2007	Exploring the role of large ncRNAs in vertebrate immunity	**Dr. Igor V. Kurochkin**	Computational and Experimental Systems Biology group, RIKEN Genomic Sciences Center, Japan

14-Nov-2007	Antibodies and their receptors: Coupling innate and adaptive Immunity	**Dr. Jeffrey V. Ravetch**	Laboratory of Molecular Genetics and Immunology, The Rockefeller University, USA
19-Nov-2007	What poxviruses teach us about the immune system	**Dr. Wayne M.Yokoyama**	Howard Hughes Medical Institute Rheumatology Division, Washington University Medical center), USA
19-Nov-2007	Starting from the end. Progenitors and precursors of B cells in bone marrow	**Dr. Fritz Melchers**	Max Planc Institute for Infection Biology, Berlin,Germany
26-Nov-2007	Rebuilding the Immune System. New Differentiation Pathways and Environmental Cues	**Dr. Paul W. Kincade**	Immunobiology and Cancer Research Program, Oklahoma Medical Research Foundation, USA
26-Nov-2007	Central Tolerance: How much affinity can you tolerate?	**Dr. Ed Palmer**	University Hospital-Basel, Switzerland
30-Nov-2007	Cell biology of antigen processing by dendritic cells	**Dr. Ira Mellman**	Research Oncology Genentech, Inc., USA
5-Dec-2007	Cytokine Gene Regulation	**Dr. Mark Bix**	St. Jude Children's Research Hospital, Affiliate Assistant Professor of University of Tennessee, USA
6-Dec-2007	Novel approaches for vaccine design:Bypassing Treg activity enhances Tcell-mediated immunity.	**Dr. Tim Sparwasser**	Institute for Medical Microbiology, Immunology & Hygiene, Technical University Munich, Germany
10-Dec-2007	Mechanisms of CD4 Downregulation by the Nef protein of Immunodeficiency Viruses	**Dr. Juan S. Bonifacino**	Cell Biology and Metabolism Branch, NICHD, NIH, USA
12-Dec-2007	Novel roles for mouse Polycomb group proteins in pericentric heterochromatin formation and genomic imprinting	**Dr. Antoine Peters**	Friedrich Miescher Institute for Biomedical Research, Switzerland
28-Jan-2008	Cellular and functional aspects of thymus development: How to make and how to break a thymus	**Dr. Georg Hollander**	Molecular Medicine in Paediatrics, University of Basel, Switzerland
31-Jan-2008	The Sublingual Mucosal : an efficient delivery route for inducing productive immune responses	**Dr. Mi-Na Kweon**	Mucosal Immunology Section, International Vaccine Institute, Seoul, Korea
31-Jan-2008	Regulation of T cell responses by OX40 ligand and ICOS ligand on human dendritic cell subsets	**Dr. Tomoki Ito**	Kansai Medical University, Japan
7-Feb-2008	Genome-wide technologies and FANTOM	**Dr. Yoshihide Hayashizaki**	Project director, Genome Exploration Research Group, RIKEN Genomic Sciences Center (GSC), Japan
26-Feb-2008	M cell-targeted and rice-based mucosal vaccine	**Dr. Tomonori Nochi and Hiroshi Kiyono**	Division of Mucosal Immunology, The Institute of Medical Science, The University of Tokyo, Japan
29-Feb-2008	Inducers and organizers in human fetal lymph nodes	**Dr. Tom Cupedo**	Department of Hematology Erasmus Medical Center, Rotterdam, The Netherlands
6-Mar-2008	Mechanisms of imprinting and epigenetic reprogramming in mammalian development	**Dr. Wolf Reik**	Laboratory of Developmental Genetics and Imprinting, Babraham Institute, Cambridge, UK
17-Mar-2008	Development and Function of Memory B Cells	**Dr. Mark Shlomchik**	Laboratory Medicine and Immunobiology, Yale University School of Medicine and Graduate School, USA
18-Mar-2008	A regulatory axis between subsets of NKT cells controlling tumor immunity	**Dr. Jay Berzofsky**	Vaccine Branch, Center for Cancer Research, National Cancer Institute, NIH, USA
18-Mar-2008	Making Daughter T Cells Different during Immunity	**Dr. Steven L. Reiner**	Department of Medicine (Section of Infectious Diseases), Abramson Family Cancer Research Institute, University of Pennsylvania, USA
19-Mar-2008	A Tale of Two T-box Transcription Factors in Host Defense and Disease	**Dr. Steven L. Reiner**	Department of Medicine (Section of Infectious Diseases), Investigator Abramson Family Cancer Research Institute, University of Pennsylvania, USA
21-Mar-2008	Definition of germinal center B cell migration patterns in vivo with intravital multiphoton microscopy	**Dr. Ann Haberman**	Department of Laboratory Medicine, Yale School of Medicine, USA

Budgets and Personnel

RCAI Budget (JPY 100 Million)

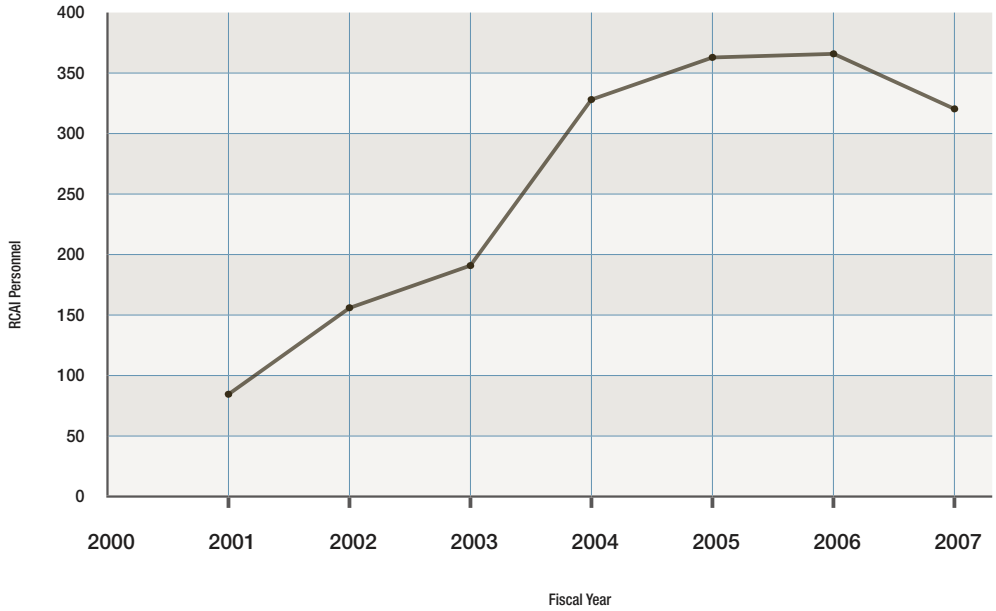


RCAI Budget FY2001-2007 (JPY 100 Million)

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

RCAI Personnel

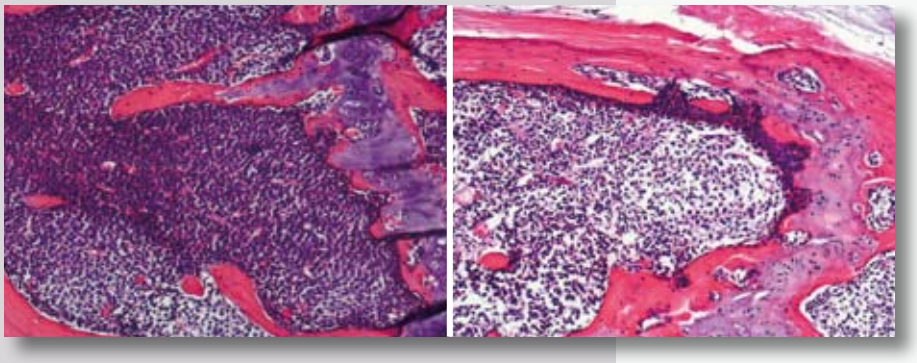


RCAI Personnel	
2001	84
2002	156
2003	191
2004	328
2005	363
2006	366
2007	339

RCAI Staff Composition
(as of Nov. 2007)

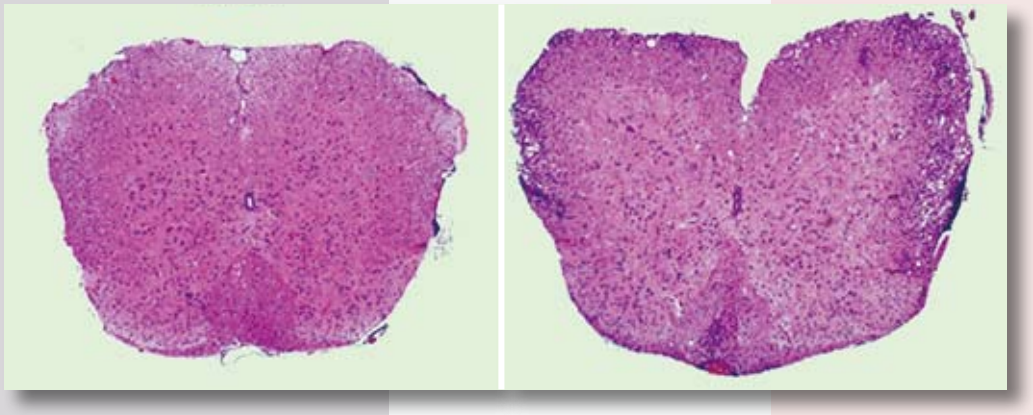
Category	Number
Director	1
Special Advisor	1
Principal Investigators	31
Science Advisor	1
Coordinator	1
Senior Scientists	8
Scientists	48
Senior Technical Scientist	1
Technical Scientists	4
Technical Staff I	29
Technical Staff II	57
Graduate Students	35
Research Associates	16
Interns	2
Visiting Scientists	31
Research Consultatnts	2
Research Fellows	2
Assistants	21
Part-time Staff	10
Temporary Employment	38
Total	339

Original Photos of the Cover and Front Pages



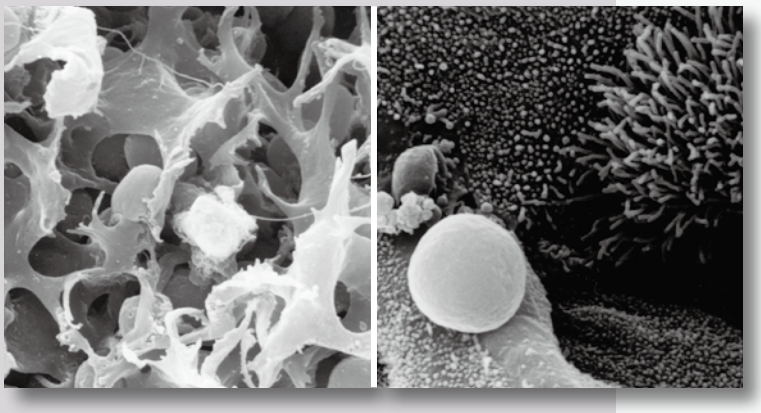
Front Cover :

Bone sections derived from acute myelogenous leukemia (AML)-engrafted mice before (*left*) and after (*right*) chemotherapy. At day 3 after cytotoxic Ara-C chemotherapy, there is a significant decrease in cellularity in the bone marrow space, but leukemic stem cells survive at the endosteal surface of the bone, which explains the major mechanism for AML relapse. (*See Research Highlights*)
Image courtesy of Research Unit for Human Disease Model



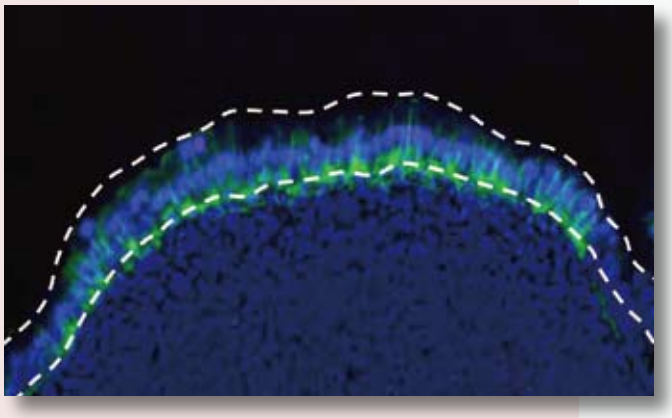
Front page of Research Highlight section:

Critical role of marginal zone (MZ) macrophages in the tolerance induction to cell-associated antigens. When apoptotic cell corpses expressing myelin oligodendrocyte glycoprotein were injected intravenously, experimentally-induced inflammation is suppressed in normal mice (*left*). However, in the mice depleted with MZ macrophages, there is a dramatic delay in the clearance of the apoptotic corpses inducing inflamed spinal cord (*right*). Laboratory for Innate Cellular Immunity found that $CD11b^{+}$ dendritic cells aberrantly 'eat' the cell corpses in the absence of MZ macrophages, although normally these corpses are eaten by $CD8\alpha^{+}$ inflammation-suppressing dendritic cells. (See *Research Highlights*)
Image courtesy of Laboratory for Innate Cellular Immunity



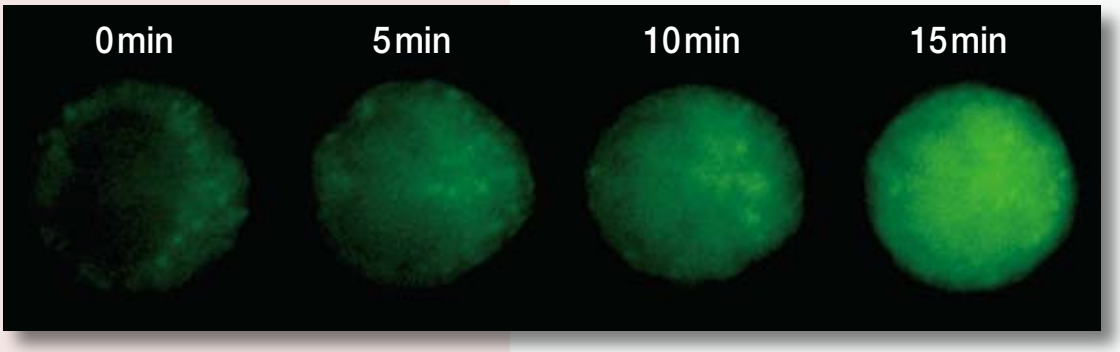
Front page of Research Activities section:

Three-dimensional (3-D) organization of thymic epithelial cells. Thymic microenvironments critically support the development of T lymphocytes. The stromal cell types composing these microenvironments are epithelial in origin, and form a 3-D organized network (*left*). However, removal of thymocytes from the thymic environment, either by genetic manipulation, or by biochemical methods, induces the generation of 2-D organized epithelial cells, forming thymic cysts lined by ciliated cells, goblet cells and absorptive cells, as normally occurring in the respiratory and intestinal tracts (*right*).
Thus, thymic epithelial cells and thymocytes are mutually dependent, and their crosstalk promotes the integrity and maintenance of the thymic environment.
Image courtesy of Research Unit for Lymphocyte Development



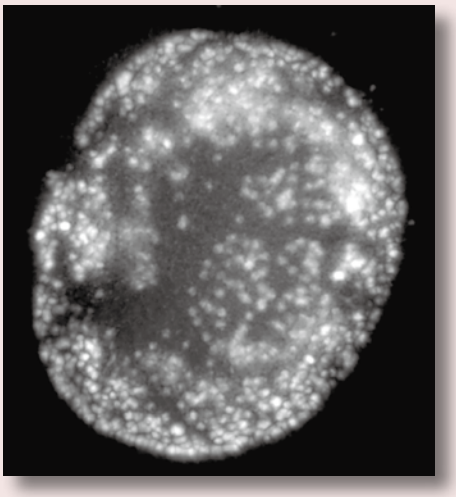
Front page of Collaborative Networks section:

Pregnancy-specific glycoprotein (Psg) 18 is a specific marker protein that promotes the interplay between follicle-associated epithelium (FAE) and immune cells in the gut. Laboratory for Epithelial Immunobiology discovered that the Psg18 mRNA was exclusively expressed in FAE in adult mice. Immunofluorescence staining of Psg18 indicated that Psg18 is secreted basolaterally by FAE toward subepithelial dome region (SED), beneath the FAE, where antigen-presenting cells accumulate.



Front page of Events section:

Intracellular zinc wave. When bone marrow derived mast cells were stimulated by IgE receptors, intracellular wave of free zinc (*green*) was released from the vicinity of the ER within several minutes. Calcium and activated MAP kinase were necessary for this 'zinc wave', and total tyrosine phosphorylation was elevated, suggesting zinc as a new intracellular second messenger. (See *Research Highlights*)
Image courtesy of Laboratory for Cytokine Signaling



Front page of Data and Statistics section:

Three-dimensional (3-D) image of nuclear pore complexes. Highly inclined laminated optical sheet (HILO) microscopy developed by Dr. Tokunaga and Dr. Sakata-Sogawa enabled us to observe and trace single molecules inside cells. To evaluate this technique, they visualized single molecules of GFP-labeled importin β, that mediates the import of cargo through nuclear pores in cells. The interaction time of single molecules with nuclear pore complexes was determined based on the duration of the fluorescent spots, and the rate of nuclear import was obtained as the maximum slope in the time course of nuclear accumulation, proving this technique supports the quantification of molecular kinetics and interactions in cells. (See *Research Highlights*)
Image Courtesy of Research Unit for Molecular Systems Immunology

RIKEN Research Center for Allergy and Immunology

http://web.rcai.riken.jp/en/

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