

RIKEN RCAI Annual Report 2009

RIKEN Research Center for Allergy and Immunology

RIKEN Research Center for Allergy and Immunology (RCAI)

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Special Advisor
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Director
Masaru Taniguchi M.D., Ph.D.

Deputy Directors
Takashi Saito Ph.D.
Toshio Hirano M.D., Ph.D.

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Team

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Lab. for Signal Network **Masato Kubo Ph.D.**
Lab. for Immunchaperones **Hei-ichiro Udono M.D., Ph.D.**
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Lab. for Epithelial Immunobiology **Hiroshi Ohno M.D., Ph.D.**
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Lab. for Transcriptional Regulation **Ichiro Taniuchi M.D., Ph.D.**
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Lab. for Vaccine Design **Yasuyuki Ishii Ph.D.**
Lab. for Cellular Systems Modeling **Mariko Okada Ph.D.**



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Toshitada Takemori M.D., Ph.D.
 Haruka Iwano D.V.M., Ph.D.
 Peter Burrows Ph.D.

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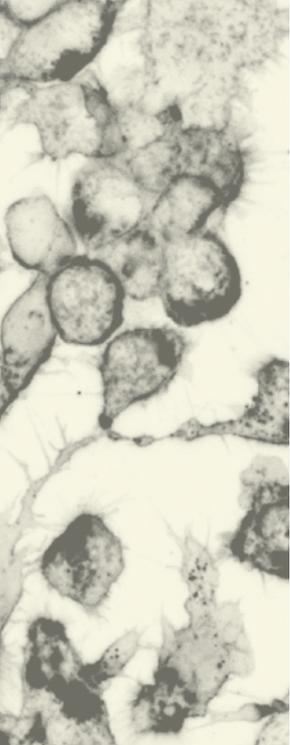
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Director's Report

This is the sixth annual report of the RIKEN Research Center for Allergy and Immunology (RCAI). Six years have passed since all research groups shifted in 2004 from universities into a new building on the RIKEN Yokohama campus. As we move into the second half of our initial ten year term, significant changes have occurred that have necessitated strategic changes. There has been an ongoing decline in government support, but, as I have summarized in this report, the Center nevertheless has continued to be successful in its core mission – cutting edge immunology research.



Change of the government affects RIKEN/RCAI

In August, 2009, the Democratic Party of Japan (DPJ) won the Lower House election and secured a significant majority. The new government, lead by Prime Minister Yukio Hatoyama, has focused on the more efficient use of the government budget. To eliminate needless or excessive projects, a special Government Revitalization Unit was formed in November, 2009 to reevaluate 220 already funded government projects. These included a number of projects at RIKEN institutes: the Super Computer Project, the Program of Founding Research Centers for Emerging and Reemerging Infectious Diseases, the Bioresource Project, and several Plant Science projects. This government initiative reminded us of how essential it is to explain the importance of scientific research to non-scientists. In order to have the support of the people, we have to help them become more familiar with the scientific approach to questions, and also to make it very clear that even basic science research can have unexpected long term practical benefits for human health and society.

The new government's strategies focus on innovation and technology that promote environmental conservation, health care and the economy. Based on this emphasis, RCAI is facing difficulties in obtaining funding for our basic science programs. The government also aims to reduce or eliminate any overlap in projects being carried out at institutions, universities and ministries. Overlap, in terms of scientific projects, may be difficult to quantify. Because of the importance of a particular area, several groups may be working on seemingly similar projects, but be utilizing many different experimental approaches. It will be important for our future that we make this feature of the scientific research endeavor very clear to the various governmental agencies.

The future direction of RCAI continues to be the focus of intensive discussions.

Last year we formulated a new organizational plan, and the basic premises and the new research directions proposed in the plan were supported by the RCAI External Advisory Council in December, 2008. Three areas were given the highest priority: molecular and cellular movement (single molecule imaging and *in vivo* imaging), human immunology (humanized mouse models, artificial lymph nodes and immune iPS cells), and systems biology. Following this direction, the Center began a reorganization process in 2009. This process will continue until 2013, when RCAI celebrates its 10th anniversary and enters its new term.

Platforms for Translational Research and Drug Development

In 2009, we established a platform for bridging basic and translational, patient-oriented research. This model aims to support the development of orphan drugs or drugs that private companies would otherwise hesitate to risk investing in. This is a two stage plan: 1) RIKEN, in collaboration with universities, will analyze the mechanisms and pharmacological effects of a particular new drug, and conduct preliminary studies of efficacy and safety as translational research. 2) Based on promising results in the phase 1/2 clinical studies, companies will be invited to develop the drug and conduct clinical trials. As an example of this platform in action, in December, 2009, RIKEN and Torii Pharmaceutical Co., Ltd. agreed to collaborate in the development of cedar pollen allergy vaccines.

Collaborative projects

With the aim of developing new paradigms in immunology, in 2009 RCAI launched a support program for multidisciplinary projects. Five out of eight collaborative projects were selected. This program supports several collaborations between “wet” and “dry” laboratories, and we hope it will be the first step in integrating systems biology approaches with experimental immunology.

We also launched the “Open Laboratories for Allergy Research” projects. A patient cohort study project combined with humanized mouse studies was started in collaboration with Chiba University and the National Center for Child Health and Development. A project on oral tolerance for the treatment of allergy will be performed in collaboration with Advanced Industrial Science and Technologies and also with the research group supported by the Ministry of Health, Labour and Welfare.

Coordination Office

In 2009, we reorganized the RCAI administrative staff and established the coordination office. All the assistants will now work together as a team to support RCAI and the laboratories. This was not a trivial reorganization, but became necessary because of the austere and uninterrupted budget cuts faced by the Center. Using this centralized approach, we hope to be able to provide sufficient administrative support uniformly to all RCAI laboratories under these difficult circumstances. The new office will begin its activity in April, 2010.

Significant research achievements

Several high impact publications since the last annual report are grounded in fundamental research but have significant translational implications.

Two of these publications deal with a newly designated research priority, the humanized mouse model. These studies are also potentially highly translational in nature, since they deal with new strategies for the cure of human leukemia. Drs. Saito and Kitamura were able to identify important therapeutic targets in human acute myelogenous leukemia (AML). The leukemic stem cells (LSC) in this disease are quiescent, and therefore resistant to most forms of chemotherapy. Unfortunately, it is these LSC that are responsible for disease relapse and the poor prognosis in AML (*Science Translational Medicine*). Drs. Saito and Ishikawa were able to induce the LSC to enter cell cycle by treatment with cytokines, thereby promoting their elimination (*Nature Biotechnology*).

Since many pathogens, including HIV and the influenza virus, gain entry through mucosal surfaces, the development of effective mucosal vaccines has been a long sought but elusive goal of immunologists. Drs. Hase, Kawano and Ohno have identified a molecule, Glycoprotein 2 (GP2), which may be a very important new target for mucosal vaccines. GP2 is expressed on the M cells that overlay Peyer's patches, structures that behave somewhat like lymph nodes for mucosal immunity. These investigators found that the fimbriae on certain pathogenic bacteria are specifically recognized by GP2, initiating a potent mucosal immune response (*Nature*). This group also discovered a novel method of intercellular communication via tunneling nanotubes (*Nature Cell Biology*).

The therapeutic efficacy of NKT cells has been verified in many experimental settings, but there are practical problems in applying this therapy to human disease. Among the most vexing is the low frequency of these cells and the fact that they may be functionally impaired in diseases like cancer. Drs. Watarai, Rybouchkin, Hongo and Taniguchi have found that large numbers of functional NKT cells can be generated *in vitro*. The precursors of these cells were initially derived from somatic cell nuclei that were then converted to embryonic stem cells, thereby bypassing important ethical issues (*Blood*).

As described in last year's report, Drs. Nishida, Hasegawa and Hirano have been studying the role of zinc in allergic diseases and embryonic development. Since then, they have made an important discovery, that one of the Zinc transporters, Znt5/Slc30a5, is required for the mast cell-mediated delayed-type allergic reaction, but not the immediate-type reaction. These two phases of the allergic response were known to have different, but unknown mechanisms, and to require different therapies. Znt5 is thus a new target for allergy therapy (*J. Exp. Med.*).

The International Collaboration Award, which teams RCAI and foreign researchers, was established at the start of RCAI and has led to many significant publications. The tradition continued this year, with the finding by Dr. Bix (St. Jude Children's Research Hospital, Memphis, TN, USA) and Dr. Kubo (RCAI) that Mina, an IL4 transcriptional repressor, controls T helper type 2 bias, an issue that has puzzled immunologists for decades (*Nature Immunology*).

The ENU mutagenesis project is a long term, labor intensive effort to identify genes important in immunological diseases. Several genes have already been identified and this year there was another milestone paper. Drs. Kakugawa, Yasuda, Kawamoto, and Yoshida discovered a gene essential for the development of single positive thymocytes (*Mol. Cell. Biol.*). The conversion of double positive to single positive thymocytes is crucial for the development of functional T cells, and these studies are likely to shed new light on human primary immunodeficiency diseases, another focus of RCAI translational studies.

The quality of research at RCAI depends on a number of factors, the creativity of the investigators, the scientific interactions with their colleagues at RCAI and other institutions in Japan and abroad, as well as financial and administrative support. Also important is the work of the RCAI technical staff. This effort is clearly indicated in a publication in *Blood* from Dr. Sato's laboratory that had two technical staff members, Ms. Kaori Sato and Ms. Kawori Eizumi, as sole co-authors. This publication described naturally occurring regulatory dendritic cells and their regulation of cutaneous chronic graft-versus-host disease, a severe clinical problem in human bone marrow transplant recipients.

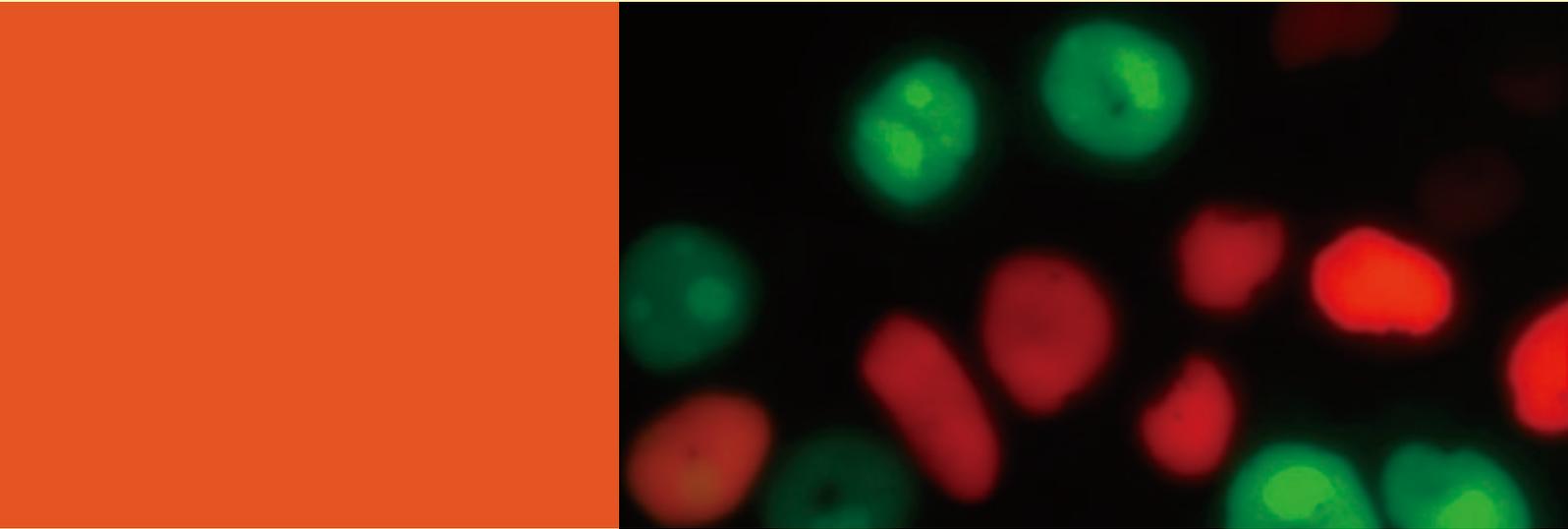


Masaru Taniguchi

March, 2010

2009

Research Highlights



Sights set on immunization target



Hiroshi Ohno

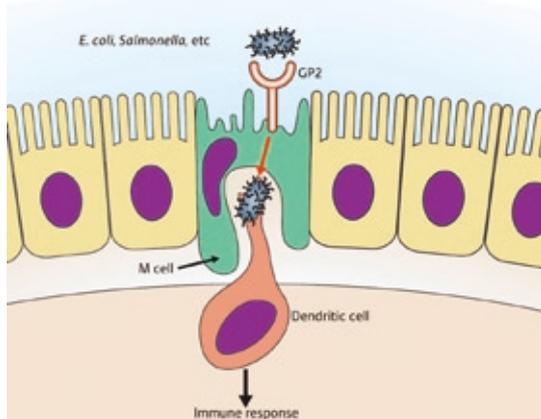


Figure : A schematic diagram depicting the role of GP2 in the immune response of the gut.

ARIKEN-led research team has unraveled the molecular details of a key mechanism of the immune system in the gut. The work opens the way to new possibilities for developing versatile, inexpensive vaccines that are swallowed, rather than injected.

Ohno and colleagues from Laboratory for Epithelial Immunobiology, in collaboration with biologists from Yokohama City University, several other Japanese universities and Stanford University in the US, uncovered a particular receptor molecule on M cells that stimulates the immune response by binding to a protein on the hair-like projections or pili of bacteria. The epithelial M cells engulf foreign bodies in the gut cavity, surrounding them with the cell membrane. These membrane-sealed packages or vesicles are then passed through the body of the M cell to waiting immune system cells in a pocket on the underside (Fig.).

The researchers speculated that the M cells may have specific molecular receptors to bind to antigen targets. So, working in mice, Ohno and colleagues used the microarray technology to scan the genome for receptor molecules specific to M cells. Glycoprotein 2 (GP2) was one such molecule and the microarray analysis showed it was highly associated with epithelium close to Peyer's patches. When they stained both GP2 and M cells, they discovered that not only was GP2 restricted to M cells on Peyer's patches in the gut, but that it was also found on M cells associated with

immune system tissues in other parts of the body in humans as well as mice. In fact, GP2 is a universal marker for M cells.

Moving in closer, the researchers employed electron microscopy to determine where GP2 was distributed on the M cells. They found it was localized on the membrane facing the gut cavity. By using an antibody that binds only to GP2, they discovered that some was incorporated into vesicular structures in the body of the M cells. This provided further evidence that the receptors were involved in the mechanism for transporting antigens to cells of the immune system beneath.

The next step was to determine what compound or compounds linked to the GP2 receptor. Ohno and colleagues experimented by mixing GP2 with *E. coli*, and found that it bound to the protein FimH on the bacterial pili. In fact, GP2 binds only to bacteria that carry FimH, and not to bacteria that lack this protein, such as *Pseudomonas* and *Listeria*.

Using an intact mouse intestine, the researchers tracked *E. coli* expressing green fluorescent protein. GP2 accumulated around the bacteria, which then could be followed moving inside the M cells. More than 90% of the bacteria transported through the M cells are captured by dendritic cells. They also showed that this process was severely hampered in *E. coli* lacking FimH or mice lacking GP2.

"It is reasonable to assume there are other molecules on the M-cell surface responsible for binding and uptake of bacteria lacking FimH," Ohno says. "This is one of the projects we are now working on. Another is to screen for small compounds that bind tightly to GP2. We can then use these molecules to target to GP2 on M cells in an effort to develop efficient oral vaccines."



Masumi Oomae (left) and Koji Hase (right)

ORIGINAL RESEARCH PAPER

Hase, K., Kawano, K., Nochi, T., Pontes, G.S., Fukuda, S., Ebisawa, M., Kadokura, K., Tobe, T., Fujimura, Y., Kawano, S., et al. Uptake through glycoprotein 2 of FimH⁺ bacteria by M cells initiates mucosal immune response. *Nature* 462, 226–232 (2009).

Helping cells reach out

An unexpected result leads to new insights about a poorly understood mode of communication between cells



Hiroshi Ohno

Remarkably little is known about M cells, which reside in the intestine and participate in immune surveillance of potential pathogens. However, Hiroshi Ohno was taken aback when Koji Hase, a staff scientist in his Laboratory for Epithelial Immunobiology, RCAI, identified a highly active M cell gene that triggered the formation of unusually long and thin membrane projections when expressed in other cell types.

"I thought it was an artifact," recalls Ohno, "but Koji insisted on its importance." Subsequent experiments proved Hase correct; a mammalian protein called M-Sec appears to drive formation of an enigmatic class of intercellular connections known as 'tunneling nanotubes' (TNT). TNTs are known to participate in the trafficking of a variety of cargoes, ranging from ions to proteins to cargo-laden membrane vesicles—and, less beneficially, viruses and prions. However, these findings offer the first real insights into the underlying mechanisms of TNT growth.

In a series of experiments using mouse immune cells known as macrophages that also express endogenous M-Sec, Ohno's group showed that formation of TNTs was inhibited upon knockdown of M-Sec expression (Fig.). These TNTs were also shown to be physiologically active, enabling signals to be transmitted from cell to cell via the movement of calcium ions.

Cells rely on a protein-based framework known as the cytoskeleton to provide infrastructure, and major morphological changes typically involve cytoskeletal rearrangements mediated by molecular switches known as small GTPases. Ohno and colleagues found that M-Sec associates with RalA, a small GTPase, and a membrane-bound protein complex known as the exocyst, and that these interactions are essential to the formation and growth of membrane protrusions that will ultimately form TNTs.

The researchers are now delving deeper into the bigger picture of M-Sec function by studying mice that have been genetically engineered to lack this protein, but are also exploring more direct ways to target M-Sec activity. "We have started screening M-Sec-binding

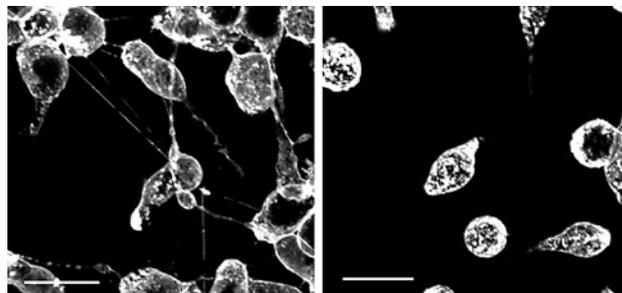


Figure : The formation of TNTs connecting mouse macrophage cells (*left*) is blocked when M-Sec is depleted by knockdown of gene expression (*right*). (Scale bar, 20 μ m).

small-molecular-weight chemicals that could inhibit M-Sec-mediated TNT formation," says Ohno.

He suggests that such drugs could ultimately be used *in vivo* to block the transmission of viruses via these intercellular conduits, but sees the most immediate benefits in simply understanding why these connections exist in the first place. "Virtually nothing is known about their *in vivo* relevance," he says. "In this regard, the discovery of M-Sec as a promoter of TNT formation will accelerate the understanding of their physiological role."



Yumiko Fujimura (*left*) and Koji Hase (*right*)

ORIGINAL RESEARCH PAPER

Hase, K., Kimura, S., Takatsu, H., Ohmae, M., Kawano, S., Kitamura, H., Ito, M., Watarai, H., Hazelett, C.C., Yeaman, C. & Ohno, H. M-Sec promotes membrane nanotube formation by interacting with Ral and the exocyst complex. *Nature Cell Biology* 11, 1427–1432 (2009).

Bringing leukemia out of hiding

Two new strategies target a deadly cancer that eludes conventional chemotherapy

Fumihiko Ishikawa

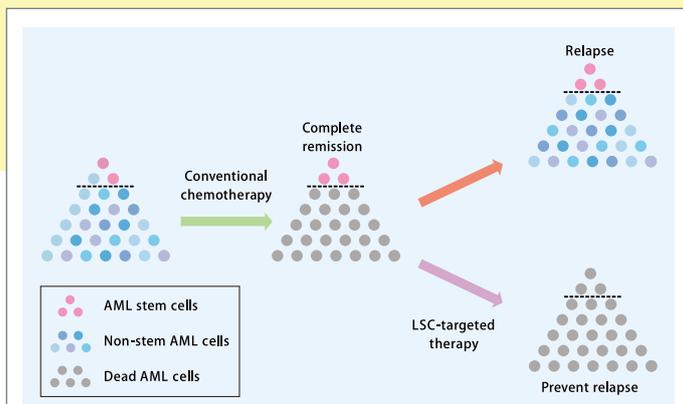


Figure : AML (left) can initially be driven into remission with chemotherapy, but this leaves behind a subpopulation of resistant LSCs (middle), with a high potential for initiating a fatal relapse (top right). Complete elimination of AML from patients may eventually be possible by directly targeting LSCs through more effective chemotherapeutic strategies or the identification of clinically useful LSC-specific markers (bottom right).

Acute myeloid leukemia (AML) is the most common form of adult leukemia, and with an estimated five-year survival rate of 20%, the long-term prognosis for many patients is relatively grim.

“Current treatments for AML can initially reduce the number of AML cells to undetectable levels, a state referred to as ‘complete remission’,” says Fumihiko Ishikawa of RICA’s Research Unit for Human Disease Model. “Unfortunately, in a substantial proportion of these patients, AML eventually comes back—and many that relapse succumb to the disease.” The need for an improved arsenal to fight AML has guided much of Ishikawa’s work, and two recently published articles from his team present promising strategies for tackling this dreaded cancer.

AML originates in bone marrow, and relapse is initiated from small pockets of chemotherapy-resistant ‘leukemia stem cells’ (LSCs) within the marrow, which can be identified by their distinctive profile of cell-surface markers. In an effort to identify other features of LSCs that might offer useful therapeutic targets, Ishikawa, Yoriko Saito and team performed a thorough comparative analysis between LSCs and normal blood stem cells to identify genes with functional characteristics pertinent to cancerous growth whose expression is specifically elevated in LSCs.

ORIGINAL RESEARCH PAPER

Saito, Y., Kitamura, H., Hijikata, A., Tomizawa-Murasawa, M., Tanaka, S., Takagi, S., Uchida, N., Suzuki, N., Sone, A., Najima, Y. et al. Identification of therapeutic targets for quiescent chemotherapy-resistant human leukemia stem cells. *Science Translational Medicine* 2, 17ra9 (2010).

Saito, Y., Uchida, N., Tanaka, S., Suzuki, N., Tomizawa-Murasawa, M., Sone, A., Najima, Y., Takagi, S., Aoki, Y., Wake, A. et al. Induction of cell cycle entry eliminates human leukemia stem cells in a mouse model of AML. *Nature Biotechnology* 28, 275–280 (2010).

Their analysis revealed two candidate cell-surface proteins, CD25 and CD32; both of these are commonly overexpressed in chemotherapy-resistant LSCs, but can also be therapeutically targeted without negatively affecting blood cell development from healthy hematopoietic stem cells, making them potentially promising targets for thwarting relapse.

In parallel, Ishikawa and colleagues have also explored methods for boosting the efficiency of chemotherapy. Standard AML drugs such as cytosine arabinoside (Ara-C) work by targeting actively dividing cells, and LSCs are believed to elude chemotherapy by entering a quiescent, non-dividing state. The researchers hypothesized that LSCs could be made more vulnerable to Ara-C via simultaneous treatment with cytokines—naturally-occurring cell signaling molecules—that stimulate them into active division. In fact, this two-pronged treatment led to a ten-fold increase in survival rate relative to chemotherapy alone for mice that had been transplanted with human LSCs.

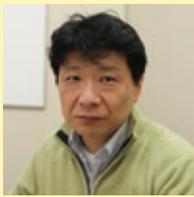
Previous studies have suggested that despite some risk of toxicity, cytokine treatment is relatively safe for patients, and Ishikawa’s team is actively investigating the practicality and safety of interventions based on both of their recent discoveries (Fig.). “We have been putting our best effort into the translation of these findings into medicine,” he says. “At the same time, we are continuing to try to identify unknown aspects of human AML and AML stem cells.”



Yoriko Saito (left), Mariko Murasawa (middle) and Hiroshi Kitamura (right)

Tipping the balance of immune response

Hybrid mice help researchers zoom in on a gene with a potential role in controlling allergic responses



Masato Kubo

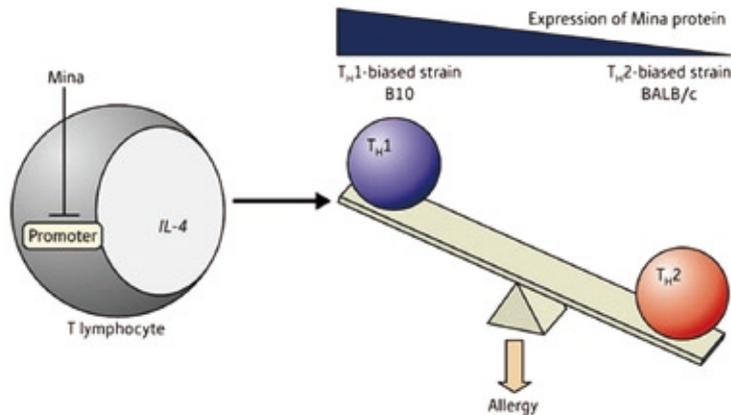


Figure: Schematic showing the role of the Mina protein in TH2 bias. The extent to which the IL-4 gene is being inhibited by Mina protein (left) affects the maturation of naïve T lymphocytes (right). Low levels of Mina mean a greater bias towards TH2 production, as in BALB/c mice, while higher levels lead to relatively higher TH1 production, as in B10 mice. Strong TH2 bias is thought to be a risk factor for allergy.

When the immune system encounters a potential threat, antigen-presenting cells deliver chunks of protein from the invading pathogen to naïve helper T cells. These naïve cells respond by differentiating into one of two classes of mature helper T cells: T_H1 cells, which mobilize the immune system against viruses and other intracellular pathogens, and T_H2 cells, which drive the response against blood-borne threats, such as the parasitic disease leishmaniasis.

Interleukin-4 (IL-4), one of a class of signalling factors known as cytokines, drives T_H2 differentiation and triggers secretion of additional IL-4, resulting in a positive feedback loop that fuels T_H2 production while suppressing T_H1 production. The degree of initial IL-4 production varies considerably between individuals and the resulting ‘ T_H2 bias’ can have serious clinical implications. “ T_H2 bias is thought to be a mirror of allergic response, because many T_H2 cytokines tightly associate with pathology of allergy,” says Masato Kubo, RICA’s Laboratory for Signal Network.

T_H2 bias also varies between different mouse strains, a fact that Kubo, Mark Bix of St. Jude’s Children’s Research Hospital in Memphis, USA, and colleagues exploited in a recent effort to identify determinants for this trait. It was known that BALB/c strain mice have a high T_H2 bias—producing large quantities of IL-4 following T cell activation—while B10.

D2 mice have a 50-fold lower bias. However, a hybrid BALB/c strain generated by Kubo and Bix that contains a chunk of chromosome 16 from the B10.D2 strain also exhibited low bias, suggesting that this segment includes a gene pertinent to this characteristic.

Closer analysis spotlighted the *Mina* gene as a likely suspect; analysis of various mouse strains revealed that *Mina* gene activity and levels of Mina protein were inversely correlated with T_H2 bias. Bix and Kubo’s team subsequently determined that Mina assembles into a larger multi-protein complex that directly binds to and inhibits the gene encoding IL-4, supporting a key role for this factor in T_H2 bias (Fig.).

The team’s analysis also identified nearly two dozen sequence variations in *Mina* that correlate with gene activity levels. These so-called single-nucleotide polymorphisms (SNPs) could provide useful diagnostic tools, and Kubo and Bix are now exploring this potential. “We have already done large-scale SNP analysis with Japanese and US populations,” says Kubo. “The human *Mina* locus has several SNPs, and some of them have weak correlation with atopic asthma in the Japanese population, but not in the US [population].”



Mariko Okamoto

ORIGINAL RESEARCH PAPER

Okamoto, M., van Stry, M., Chung, L., Koyanagi, M., Sun, X., Suzuki, Y., Ohara, O., Kitamura, H., Hijikata, A., Kubo, M. & Bix, M. Mina, an *IL4* repressor, controls T helper type 2 bias. *Nature Immunology* 10, 872–879 (2009).



Toshio Hirano

A zinc transporter is selectively required for one type of mast cell-mediated allergic response

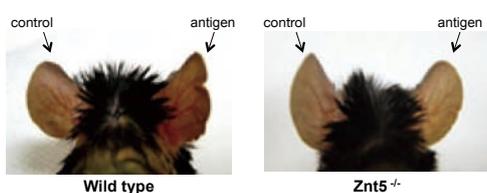
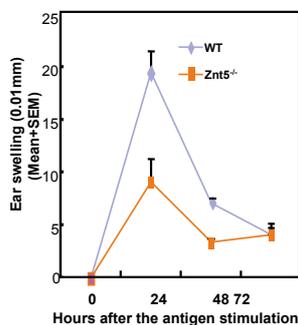


Figure: Contact hypersensitivity response (CHS) in wild type and *Znt5* deficient mice. Mice were sensitized with experimental hapten such as FITC. Ear swelling was measured at the indicated times after hapten challenge. WT mice developed robust CHS responses by tissue swelling at the site of hapten challenge (lower left), which reached a maximum 24 h after antigen stimulation (upper panel). The *Znt5* deficient mice exhibited defective CHS reaction (lower right). Even 24 h after the antigen challenge, the increase in ear thickness of the *Znt5* deficient mice was <45% of that of WT mice.

There are two types of allergic responses mediated by allergen-specific IgE antibodies bound to IgE receptors (FcεRI) on mast cells. The first is rapid, occurring immediately after the allergen crosslinks the FcεRI, and is caused by mast cell degranulation and release of preformed mediators such as histamine. The second type of response is delayed since it requires that the mast cell synthesize new proteins, in particular cytokines and chemokines. The symptoms of the immediate response can be treated with over the counter medications such as antihistamines, but the delayed-type response is more intractable. Therefore, understanding of how these responses are regulated is of great clinical significance and has been the focus of intense investigation. Toshio Hirano and his colleagues in the Laboratory for Cytokine Signaling have recently made the unexpected discovery of an essential role for a zinc transporter in the delayed-type response.

Zinc (Zn) is an essential trace element that is a co-factor in many enzymes and transcription factors. Hirano's group had previously described a novel role for Zn as an intracellular signaling molecule, akin to calcium. Furthermore, they also found that treatment

with a Zn chelator inhibited mast cell activation, suggesting an important role for this trace element in allergic responses.

Zn deficiency causes growth retardation, immunodeficiency, and other health problems. Therefore cells have evolved a complex system to maintain appropriate Zn levels through a combination of transporters for uptake, intracellular storage, and efflux. In their studies published in the *Journal of Experimental Medicine*, Nishida, et al. performed genetic ablation of one of the Zn transporter genes, *Znt5/Slc30a5*, and found that it is required for the mast cell-mediated allergic response such as contact hypersensitivity response (CHS) (Fig.). Intriguingly, *Znt5/Slc30a5* is only needed for the delayed-type response; degranulation of the mast cells from the zinc transporter-deficient mice was normal. They went on to study possible mechanisms for the defect in the knockout mice. Protein kinase C (PKC) is a serine/threonine kinase that plays an important role in multiple signaling cascades. In mast cells, PKC is required for cytokine production in the delayed-type response. The Hirano group found that the zinc transporter is involved in the translocation of PKC to the plasma membrane, a process important for its function. Zinc fingers are protein motifs that coordinate Zn ions, and PKC contains a zinc-finger-like motif. The authors found that this motif is required for translocation of PKC to the plasma membrane, thus explaining the requirement for Zn. They also found a defect in the nuclear translocation of nuclear factor (NF)-κB in the knockout mast cells. NF-κB is a transcription factor involved in upregulating the expression of proinflammatory cytokine genes such as IL-6 and TNFα. Their findings suggests that *Znt5/Slc30a5* is a novel player of PKC / NF-κB signaling.

These studies shed new light on the Zn-mediated regulation of the allergic response, specifically of the delayed type, and may provide new therapeutic targets for treatment of allergic diseases.



Keigo Nishida (left) and Aiko Hasegawa (right)

ORIGINAL RESEARCH PAPER

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

A novel gene essential for the development of single positive thymocytes

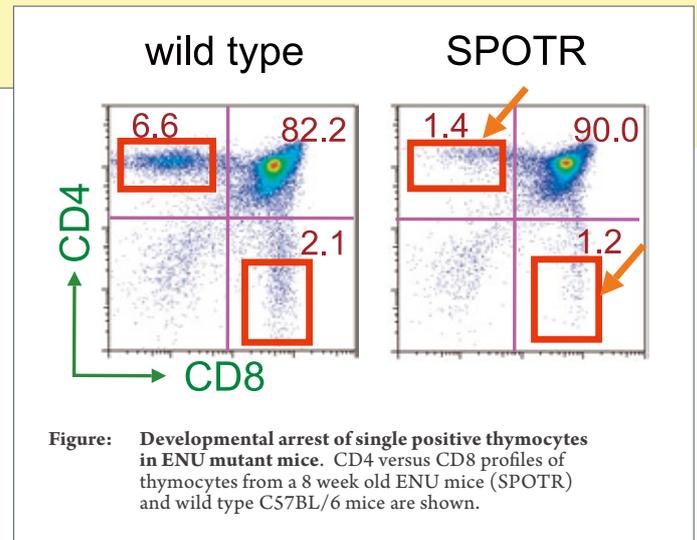


Hiroshi Kawamoto

Hisahiro Yoshida

As they develop in the thymus, T cells undergo a stepwise progression that can be traced by their expression of CD8 and CD4, co-receptors for the Major Histocompatibility Complex Class I and Class II molecules, respectively. Prior to rearrangement and expression of genes encoding the T cell receptor (TCR) the cells are CD4⁺/CD8⁻ (DN, double negative) and then become CD4⁺CD8⁺ double positive (DP). As they transition through these stages, the developing T cells undergo positive selection for recognition of self MHC and negative selection to eliminate cells that react too strongly. A critical step during intrathymic T cell development is the transition of cells to the MHC I-restricted CD4⁺CD8⁺ and MHC II-restricted CD4⁺CD8⁻ single positive (SP) cell stage. The cells that leave the thymus to constitute the T cell arm of the immune system in the periphery retain this SP phenotype and consequent recognition of antigens presented by MHC I or MHC II molecules.

RCAI investigators have now identified a gene that is essential for the critical transition from the DP to the SP thymocyte stage. This collaborative effort between the research groups of Hisahiro Yoshida, Laboratory for Immunogenetics and Hiroshi Kawamoto, Laboratory for Lymphocyte Development was published by Kiyokazu Kakugawa et al. in *Molecular and Cellular Biology*. In order to identify the genetic basis for immune disorders, Yoshida and his team have been screening a pool of mutant mice generated by random chemical mutagenesis with *N*-ethyl-*N*-nitrosourea. Through T cell phenotype-based screening, they established a mouse line in which numbers of CD4 and CD8 SP thymocytes as well as peripheral CD4 and CD8 T cells were dramatically reduced. They named this mouse line SPOTR (Single Positive Thymocyte Reduction), and by using linkage analysis and DNA sequencing, they identified a missense point mutation in a predicted gene, *E430004N04Rik*, of unknown function. This orphan gene was shown to be specifically expressed in DP and SP thymocytes and peripheral T cells, whereas the levels of protein encoded by this gene were drastically reduced in mutant thymo-



cytes. Kakugawa et al. next generated *E430004N04Rik*-deficient mice and their phenotype was essentially identical to that of the ENU mutant mice. This result conclusively demonstrated that *E430004N04Rik* is essential for the development of SP thymocytes.

While the RCAI investigators were in the process of manuscript submission, they became aware of the fact that several other groups had found this gene and had named it *Themis*, after the Greek goddess of justice and natural order and, in this case, for “thymus-expressed molecule involved in selection”. The RCAI group graciously agreed to use *Themis* as the name for their new gene. *Themis* is a member of a small gene family that contains two other members of unknown function. Apart from a nuclear localization signal and a carboxy terminal proline-rich sequence, the domains in the predicted *Themis* protein do not correspond to any known motifs and provide no clue as to its function. Further studies will likely reveal novel mechanisms in T cell development. Although no mutations in the human *Themis* ortholog have been reported yet, this gene will also be a good candidate for a causative gene in human primary immunodeficiency.



Kiyokazu Kakugawa (left) and Takuwa Yasuda (right)

ORIGINAL RESEARCH PAPER

Kakugawa, K., Yasuda, T., Miura, I., Kobayashi, A., Fukiage, H., Satoh, R., Matsuda, M., Koseki, H., Wakana, S., Kawamoto, H. & Yoshida, H. A novel gene essential for the development of single positive thymocytes. *Mol Cell Biol* 29, 5128-5135 (2009).



Katsuaki Sato

Natural suppressors of a treatment-induced disease

A naturally occurring population of dendritic cells reduces the incidence and severity of graft-versus-host-disease in mice

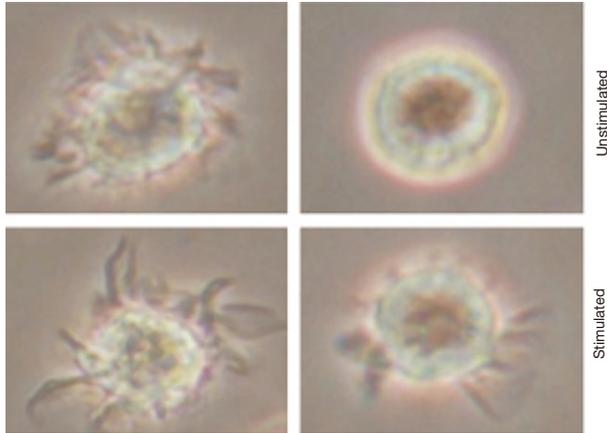


Figure : Phase contrast microscopy images (original magnification × 400) showing the morphology of non-regulatory (CD11c⁺) DCs (left column) and CD200R3⁺ DC_{regs} (right column) before and after stimulation with a pro-inflammatory agent.

Researchers in RCAI have shown that mouse dendritic cells (DCs), which can promote or inhibit inflammation depending on the proteins displayed on their surface, include a subpopulation that exerts beneficial effects during a treatment for leukemia and other malignancies. The treatment—known as allogeneic hematopoietic stem cell transplantation (alloHSCT)—can, in some situations, result in graft-versus-host-disease (GVHD). Acute and chronic GVHD occurs when donor immune cells called T lymphocytes recognize and become activated by proteins present on recipient but not donor tissues. The resulting T lymphocyte-driven immune response can result in severe damage to the recipient skin, gastrointestinal tract and liver.

Previous work by these researchers described the generation of regulatory DCs from mouse bone marrow (BM-DC_{regs}) that, when injected after alloHSCT, reduce the severity and incidence of acute and chronic GVHD. The team, led by Katsuaki Sato at the Laboratory for Dendritic Cell Immunobiology, has now shown that naturally occurring counterparts of BM-DC_{regs} exist and influence the outcome of alloHSCT in mice.

The researchers started by searching for genes associated with immunosuppressive DC function. A comparison of the genes expressed in BM-DC_{regs} and

non-regulatory DCs revealed that the gene encoding the surface protein CD200R3 is expressed exclusively in BM-DC_{regs}. They found that blockade of CD200R3 impaired the ability of BM-DC_{regs} to suppress proliferation of T lymphocytes, whereas forced expression of CD200R3 in non-regulatory DCs reduced their ability to promote T lymphocyte cell division. This indicates that CD200R3 contributes to the immunosuppressive function of BM-DC_{regs}.

Reasoning that naturally occurring regulatory DCs might also express CD200R3, the researchers screened blood and spleen cells for CD200R3 expression. They identified a small population of CD200R3-expressing cells that, like BM-DC_{regs}, produced immunosuppressive cytokines, which are regulators of the immune system, and inhibit T cell proliferation. These CD200R3⁺ DCs exhibited a different morphology than non-regulatory DCs (Fig.).

When injected after alloHSCT, Sato and colleagues found that these CD200R3⁺ DCs—like BM-DC_{regs}—suppressed the onset and severity of GVHD. Recipients of CD200R3 DCs contained lower amounts of serum proinflammatory proteins, and higher numbers of immunosuppressive regulatory T lymphocytes. Further highlighting the biological importance of CD200R3⁺ DCs, pre-treatment with a CD200R3-blocking antibody prior to alloHSCT exacerbated GVHD.

“The functional identification of naturally occurring human DC_{regs}, as well as their counterparts generated the laboratory, may provide an advantageous means of intervening to prevent chronic GVHD after alloHSCT,” says Sato.



Kawori Eizumi (left) and Kaori Sato (right)

ORIGINAL RESEARCH PAPER

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

Generation of functional NKT cells in vitro from embryonic stem cells



Masaru Taniguchi

Several features of NKT cells make them unique among the T cell subsets. Instead of recognizing peptide antigens presented by proteins of the Major Histocompatibility Complex (MHC), NKT cells recognize lipid antigens presented by the MHC Class I-like molecule CD1d. The prototypical NKT cells are known as invariant NKT (*i*NKT) cells because they express an invariant T cell antigen receptor (TCR) α chain. The response of NKT cells is also unique in that they are poised to rapidly secrete large amounts of TH1 and/or TH2 type cytokines. The nature of the response is dictated by the initial cytokine environment: interaction with IL-10-producing cells induces negative regulatory Th2 T cell-type NKT cells, while that with IL-12-producing cells results in pro-inflammatory Th1-type responses. As a result of their potent and varied cytokine profile, *i*NKT cells can have seemingly paradoxical functions, including enhancement of responses to pathogens and tumors, suppression of certain autoimmune diseases, induction of transplant tolerance, and promotion of allergy and asthma. These CD1d-restricted invariant natural killer T (NKT) cells bridge innate and acquired immunity and thus play an important role in both protective and regulatory responses, but may also be pathogenic. Autologous NKT cell therapy has shown promise in treating cancer in clinical trials. However, full realization of this promise will require a better understanding of how NKT cells develop, how to obtain sufficient numbers of these cells for therapeutic use, and how to control their differentiation along Th1- or Th2-type pathways. This is particularly relevant to the anti-tumor response; NKT cells mediate adjuvant activity by their production of IFN- γ , which is a Th1-type response. Masaru Taniguchi and his colleagues in the Laboratory for Immune Regulation have developed a new mouse model system that should help close the gaps in our knowledge of NKT cells.

In a paper published in *Blood*, Hiroshi Watarai et al. used a combination of cutting-edge techniques to generate NKT cells *in vitro*. They first transferred nuclei isolated from liver NKT cells into unfertilized eggs and then derived embryonic stem cell (ES) lines from the

resulting blastocyst stage of embryonic development (Fig.). The use of this approach meant that

all of the ES cells (NKT-ES cells) already had productively rearranged TCR α and TCR β chain genes to encode the invariant NKT cell TCR. Because of their genotype, the cells were expected to preferentially generate NKT cells *in vitro* if they could identify the proper culture conditions. They succeeded in achieving this goal. They were able to identify cells with NKT-cell potential as well as to obtain NKT cells with different cytokine profiles. Early NKT progenitors first appeared on day 11 in cocultures of NKT-ES cells with OP9 cells expressing a Notch ligand, delta-like. In the continued presence of Notch signals, the NKT-ES cells differentiated only into thymic-type CD44^{lo} CD24^{hi} NKT cells producing mainly interleukin-4 (IL-4), a Th2 phenotype. By contrast, NKT cells resembling CD44^{hi} CD24^{lo} liver NKT cells producing mainly IFN- γ , a Th1 phenotype, developed in switch cultures starting at day 14 in the absence of Notch signaling. These Th1-like cells also exhibited strong adjuvant activity *in vivo*, one of the hallmarks of naturally occurring Th1-type NKT cells. This newly developed cloned ES cell culture system offers a new opportunity for the elucidation of the molecular events during NKT-cell development. It will also allow the establishment of an animal model for NKT-cell therapy, which may ultimately be translatable into the clinic.

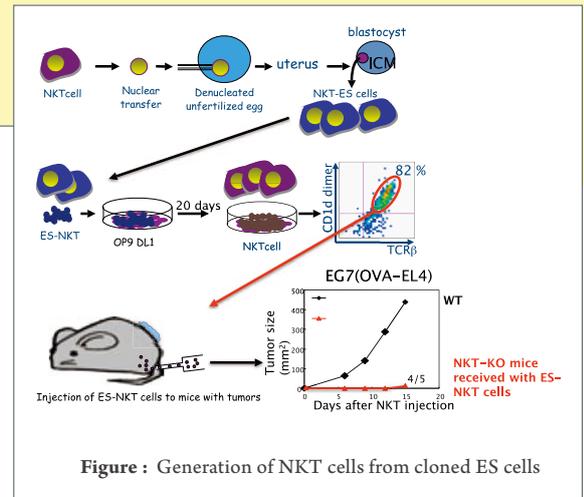


Figure : Generation of NKT cells from cloned ES cells



Hiroshi Watarai (left), Andrei Rybouchkin (middle) and Naomi Hongo (right)

ORIGINAL RESEARCH PAPER

Watarai, H., Rybouchkin, A., Hongo, N., Nagata, Y., Sakata, S., Sekine, E., Dashtsoodol, N., Tashiro, T., Fujii, S., Shimizu, K., Mori, K., Masuda, K., Kawamoto, H., Koseki, H. & Taniguchi, M. Generation of functional NKT cells in vitro from embryonic stem cells bearing rearranged invariant Valpha14-Jalpha18 TCRalpha gene. *Blood* 115, 230-237, (2010).

Outstanding Contribution of the Year 2009

RIKEN RCAI's Outstanding Contribution of the Year Award was established in 2006 to recognize staff members who have made outstanding contributions to the Center. The awardees are determined by the Director and receive trophies and a monetary prize. The awards ceremony was held on March 26, 2010. Koji Hase and Hiroshi Ohno (Laboratory for Epithelial Immunobiology), Yoriko Saito and Fumihiko Ishikawa (Research Unit for Human Disease Models), and Takanori Hasegawa and his team (Animal Facility, Laboratory for Developmental Genetics) received the award.

Yoriko Saito (Photo 1) and Fumihiko Ishikawa received the award for their contribution to the understanding of human immunity and diseases through the creation of a humanized mouse system.



Photo 1 : Yoriko Saito

Their research focuses on studying human hematopoiesis and immunity by using the humanized mouse model. By isolating hematopoietic stem cells (HSCs) and transplanting them into immunodeficient NOD/SCID/IL2 γ KO newborn mice, they succeeded in generating mice reconstituted with human hematopoietic and immune systems (Ishikawa, F., et al. 2005). Using this system, they created *in vivo* models of human acute myeloid leukemia (AML). AML is an extremely difficult disease. Typically, a patient presents with a high level of total body AML burden that is treated by highly toxic combination chemotherapy. In many cases, this results in what is referred to as complete remission, meaning that AML cells are no longer detectable in the blood and bone marrow of the patient. However, in many patients, AML relapses over time. Therefore, the RCAI investigators thought that the humanized mouse would be the key to understand what is happening in humans during this period.

In 2007, by establishing a robust *in vivo* model

that recapitulate human AML in mice, they found that leukemia stem cells are cell cycle quiescent and thus exhibit chemotherapy resistance in the bone marrow niche, whereas non-stem AML cells can be eliminated by conventional chemotherapy. It is these chemotherapy-resistant leukemic stem cells (LSCs) that eventually lead to disease relapse (Ishikawa, F., et al. 2007). Therefore, the researchers decided to explore effective anti-LSC therapy to improve patient outcomes. To target LSCs, they took a multi-faceted approach. First, they looked for ways of interfering with LSC function

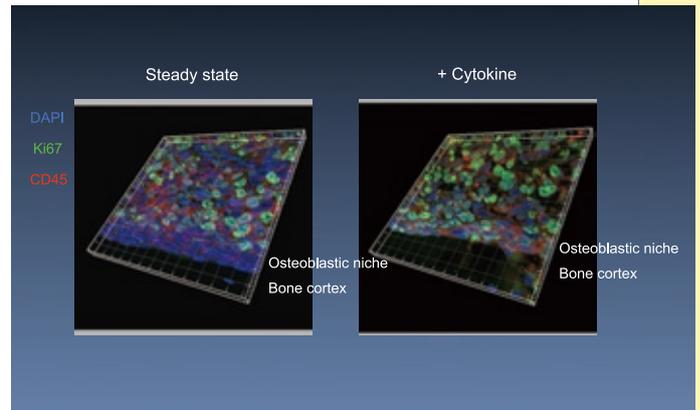


Figure 1: Quiescent LSCs within the osteoblast niche in bone marrow enter the cell cycle after *in vivo* G-CSF treatment. (left) At steady state, the LSCs adjacent to the bone marrow endosteum are cell cycle quiescent (Ki67⁻), whereas in the perivascular region, CD45⁺ leukemic cells are Ki67⁺ (cycling). (Right) After *in vivo* G-CSF treatment, LSCs within the bone marrow endosteal region became Ki67⁺, indicating that they had entered the cell cycle. CD45 (red), Ki67 (green), DAPI (blue).

and survival *in vivo*. They were able to induce cell cycle in LSCs by treatment with granulocyte colony-stimulating factor (G-CSF). In combination with cell cycle dependent chemotherapy, G-CSF pretreatment sig-

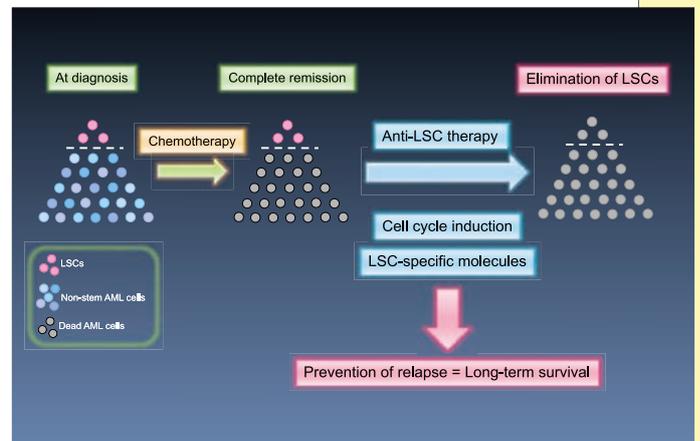


Figure 2: Two strategies to target LSCs to prevent AML relapse. The first strategy is interfering with LSC survival by induction of cell cycles, and the second is targeting LSC-specific molecules.

nificantly enhanced the apoptosis and elimination of LSCs in the humanized mouse (Fig. 1). Secondly, they looked for LSC-specific molecules as potential therapeutic targets. In collaboration with Osamu Ohara's lab in RCAI, they compared global gene expression in LSCs and normal HSCs. They succeeded in identifying 25 molecules expressed only in LSCs, including cell surface markers CD32 and CD25, kinases and transcription factors. Thus, these two lines of investigation led them to propose two potential therapeutic strategies that target leukemia stem cells: Cell cycle-modified chemotherapy (Saito, Y., et al. 2010) and LSC-specific molecular targets (Saito, Y., et al. 2010) (Fig. 2, See also *Research Highlights*).

"We hope that in the near future, we can begin to offer effective therapies for patients with AML. We deeply thank the patients and physicians at the Toranomon Hospital for their generous cooperation in providing samples for these studies." said Saito.

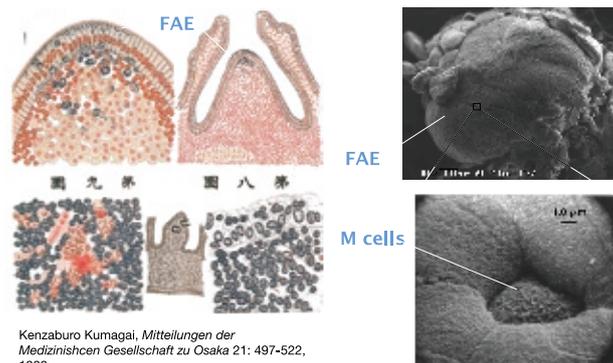
Koji Hase and Hiroshi Ohno (Photo 2) received the award for their research on the functions of M



Photo 2 : Koji Hase (left) and Hiroshi Ohno (right)

cells. M cells, specialized epithelial cells in follicle-associated epithelium (FAE), were discovered by Cooper in 1973, and named M cells by Owen in 1974. Intriguingly, however, 50 years before them, a Japanese physician, Kumagai, had described the follicle-associated epithelium (FAE) how it was responsible for taking up macromolecules such as bacteria (Fig. 3). When Ohno started his study on M cells in 2002, almost nothing was known about these cells molecularly. "I studied immunology and worked on membrane traffic at NIH. So I always wanted to combine these fields. When I heard at an immunology meeting that M cells were active in transcytosis, I thought this should be something I could get involved in, a fusion of immunology and cell biology," Ohno recalled.

When Koji Hase joined Ohno's lab in 2002, Hase established the method to isolate FAE from the vast majority of conventional epithelial cells of the intestinal



Kenzaburo Kumagai, *Mitteilungen der Medizinischen Gesellschaft zu Osaka* 21: 497-522, 1922

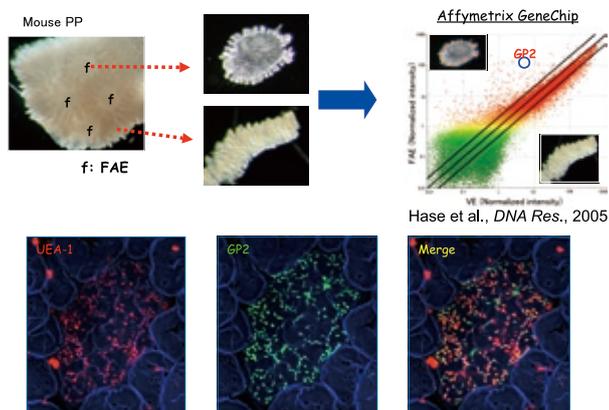
熊谷謙三郎, 「形態的成分の腸管吸収機転に就て」
大阪医学会雑誌 21: 497-522, 1922 (大正11年)

Bockman, D.E. and Cooper, M.D., *Am J Anat* 136:455-477, 1973
Owen, R.L. and Jones A.L., *Gastroenterology* 66: 189-203, 1974

Figure 3: Identification of FAE and M cells. In 1922, a Japanese doctor reported that bacterial antigens are taken up by dome-shaped epithelium covering lymphoid follicle, which is now called follicle-associated epithelium (FAE). Fifty years later, M cells were identified by Max Cooper and Robert Owen.

villi and identified several M cell-specific genes (Hase, K., et al. 2005). One of them was GP-2 (Fig. 4), which turned out to be an uptake receptor for *E-coli* and *Salmonella* to initiate the mucosal immune response (see *Research Highlights*).

However, the road to publication was not easy. They submitted the paper to *Nature*, *Science* and *Nature Cell Biology*, but it kept being immediately rejected. "So, we decided to add functional data," said Ohno. They had been collaborated with Shin-ichi Fukuoka in Aoyama Gakuin Univ., and started an additional collaboration with Hiroshi Kiyono in the Univ. of Tokyo and Anson Lowe in Stanford Univ. After two



Hase et al., *DNA Res.*, 2005

Figure 4: Methods to explore M cell-specific genes. FAE and villous epithelium (VE) were isolated from Peyer's Patches. Total RNAs prepared from FAE or VE were used for microarray analysis. Expression of the candidate molecules was confirmed by quantitative real-time PCR and in-situ hybridization.

years of collaboration, they finally found differences in the immune responses to oral *Salmonella*-ToxC in GP-2 knockout versus wild type mice. “With the efforts by all these four groups, the paper was finally accepted (Hase, K., et al. 2009). It took nearly three years from the first submission,” said Ohno in his speech.

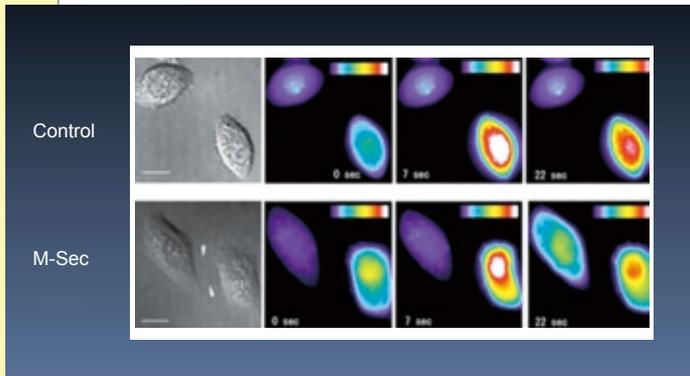


Figure 5: Intercellular calcium signalling between remote cells is observed only in physically connected cell pairs. HeLa cells stably expressing M-Sec were loaded with fluo-4. Cell pairs were randomly selected and a cell on one side in each pair was mechanically stimulated to evoke a calcium activation signal. The propagation of calcium flux was undetectable between unconnected pairs (*upper panels*). In contrast, it was frequently observed between connected pairs (*lower panels*). Arrowhead indicates a TNT interconnecting remote cells. Scale bars, 15 μ m.

They also made a surprising discovery of a molecule, named M-sec, which was expressed specifically in M cells and myeloid cells. When its expression was forced in other cell types, M-sec triggered the formation of thin tubular connections between remote cells, called tunneling nanotubes (TNT) (see *Research Highlights*). After stimulation of one of the cells, the calcium flux was promptly propagated from the stimulated cell to the surrounding cells via interconnecting TNTs (Fig. 5) (Hase, K., et al. 2009). They have further shown that

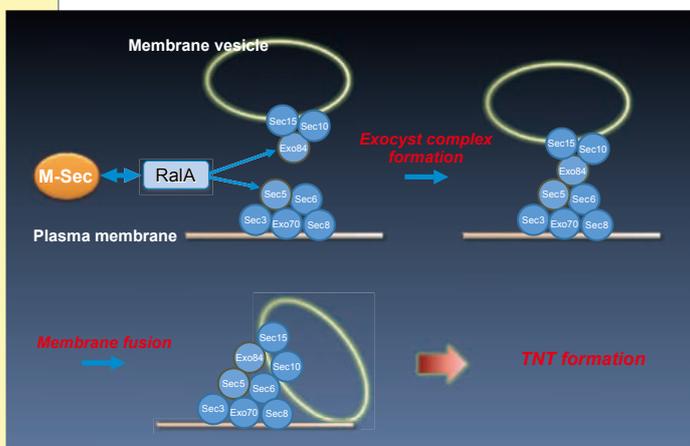


Figure 6: Model for M-Sec to promote TNT formation. M-sec associates with the small GTPase, RalA, a regulator of actin remodeling. RalA interacts with two exocyst subcomplexes, and promotes the full complex formation, which leads to membrane fusion

M-sec associates with the small GTPase, RalA, a regulator of actin remodeling. RalA then interacts with two exocyst subcomplexes, and promotes the full complex formation, which leads to membrane fusion (Fig. 6). “We hope to clarify how M cells differentiate, how M cells work and hopefully, prove that M cells are really important in gut immunity,” said Ohno, “We appreciate all the collaborators, and of course we thank the people of RCAI for their advice and support.”



Photo 3 : Takanori Hasegawa

Takanori Hasegawa and his Animal Facility team received the award for their continuous and skillful contributions to promote the best quality animal experiments at RCAI. Their contributions cover almost all aspects of animal experiments, including maintenance of animals under stable and hygienically well-controlled conditions, cryopreservation and generation of genetic resources, compliance and education for animal experiments, and many others. Moreover, their efforts enabled very flexible management of the animal facility, which is not an easy goal to be realized in a research institution.

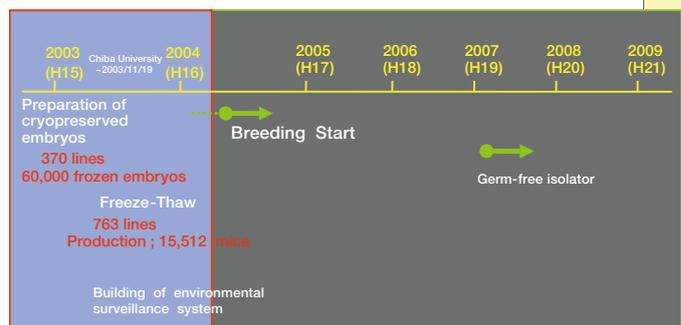


Figure 7: Preparations for the start-up of RCAI

RCAI started its activity in 2004, but before then, the original team members, Mochizuki, Ishikura, Matsuda and Hasegawa who were at Chiba University at that time, had started preparation under Haruhiko Koseki. Their last day at Chiba University was going to

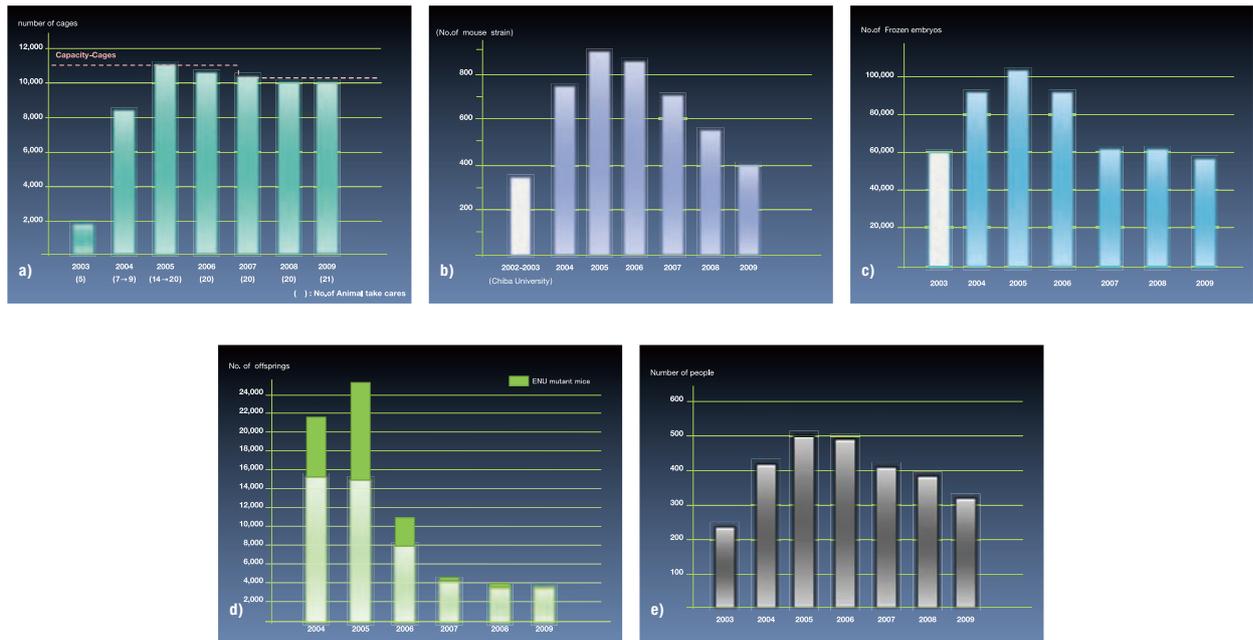


Figure 8: Annual statistics of the animal facility a) Change in the number of cages, b) clean-up of mouse strains by *in vitro* fertilization, c) cryopreservation of the embryos, d) providing cleaned-up offspring by thawing the frozen embryos, e) change in the number of users

be Nov. 19, 2003, so in the summer of 2002, they started cryopreservation of embryos. “We worked like a wind-up toy whose spring was tightened to the end,” Hasegawa recalled. The numbers reached 370 lines or 60,000 embryos. They packed everything and set out to RIKEN on Nov. 19. Then, at RCAI, they had to do the reverse, but this time, including the mice from other universities, too. They thawed 763 lines and produced 15,512 mice before the launch of RCAI in 2004 (Fig. 7). “Animal facility members were already so exhausted when RCAI marked its start in April, 2004,” said Hasegawa.

“We thought that our work load at the new facility would increase slowly, but it did not shift like that,” Hasegawa says. In 2004, the number of cages reached 8,000, and the next year, it already hit the upper limit. In addition to that, generation of SPF mice, clean-up of mouse strains by *in vitro* fertilization, cryopreservation of the embryos and providing cleaned-up offspring by thawing the frozen embryos, hit a peak in 2005. The number of the facility users reached its peak in 2005, too (Fig. 8a-e). To catch up, the number of team members was increased from 5 in 2003 to 20 in 2005. Besides these services, the team generates transgenic and chimeric mice, human BAC clone-transgenic mice, cryopreservation of sperm, and support of the ENU mutant mice and humanized mice projects. “To some extent, every member of RCAI has profited from the efforts of Hasegawa and his colleagues,” said the Director Taniguchi at the awarding ceremony.

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Toshio Hirano awarded the Crafoord Prize 2009

Crafoord Prize is a science prize intended to promote international basic research in the disciplines of astronomy and mathematics, geosciences, biosciences, and polyarthritis. The prize was established in 1980 by Anna-Greta and Holger Crafoord and is administered by the Royal Swedish Academy of Sciences. The prize is awarded to one discipline each year, but a prize for polyarthritis research is only awarded when a special advance is made. The Crafoord Prize 2009 was awarded to Charles A. Dinarello for identification of interleukin-1 (IL-1), and Tadimitsu Kishimoto and Toshio Hirano for the identification of interleukin-6 (IL-6) (Photo 1). Both these cytokines are found in high concentrations in the joints of people with polyarthritis. Drugs that mitigate the effect of IL-1 are already in use, and a drug against IL-6 has been approved in Japan and all over the world including countries in Europe and the US.



Photo 1: The Crafoord Prize 2009 awardees
The Crafoord Prize 2009 was awarded to Tadimitsu Kishimoto (*left*), Toshio Hirano (*middle*) and Charles A. Dinarello (*right*).

On May 11, Hirano received the award from the hands of His Majesty the King of Sweden (Photo 2). "We do not know what the view from a mountain top will be. Life creates many challenges and it is not until we have reached one summit that we can see what lies before us. Only the one who stands on the top of the mountain can know what this view is, how extensive it is, how beautiful it is and what it will lead to." Hirano explained in his Crafoord Prize lecture.

When Hirano worked at Osaka Prefectural



Photo 2: Hirano received the award from the hands of His Majesty the King of Sweden.

Habikino Hospital in 1978, he saw many patients with tuberculous pleurisy. He found that pleural effusion cells from these patients produce an active factor that induces immunoglobulin production by B cells. He decided to isolate this factor. The effort continued although he moved to Kumamoto University Medical School in 1980 and to Kishimoto's laboratory in Osaka University in 1984. After 8 years, on the Sunday morning of May 25th, 1986, he finally cloned the activation factor. To his surprise, the factor activated not only B cells, but also a variety of cells and tissues. The factor, named IL-6, was a multifunctional cytokine that plays roles in immune and inflammatory responses, as well as in hematopoiesis and the endocrine and nervous systems (Fig. 1). Then, the receptor for IL-6 was isolated. It is composed of two subunits, the IL-6 binding subunit and a signal transducer, gp130. Gp130 was not only part of the IL-6 receptor, but also a signal transducer for other cytokines such as IL-11, OSM, LIF, CT-1, CNTF, and IL-27. "The view I saw was far beyond my expectation," said Hirano.

That was not the end of the story. They found that a benign cardiac tumor, myxoma, produced IL-6 in large quantities. Patients with myxoma have symptoms resembling those of rheumatic diseases: fever and joint pain, for example, but as soon as the tumor is surgically removed the symptoms vanish. With this discovery, they began to suspect that IL-6 is involved in autoimmune diseases, and in 1988, they also found overproduction of IL-6 in the joints of patients with

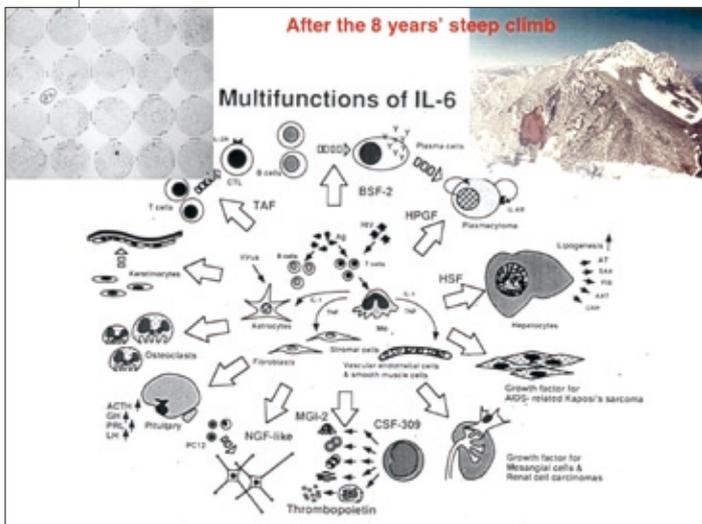
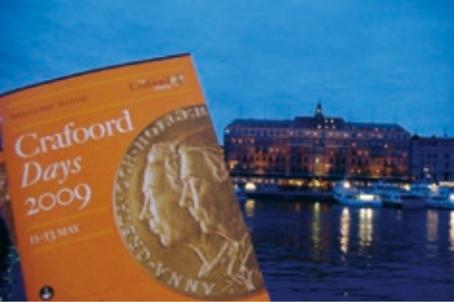


Figure 1: Multifunction of IL-6

rheumatoid arthritis (RA). Patients with RA show a variety of symptoms: polyclonal plasmacytosis accompanied by production of rheumatoid factor, increase of acute phase proteins, enhanced bone resorption activity, and increase of platelets, none of which had any apparent relationship to each other. However, when one considered the multiple functions of IL-6, this puzzle was solved, Hirano thought.

In the early 1990s, he proposed a new hypothesis about the possible mechanisms that operate in chronic inflammatory proliferative diseases (CIPD) like RA; The interaction between the immune system and non-immune tissues plays a role in autoimmune diseases. Hirano and his colleague have extensively revised this original hypothesis based on extensive experiments using F759 knockin mice, which express a mutated gp130 showing enhanced IL-6-induced STAT3 activation and spontaneously develop RA-like arthritis (F759 arthritis). They demonstrated that the interaction between non-immune tissues and the immune system plays a critical role in the pathogenesis of autoimmune F759 arthritis and that this interaction is mediated at least in part by an "IL-6 amplifier". IL-17 and IL-6 synergistically induce IL-6 gene expression through activation of NF- κ B and STAT3 in Type I collagen positive non-immune tissue. Enhanced IL-6 production results in enhanced TH17 development,

giving rise to further enhanced production of IL-6. The "IL-6 amplifier" is required for autoimmune arthritis in F759 mice as well as in experimental autoimmune encephalomyelitis (EAE) where antigen-specific T cells are involved. They hypothesize that any event, including autoantigen-specific T cells, virus infection, injury and physical stimulation capable of activating the "IL-6 amplifier" through either STAT3 activation or NF- κ B activation or both plays a critical role in causing autoimmune diseases. (Fig.2).

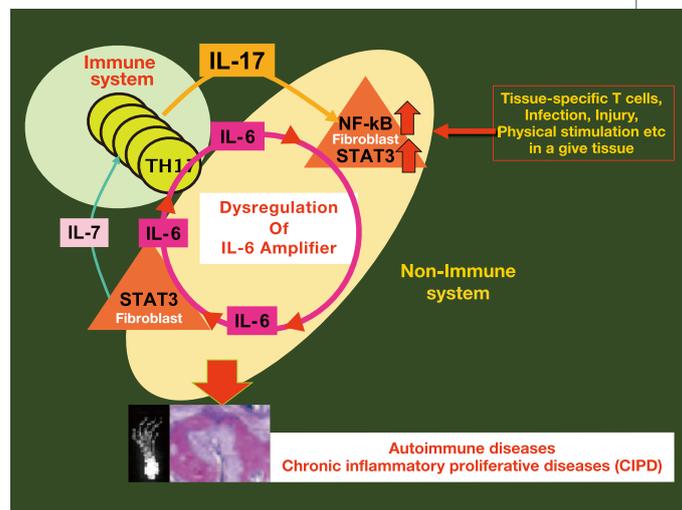


Figure 2: The model of IL-6 amplifier

"Now, I am standing on top of the mountain and I can finally enjoy the view, the functions of IL-6 that I could not imagine 30 years ago. For me, IL-6 is a gift from patients with tuberculosis. Anti-IL-6 receptor antibody treatment, which inhibits IL-6 function, has been found to be beneficial for many patients. Thus our basic studies contributed toward an understanding of the immunological mechanisms of RA and paved the way to develop a new drug beneficial for the patients and hopefully other autoimmune diseases and inflammatory diseases," Hirano said in his speech.

RIKEN's staff celebrated his receiving the award on Dec. 16, 2009. Sixty people gathered for the celebration party.

Other Prize Winners 2009



Photo 1: Fumihiko Ishikawa

Fumihiko Ishikawa, Leader of the Research Unit for Human Disease Model, received the Minister of Education, Culture, Sports, Science and Technology (MEXT) Prize for Young Investigators, 2009 (photo 1). This prize is awarded to young scientists (under 40 years of age) in recognition of creative and original research and outstanding ability to develop scientific research projects. Ishikawa was awarded for his research on hematopoietic stem cells and leukemic stem cells utilizing the newly developed humanized mouse model.

Tsuneyasu Kaisho (photo 2), Leader of the Laboratory for Host Defense, received the 12th Japanese Society for Immunology Award during the society's annual meeting in December. Kaisho was awarded for his research on molecular mechanisms for the functional regulation of dendritic cells (DC). He discovered a MyD88 independent TLR signaling pathway that causes DC maturation, and found functional abnormalities of dendritic cells that shifted the Th1/Th2 balance towards Th2 in MyD88 KO mice. He later discovered the importance of IKK α in innate immune responses. He found that inactivation of IKK α inhibits IFN- α gene induction by nucleic acid sensors, TLR7/9, in plasmacytoid DC, and he further proved that IKK α actually cooperates with IRF-7 to control IFN- α gene expression. This award is given to a researcher, age 50 or younger, whose contribution to the advances in immunology has been original and extraordinary and further research achievements are expected in the future.



Photo 2: Tsuneyasu Kaisho



Photo 3: Hisaaki Shinohara

Hisaaki Shinohara (photo 3), Researcher in the Laboratory for Lymphocyte Differentiation, received the Research Encouragement Award of the Japanese Society for Immunology during its 39th annual meeting. Shinohara was awarded for his research on B cell signaling, especially the discovery of TAK1 involvement in B cell antigen receptor mediated NF- κ B activation. His current work on mathematical modeling of NF- κ B activation by the B cell antigen receptor is expected to develop a new field. This award is given to young investigators, age 40 or younger, who have conducted distinguished immunological studies.



Photo 4: Masahiro Kitano

Masahiro Kitano (photo 4), Special Postdoctoral Researcher in the Research Unit for Immunodynamics, received the GE & *Science* Prize for Young Life Scientists. He was awarded for his essay "Imaging of Rab5 Activity Identifies Essential Regulators for Phagosome Maturation". His strong interests in imaging led him to complete a Ph.D. at Osaka University, where he developed a biosensor to identify regulators of the phagosome maturation process, and he then joined Takaharu Okada's lab in RCAI to study dynamics of immune cells *in vivo*.

The GE & *Science* Prize for Young Life Scientists was established in 1995 to recognize outstanding Ph.D.s from around the world and reward their research in the field of molecular biology. Entrants write a 1000-word synopsis of their thesis and submit it to the journal *Science* for judging by an executive panel. Prizes are awarded each year to finalists in four geographic regions, North America, Europe, Japan and other countries. Each winner receives US\$ 5,000 and a trip to Stockholm, Sweden to accept their prize and meet with the current Nobel laureates.

Toshio Hirano (photo 5), Leader of the Laboratory for Cytokine Signaling, received the Suita City Mayor Award on June 15 from the mayor, Yoshio Sakaguchi. He was awarded for the discovery of Interleukin-6 and the elucidation of IL-6 function in rheumatoid arthritis and other inflammatory diseases.



Photo 5: Toshio Hirano



Photo 6: Koji Hase



Photo 7: Yasuaki Murahashi

Koji Hase (photo 6) and **Yasuaki Murahashi** (photo 7) received RIKEN Research and Technology Incentive Awards 2009. Koji Hase, Researcher in the Laboratory for Epithelial Immunobiology, received the award for his study on the biological significance of intestinal M cells. Yasuaki Murahashi, Technical Scientist of RICA, received the award for construction of 'PID Japan', an

integrative database of primary immunodeficiency diseases. RIKEN Research and Technology Incentive Awards were newly established in FY2009 and are given to young researchers and technicians under age 40 who have contributed to furthering RIKEN's ideals by achieving exemplary results in their research or research support activities.



Photo 8: Shinji Fukuda

Shinji Fukuda (photo 8), Special Postdoctoral Researcher in the Laboratory for Epithelial Immunobiology, received The Best Original Paper Award from Nestle Japan for his *PLoS ONE* paper "Evaluation and characterization of bacterial metabolic dynamics with a novel profiling technique, real-time metabolotyping".

Shunsuke Kimura (photo 9), Researcher in the Laboratory for Epithelial Immunobiology, was given the CSF Award of the Year from the Japan Society for Cell Biology for his paper "Dynein-dependent Movement of Autophagosomes Mediates Efficient Encounters with Lysosomes" published in *Cell Structure and Function (CSF)*. This annual award is given to recognize an excellent original paper published in *CSF*.



Photo 9: Shunsuke Kimura



Photo 10: Gaku Nakato

Gaku Nakato (photo 10), **Shinji Fukuda** (photo 8) and **Koji Hase** (photo 6), Laboratory for Epithelial Immunobiology, received The 14th International Congress of Mucosal Immunology ICMI 2009 travel award.

Masashi Ebisawa (photo 11), Junior Research Associate in the Laboratory for Epithelial Immunobiology, received the Encouragement Award from the Hindgut Club Japan for his presentation "CCR6^{hi} CD11c⁺ B cells promote M-cell differentiation in Peyer's Patch". He also received the Chancellor's Encouragement Award from Yokohama City University.



Photo 11: Masashi Ebisawa

Excellent Paper of the Year 2009

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

In 2009, 9 excellent papers were selected from 12 candidates for this award.



Koji Hase, Kazuya Kawano and Hiroshi Ohno

Uptake through Glycoprotein 2 of FimH⁺ bacteria by M cells initiates mucosal immune response
Nature, Vol 462, pp 226-230, 2009

Yoriko Saito and Fumihiko Ishikawa

Induction of cell cycle entry eliminates human leukemia stem cells in a mouse model of AML
Nature Biotechnology, Vol. 28, pp 275-281, 2010

Koji Hase and Hiroshi Ohno

M-Sec promotes membrane nanotube formation by interacting with Ral and the exocyst complex
Nature Cell Biology, Vol. 11, pp 1427-1432, 2009

Mariko Okamoto and Masato Kubo

Mina, an *Il4* repressor, controls T helper type 2 bias
Nature Immunology, Vol 10, pp 872-879, 2009

Yoriko Saito and Hiroshi Kitamura

Identification of therapeutic targets for quiescent chemotherapy-resistant human leukemia stem cells
Science Translational Medicine, Vol 2, pp 17ra9, 2010

Keigo Nishida, Aiko Hasegawa and Toshio Hirano

Zinc transporter *Znt5/Slc30a5* is required for the mast cell-mediated delayed-type allergic reaction, but not the immediate-type reaction
J. Exp. Med., Vol. 206, pp 1351-1364, 2009

Kiyokazu Kakugawa, Takuwa Yasuda, Hiroshi Kawamoto and Hisahiro Yoshida

A novel gene essential for the development of single positive thymocytes
Mol. Cell. Biol., Vol. 29, pp 5128-5135, 2009

Kaori Sato, Kawori Eizumi and Katsuaki Sato

Naturally occurring regulatory dendritic cells regulate murine cutaneous chronic graft-versus-host disease
Blood, Vol. 113, pp 4780-4789, 2009

Hiroshi Watarai, Andrei Rybouchkin, Naomi Hongo and Masaru Taniguchi

Generation of functional NKT cells *in vitro* from embryonic stem cells bearing rearranged invariant *V α 14-J α 18* TCR α gene
Blood, Vol. 115, pp 230-237, 2010

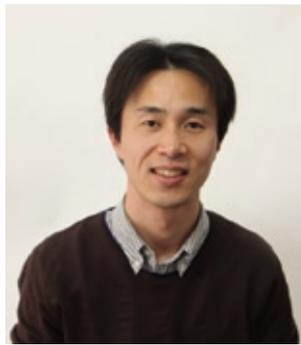
Excellent Poster Award 2009

On August 31, RCAI held two poster presentation sessions during its retreat meeting in Chiba. Among ninety posters presented, nine posters were selected for an “Excellent Poster Award 2009” by vote of laboratory heads.

Awardees of Excellent Poster Award 2009 :



Kiyokazu Kakugawa
(Lab. for Lymphocyte Development)



Kenichi Asano
(Lab. for Innate Cellular Immunity)



Hiroshi Watarai
(Lab. for Immune Regulation)



Toshiyuki Fukuda
(Lab. for Cytokine Signaling)



Kohei Kometani
(Lab. for Lymphocyte Differentiation)



Michio Tomura
(Lab. for Autoimmune Regulation)



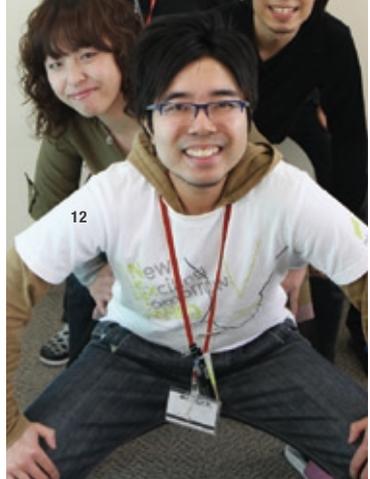
Yoriko Saito (left)
(Research Unit for Human Disease Model)

Mariko Murasawa (right)
(Research Unit for Human Disease Model)



Masahiro Kitano
(Research Unit for Immunodynamics)

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



RIKEN Special Postdoctoral Researcher (SPDR) Program

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

- Kenichi Asano** (Lab. for Innate Cellular Immunity) (01)
- Kohei Kometani** (Lab. for Lymphocyte Differentiation) (02)
- Yoshitaka Shirasaki** (Lab. for Immunogenomics) (03)
- Nayuta Yakushiji** (Lab. for Developmental Genetics) (04)
- Yuki Horisawa-Takada** (Lab. for Developmental Genetics) (05)
- Mizuho Kajikawa** (Lab. for Infectious Immunity) (06)
- Masahiro Kitano** (Research Unit for Immunodynamics) (07)
- Takashi Kanaya** (Lab. for Epithelial Immunobiology) (08)
- Shinji Fukuda** (Lab. for Epithelial Immunobiology) (09)

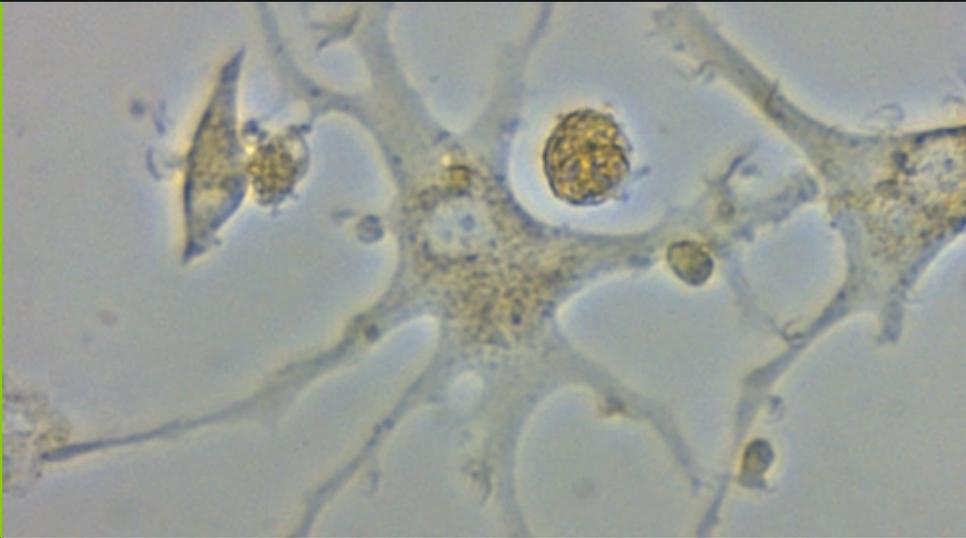
RIKEN's Junior Research Associate (JRA) Program

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, established scientists with years of experience. This program provides part-time positions at RIKEN for young researchers enrolled in university Ph.D. programs. The JRA program serves the dual purpose of fostering the development of these young scientists while also energizing RIKEN with their innovative thinking. This year, seventeen JRA students studied in RCAI.

- Gaku Nakato** (Lab. for Epithelial Immunobiology) (10)
- Toshi Yuyama** (Lab. for Epithelial Immunobiology) (11)
- Masashi Ebisawa** (Lab. for Epithelial Immunobiology) (12)
- Shintaro Hojo** (Lab. for Cytokine Signaling) (13)
- Beum-Ho Byn** (Lab. for Cytokine Signaling) (14)
- Yasutaka Motomura** (Lab. for Signal Network) (15)
- Machiko Sugiyama** (Lab. for Immunogenetics) (16)
- Hideaki Takagi** (Lab. for Dendritic Cell Immunobiology) (17)
- Tomohiro Fukaya** (Lab. for Dendritic Cell Immunobiology) (18)
- Izumi Sasaki** (Lab. for Host Defense) (19)
- Chihiro Yamazaki** (Lab. for Host Defense) (20)
- Saya Moriyama** (Lab. for Lymphocyte Differentiation) (21)
- Takanori Sawaguchi** (Lab. for Developmental Genetics) (22)
- Kazunori Kadokura** (Lab. for Epithelial Immunobiology) (23)
- Daisuke Takahashi** (Lab. for Epithelial Immunobiology) (24)
- Naomi Hongo** (Lab. for Immune Regulation) (25)

2009

Laboratory Activities



Group Director

Haruhiko Koseki

Research Scientists : **Yixin Dong, Mitsuhiro Endo, Yūichi Fujimura, Kyōichi Isono, Kyoko Masuda, Jun Shinga, Daisuke Yamada, Ruken Yaman-Deveci, Yuki Takada (SPDR), Nayuta Yakushiji (SPDR)**

Technical Staff : **Masako Fujita, Hiroshi Kajita, Iyo Kataoka, Kayoko Katsuyama, Fuyuko Kezuka, Genta Kitahara, Yōko Koseki, Tamie Morisawa, Naoko Ohnaga, Naomi Otsuka, Rie Suzuki, Momoko Okoshi, Chieko Tezuka**

Visiting Scientists : **Mutō Masahiro, Jafar Sharif**

Student Trainee : **Takanori Sawaguchi (JRA), Asami Tsuboi**

Administrative Staff : **Ryōko Moriizumi**

Laboratory for Developmental Genetics

The Developmental Genetics Research Group fulfills a dual 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 organ development and stem cell functions, with particular emphasis on epigenetic regulation mediated by Polycomb group (PcG) genes in development and stem cell functions.

PRC1 suppresses ES cell differentiation programs through PRC2-dependent and PRC2-independent mechanisms (Endoh et al., submitted)

To clarify the molecular mechanisms linking two distinct Polycomb complexes, PRC1 and PRC2, and their biological outcomes, we examined the genome-wide distributions of the PRC1 components Ring1B and Cbx2, and the PRC1-catalyzed histone modification, mono-ubiquitinated H2A (H2Aub1), in embryonic stem cells (ESCs). We also integrated data for PRC2 components and the PRC2-catalyzed modification, trimethylated H3K27 (H3K27me3). H2Aub1 localizes to silent developmental gene promoters, nearly all of which are also enriched for PRC1 and PRC2. H2Aub1 deposition and PRC1 localization were shown to depend on two parallel pathways. The first pathway is PRC2-dependent and involves recognition of H3K27me3 by Cbx2 component of PRC1. The second is independent of PRC2 and instead depends on the catalytic activity of Ring1 at key PRC1 target genes. We propose that these complementary mechanisms combine to repress critical differentiation genes in ESCs. Such combined mechanisms may be instrumental in keeping Polycomb-mediated repression at permissive levels to undergo developmental programs.

Subnuclear architecture involved in mediation of transcriptional silencing (Isono et al., in preparation; Fujimura et al., in preparation)

Recent publications

1. Harada M, Murakami H, Okawa A, Okimoto N, Hiraoka S, Nakahara T, Akasaka R, Shiraishi Y, Futatsugi N, Mizutani-Koseki Y, Kuroiwa A, Shirouzu M, Yokoyama S, Taiji M, Iseki S, Ornitz DM, Koseki H.. FGF9 monomer-dimer equilibrium regulates extracellular matrix affinity and tissue diffusion. *Nat Genet.* 41, 289-298 (2009)
2. Endoh M., Endo T.A., Endoh T., Fujimura Y., Ohara O., Toyoda T., Otte A.P., Okano M., Brockdorff N., Vidal M., Koseki H. Polycomb group proteins Ring1A/B are functionally linked to the core transcriptional regulatory circuitry to maintain ES cell identity. *Development* 135,1513-1524 (2008)



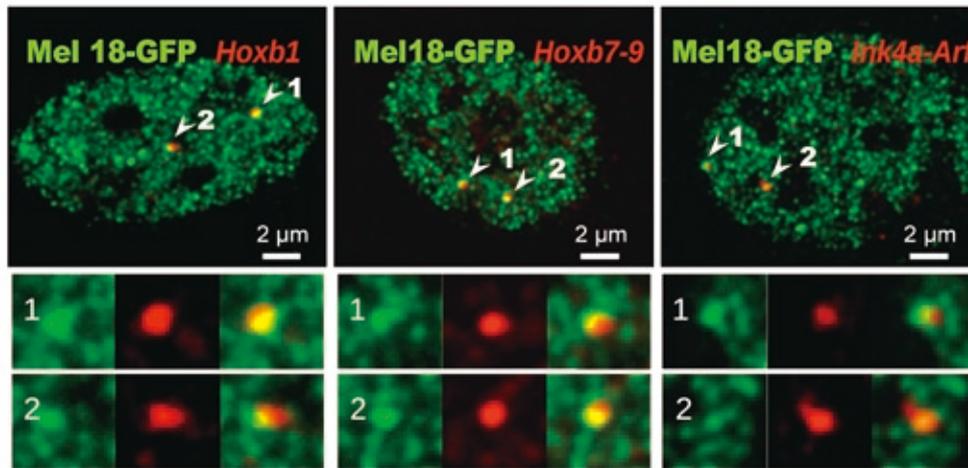


Figure: Association of *Hoxb1*, *Hoxb7-9* and *Ink4a-arf* genes with Polycomb bodies in embryonic fibroblasts (MEFs). Immuno-FISH analysis was performed by using MEFs from *Mel18-GFP* knock-in mice. Polycomb bodies represented by distribution of Mel18-GFP fusion protein (Green) were shown to colocalize with *Hoxb1*, *Hoxb7-9* or *Ink4a-arf* genes (Red) in the lower panels.

Compartmentalization in eukaryotic nuclei is important for separation and coordination of various functions that proceed simultaneously within a single nucleus. We therefore took another approach based on imaging techniques, in parallel with the above genomic approach, to understand the underlying mechanisms for Polycomb repression. PRC1 components have been shown to exhibit focal accumulations that are designated “Polycomb bodies” in various tumor cell lines, although the functions of these structures in Polycomb repression are totally unknown. We have for the first time identified Polycomb bodies in primary cells and clarified their molecular nature by combining genetic and imaging approaches. We found the role of PRC1 is to condense *Hoxb* clusters into a single Polycomb body and mediate the repression (Fig.). We further identified the role of SAM domains of polyhemic homologues to mediate Polycomb body formation by linking multiple PRC1 complexes via homophilic interactions. Polycomb body formation was further suggested to be a process to enhance recruitment of PRC1 to polycomb target genes. This study therefore identified an additional layer of Polycomb repression and potentially provides new insight into epigenetic regulation of cellular differentiation.

In the process of affinity purification of PRC1, we have repeatedly co-purified α - and β -tubulins, historically known as the principal components of microtubules. By immuno-

fluorescence analysis of ESCs after extraction by detergents and high salt concentrations, we identified a fraction of tubulins that remained persistently associated with the nuclear lamina and exhibited a filamentous structure. ChIP-chip analysis for tubulins and Lamin-B, a major component of nuclear lamina, revealed significant co-occupancy of their target genes in ESCs and thus confirmed the interaction of tubulins with the nuclear lamina. Using Vinblastin to depolymerize tubulins, we found that the lamina-associated tubulins are highly polymerized. Immuno-FISH analysis for several genes that were bound by both tubulins and Lamin-B, revealed that they were relocated from the nuclear periphery to the nuclear interior. This is accompanied by dissociation of these genes from Lamin-B, as shown by ChIP assay. These observations indicated that the role of tubulins is to link their targets to the nuclear lamina. Interestingly, tubulins extensively colocalized with HDACs at the nuclear lamina and this accumulation of HDACs depends on tubulin polymerization. Our data suggest that tubulins act as a scaffold for the convergence of chromatin-HDAC-nuclear lamina, and are necessary for maintaining the hypoacetylated status of genes that associate with the nuclear lamina.

3. 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. The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. *Nature* 450, 908-912 (2007)

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

5. 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) Nucleotide-sugar transporter

SLC35D1 is critical to chondroitin sulfate synthesis in cartilage and skeletal development in mouse and human. *Nat Med.* 13, 1363-1367 (2007)

Team Leader

Hiroshi Kawamoto

Research Scientists : **Tomokatsu Ikawa**
Kiyokazu Kakugawa
Kyoko Masuda

Research Associate : **Rumi Satoh**

Technical Staff : **Asako Shibano**
Yuri Suzuki
Chiho Matsuura

Student Trainee : **Johanne Angelina Jacobus Huijskens**

Administrative Staff : **Yuko Ochi**

Visiting Scientists : **Yoshimoto Katsura**

Laboratory for Lymphocyte Development

During hematopoiesis, pluripotent hematopoietic stem cells (HSC) are sequentially restricted 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 lymphoid progenitors and the ontogeny and phylogeny of T- and B-cell development.

Lineage commitment of hematopoietic stem cells toward unipotent T cell progenitors

T cells are generated from multipotent hematopoietic stem cells through a series of differentiation steps. Recent studies including ours (Lu et al, *J.I.*, 2002) have indicated that the first step in this pathway is the generation of progenitors that have lost erythroid/megakaryocyte potential but retain the capacity to generate other hemopoietic cells including myeloid, T and B cells. We have recently identified the next stage beyond these myelo-lymphoid progenitors, which consists of T cell progenitors that have lost B cell potential but are still able to generate myeloid cells, DC and NK cells (Wada et al, *Nature*, 2008). Therefore, the most critical step for construction of the T cell lineage is now thought to be at the point where myeloid potential is terminated.

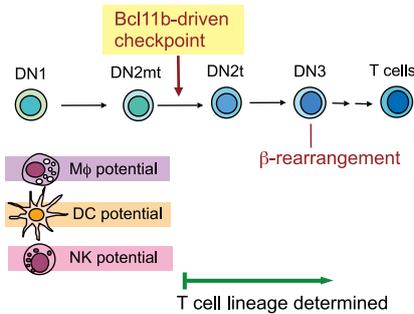
We have previously shown that the DN2 stage can be subdivided into two stages based on GFP expression controlled by the proximal Ick (plck) promoter. Cells in the earlier, GFP⁻ stage retain non-T lineage potential, including that for DC and NK cells, whereas the latter stage GFP⁺ cells are determined to the T cell lineage (Masuda et al, *J. I.* 2007). We have further shown that DN2 cells still retain myeloid potential (Wada et al, *Nature*, 2008). Here we designate these two stages DN2mt (myeloid-T) and DN2t (T-lineage determined), and term the step between these stages the DN2-determination step. Such a T cell lineage determination step is thought to be the first critical checkpoint in T cell development.

Recent publications

1. Moro K., T. Yamada, M. Tanabe, T. Takeuchi, T. Ikawa, H. Kawamoto, J.-I. Furusawa, M. Ohtani, H. Fujii, and S. Koyasu. Innate production of Th2 cytokines by adipose tissue-associated c-Kit+Sca-1+ lymphocytes. *Nature* 463: 540-544, (2010).
2. Kakugawa K., T. Yasuda, I. Miura, A. Kobayashi, H. Fukiage, R. Satoh, M. Matsuda, H. Koseki, S. Wakana, H. Kawamoto*, H. Yoshida*. (*correspondence) A novel gene essential for the development of single positive thymocytes. *Mol. Cell. Biol.* 29:5128-5135, (2009).



A Normal T cell development



B DLL4/IL-7 culture or Bcl11b^{-/-} progenitors

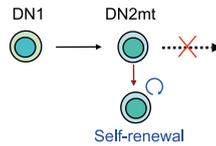


Figure 1: A novel T cell developmental checkpoint in the midst of the DN2 stage is regulated by Bcl11b. (A) Model of normal T cell lineage determination. Non-T lineage potential, including that for myeloid, DC, and NK lineages, of T cell progenitors is terminated during the DN2 stage, subdividing this stage into DN2mt and DN2t stages. Bcl11b is required for the DN2mt cells to pass through this checkpoint. (B) Sustained IL-7 signaling or Bcl11b deficiency induces developmental arrest at the DN2mt stage. The arrested DN2mt cells acquire self-renewal ability and maintain the potential to generate macrophages, NK cells and dendritic cells.

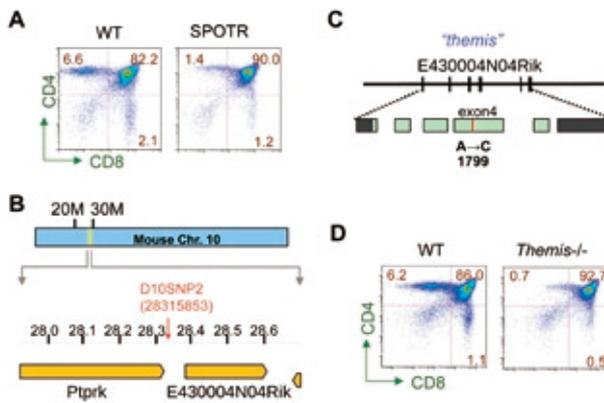


Figure 2: Identification of novel gene essential for the development of single positive thymocytes. (A) Establishment of ENU-induced mutant line SPOTR that shows reduction in T cell numbers. CD4 versus CD8 profiles of thymocytes from an 8 week-old wild type and SPOTR mouse. (B) Genetic mapping to narrow down genes responsible for the SPOTR phenotype. A total of 254 mice were examined by genetic mapping, and the responsible loci were mapped to the 0.39 centiMorgan (cM) region including D10SNP2 (shown as a red arrow on the expanded chromosome region). Calculating that 1cM \approx 2Mbp, a 0.78Mbp region was predicted to contain the candidate loci (yellow on chromosome 10). Three genes in this region in the order *Ptprk*, *E430004N04Rik* and *2310057J18Rik* are indicated, with the direction of transcription indicated by the pointed end of the rectangular bars. (C) A missense mutation was found in exon 4 of the *E430004N04Rik* gene, A1799C, leading to the amino acid substitution T512P. The coding regions of the *E430004N04Rik* gene are indicated in light green and the UTRs are indicated in black. (D) *Themis* deficient mice showed a phenotype similar to that of SPOTR mice. CD4 versus CD8 profiles of thymocytes from a 4 week old wild type and *themis* deficient mouse are shown.

We recently found that when murine fetal liver progenitors were cultured on immobilized Notch ligand DLL4 in the presence of a cytokine cocktail including IL-7, the cells were arrested at the DN2mt stage. The arrested DN2 cells did not die but retained the capacity for self-renewal as well as the potential to generate myeloid, dendritic, and NK cells. These self-renewing DN2 cells were induced to T cell lineage determination by simply reducing the concentration of IL-7, eventually giving rise to CD4⁺CD8⁺ T cells under feeder-free culture conditions. A similar arrest and self-renewal of progenitors were observed in thymocytes of mice deficient in the transcription factor Bcl11b. Our study thus identifies the earliest checkpoint of T cell development and demonstrates that it is Bcl11b-dependent (Ikawa et al, submitted).

A novel gene essential for the development of single positive thymocytes

A critical step during intrathymic T cell development is the transition of CD4⁺CD8⁺ double positive (DP) cells to the MHC I-restricted CD4⁺CD8⁺ and MHC II-restricted CD4⁺CD8⁻ single positive (SP) cell stage. In collaboration with the Immunogenetics Laboratory (Yoshida TL), we identified a novel gene that is essential for this process (Kakugawa et al,

MCB, 2009). Through T cell phenotype-based screening of N-ethyl-N-nitrosourea (ENU)-induced mutant mice, we established a mouse line in which numbers of CD4 and CD8 SP thymocytes as well as peripheral CD4 and CD8 T cells were dramatically reduced. We named this mouse line SPOTR (Single Positive Thymocyte Reduction). Using linkage analysis and DNA sequencing, we identified a missense point mutation in a gene, *E430004N04Rik*, which does not belong to any known gene family. This orphan gene is specifically expressed in DP and SP thymocytes and peripheral T cells, whereas in mutant thymocytes, the levels of protein encoded by this gene were drastically reduced. We generated *E430004N04Rik*-deficient mice and their phenotype was virtually identical to that of the ENU mutant mice, thereby confirming that this gene is essential for the development of SP thymocytes. While we were in the process of manuscript submission, we noted that several other groups have also found this gene and named it "*themis*", therefore we decided to use this name. Since *themis* is a totally unknown gene, whose functional domains are so far unknown, further studies on its role will reveal novel mechanisms in T cell development.

3. Kawamoto, H. and Katsura, Y. A new paradigm for hematopoietic cell lineages: revision of the classical concept of the myeloid-lymphoid dichotomy. *Trends in Immunology*. 30: 193-200, (2009).

4. Wada H, Masuda K, Satoh R, Kakugawa K, Ikawa, T, Katsura Y, Kawamoto H. Adult T cell progenitors retain myeloid potential. *Nature*, 452: 768-772, (2008)

5. Masuda K, Kakugawa K, Nakayama T, Minato M, Katsura Y, Kawamoto H. T cell lineage determination precedes the initiation of TCRbeta gene rearrangement. *J. Immunol*. 179: 3699-3706, (2007)

Unit Leader
Andrei Rybouchkin

Research scientist : **Aleksey Menzorov**

Technical Staff : **Raul Sakoda**

Research Unit for Lymphocyte Cloning

iPS cells are considered as one of the most advanced and promising tools for personalized and regenerative medicine. The potential of these cells will not be realized, however, until development of safe and efficient methods for their application to the treatment of diseases. It is generally accepted that before transplantation into patients, iPS cells have to be differentiated into the terminally differentiated, functional cells of a particular type, or, alternatively, into adult stem cells such as mesenchymal stem cells (MSC) or hematopoietic stem cells (HSC), which, after transplantation would differentiate into the desired cells after homing to the proper location. Taking this second approach, we are attempting to develop methods to differentiate human and mouse iPS cells into MSC, as MSC may be instrumental in formation of a supportive environment for survival of transplanted HSC and their function of regeneration of the immune system. MSC, and their derivatives the stromal cells, are also thought to modulate inflammatory responses and contribute to regeneration of many tissues by production of multiple growth stimulatory cytokines.

Developing a protocol for differentiation of human iPS into MSCs or mesenchymal precursors.

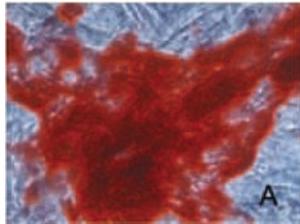
In our studies we used human iPS cell lines derived in the RIKEN BioResource Centre and in the first stage applied several recently published protocols that reportedly were effective in differentiation of human ES cells into MSCs or their precursors. Two out of four tested protocols resulted in the robust production of the cell lines with features similar, but not identical to, donor bone marrow derived MSC (BM-MSC). The cell lines have a very similar surface antigen phenotype and readily differentiated into osteoblasts in osteogenic medium (Alizarin Red staining, Fig.1A), but were unable to differentiate into adipocytes in the adipogenic medium (Oil Red O staining, Fig.1B). We concluded that the lines we received are lines of osteoprogenitor (OP) cells. As reported by others, OP lines can organize hematopoietic microenvironments (Sacchetti et al., 2007 Cell) and one of our ideas was to try to test our iPS derived OP lines for this function. In the meantime, we continued our search for a protocol for differentiation of our human iPS cell lines into

Recent publications

1. A Rybouchkin, Y Kato, Y Tsunoda. Role of histone acetylation in reprogramming of somatic nucleus following nuclear transfer. *Biol. Reprod.* 2006, 74: 1083-1089.
2. Watarai H, Rybouchkin A, Hongo N, Nagata Y, Sakata S, Sekine E, Dashtsoodol N, Tashiro T, Fujii S, Shimizu K, Mori K, Masuda K, Kawamoto H, Koseki H, Taniguchi M. Generation of functional NKT cells in vitro from embryonic stem cells bearing rearranged invariant Valpha14-Jalpha18 TCRalpha gene.



Osteogenic



Adipogenic

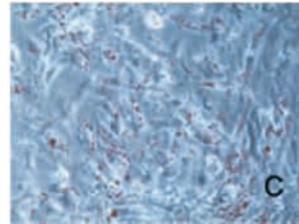
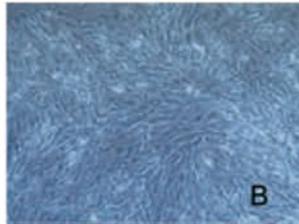


Figure 1: Differentiation of hiPS derived osteoprogenitors into (A) osteoblasts, but not into (B) adipocytes. (C) Differentiation of mesenchymal precursors derived from the same iPS line by other protocol into adipocytes.

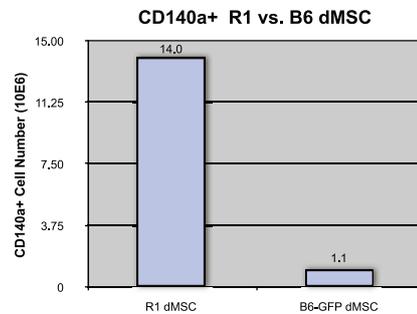
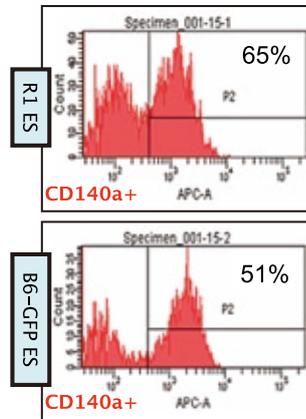


Figure 2: Percentage of CD140a⁺ mesenchymal precursors and total number of these cells derived by the same differentiation protocol from R1 (129) and B6 ES cell lines on differentiation day 9.

authentic MSC. Our last month results have finally resulted in the lines capable of differentiating into adipocytes (Fig. 1C). We hope to continue to fine-tune this protocol even after closure of this research unit on March 31, 2010.

Factors affecting differentiation of mouse ES and iPS cells into MSC

We recently derived several mouse iPS cell lines from the C57Bl/6J (B6) mouse embryonic fibroblasts. This strain was chosen because it is one of the most frequently applied strains for genetic and particularly immunological studies, and a mouse model of iPS to MSC differentiation would allow us to test properties of iPS derived cells in preclinical cell transplantation trials. However, there was no reported protocol for differentiation of B6 ES cells into MSC. Moreover, several published studies have reported significant difficulties in culture of bone marrow derived adult MSC recovered from this strain of mice.

We first tested a published ES to MSC precursors (MP) differentiation protocol on the 129 strain-derived ES cell line R1. We obtained robust results in most of the experiments. Cells were positive for MP surface markers (CD140a), readily expandable, and capable of differentiating into adipocytes and osteoblasts in the appropriate induction media. The same protocol worked very poorly for the B6 strain ES cell line. The proportion of CD140a positive cells was similar to the R1 cell cultures, but the total number of cells and therefore the number of positive cells was much lower (Fig 2). More importantly sorted CD140a positive cells had very

poor expansion potential and less efficient differentiation potential. Furthermore, results of differentiation experiments varied greatly from experiment to experiment for no obvious reasons. We have tried several approaches to improve this differentiation protocol, including varying the concentrations of serum (10% vs 20%), the differentiation inducing agent (0, 100 and 500nM of retinoic acid), and cells seeded at the start of differentiation. We also studied the effects of the survival stimulating agent dbcAMP the oxygen concentration (5% vs 20%) on cell differentiation and/or expansion of sorted cells. The sorting date of CD140a positive cells from heterogeneous starting population (day 9 versus day 11 and 13) have also been analyzed. Finally, we have also tried to identify the reasons for the alarming rate of experiment to experiment variation by testing effects of B6 ES cell culture media (DMEM based versus IMDM based), feeder cell density, as well as ES cell confluency level and passage numbers.

We have come up with a semi-optimized protocol allowing us to obtain high numbers of B6 ES derived MP cells in most experiments. Expansion of the resulting cells has remained, however, a significant problem. Interestingly, after applying the new protocol to our B6 iPS cell lines, we found out that MP from these lines are more readily expandable than their B6 ES-derived counterparts. It would be of interest to investigate whether better expandability of iPS derived MP is related to a higher level of leaky expression of pluripotency inducing transgenes such as c-myc or flk4.

Team Leader

Ichiro Taniuchi

Research Scientists : **Hirokazu Tanaka**

Taku Naito

Wooseok Seo

Mari Tenno

Technical Staff :

Sawako Muroi

Chizuko Miyamoto

Risa Chihara

Kazuko Kosuge

Student Trainee :

Sebastian Nieke (IPA)

Administrative Staff : **Aiko Iyama**



Laboratory for Transcriptional Regulation

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 a specific gene expression pattern is established during a commitment process as the cell becomes fully differentiated. We are addressing these questions by studying transcription factor networks regulating T lymphocyte development. In particular, we have been studying the transcriptional regulation of lineage choice by CD4⁺CD8⁺ double-positive (DP) thymocytes differentiating into either CD4⁺ helper- or CD8⁺ cytotoxic-lineage T cells.

Our previous studies revealed that antagonistic interplay between two transcription factors, the Runx transcriptional complexes and *ThPOK*, plays a central role in the transcription factor network that governs fate determination by DP thymocytes. While expression of the *ThPOK* is essential for helper T cell development, in part via antagonizing Runx-mediated gene regulation, repression of *ThPOK* expression by Runx complexes via activating the transcriptional silencer in the *ThPOK* locus is required for cytotoxic T cell development. We are expanding our findings to understand how expression of the *ThPOK* gene is regulated and how Runx complexes are involved in immune system development.

Mechanism regulating *Ztb7b/ThPOK* gene expression

The specificity of TCRs for self MHC molecules correlates well with the outcome of lineage decision by DP thymocytes. Cells expressing a class I-restricted TCR differentiate primarily into the CD8⁺ cytotoxic-lineage, whereas cells expressing a class II-restricted TCR differentiate into the

Recent publications

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2. Muroi S., Naoe Y., Miyamoto C., Akiyama K., Ikawa T., Masuda K., Kawamoto H., Taniuchi I. Cascading suppression of transcriptional silencers by ThPOK seals helper T cell fate. *Nat. Immunol.* 9, 1113-21 (2008)

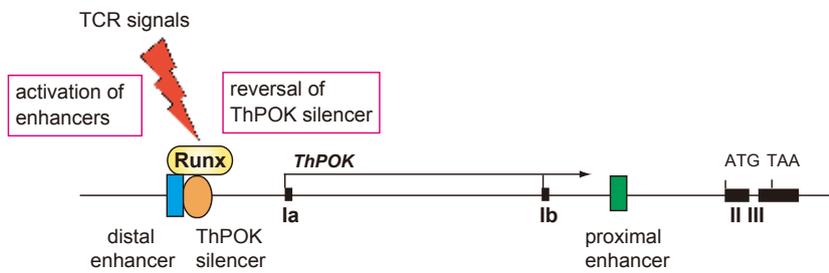


Figure 1: Cis-regulatory elements regulating expression of the *ThPOK* gene. The expression of *ThPOK* gene during T cell development is primarily regulated by the combined activation and inactivation of the silencer and the two enhancers. Binding of the Runx complexes to the silencer is essential, but not sufficient, to activate the silencer. In order to initiate *ThPOK* gene expression upon TCR engagement, both reversal of the silencer function and activation of the enhancers are necessary.

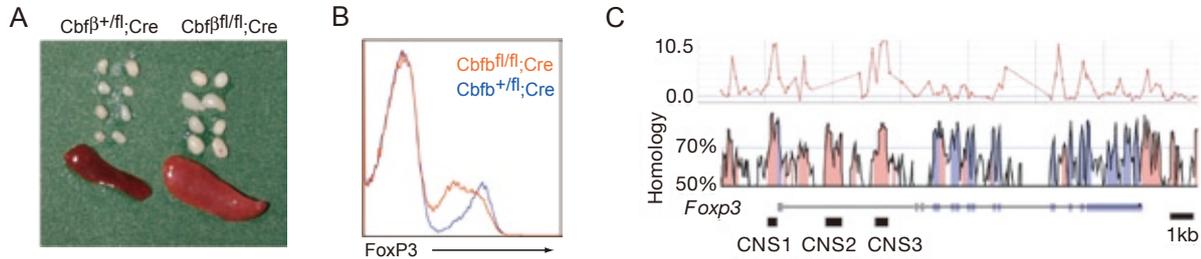


Figure 2: Autoimmune disease development following loss of the Runx1/Cbfb complex in regulatory T cells. (A) Splenomegaly and lymphadenopathy in 8-week-old mice resulting from inactivation of the *Cbfb* gene in FoxP3⁺ cells. (B) Reduced expression of FoxP3 protein in T_{reg}s after loss of *Cbfb* protein. (C) Binding of the Runx1/Cbfb complex to the regulatory regions, depicted as conserved non-coding sequences (CNS), in the *FoxP3* locus.

CD4⁺ helper-lineage. However, it remains unclear how differences in TCR signaling are sensed and integrated into developmental programming in the cell nucleus of post-selection thymocytes. Because expression of *ThPOK* is both essential and sufficient to induce helper-lineage fate, regardless of the MHC specificity of the thymocyte TCR, understanding of the mechanism that regulates helper-lineage specific expression of the *ThPOK* gene will be pivotal to address this issue. Having identified a transcriptional silencer (termed as the *ThPOK* silencer) as an essential cis-regulatory element for helper-lineage specific expression of the *ThPOK* gene, we are attempting to unravel a molecular mechanism regulating activity of the *ThPOK* silencer. Our unpublished results obtained by modifying the activity of the *ThPOK* silencer suggest that reversal of its silencer activity by an as yet uncharacterized mechanism is necessary for expression of the *ThPOK* gene in class II-selected cells. In order to identify molecules that determine specificity of the *ThPOK* silencer function, we are isolating protein complexes that bind specifically to the *ThPOK* silencer.

In addition to the reversal of the *ThPOK* silencer activity, activation of positive regulatory elements, such as enhancers and the promoter, is also necessary for expression of the *ThPOK* gene. We have identified two enhancers: a distal enhancer located in the 5' region proximal to the silencer in the *ThPOK* locus, and a proximal enhancer downstream of exon Ib. While the distal enhancer acts mainly to initiate *ThPOK* gene expression in post-selection thymocytes, the

proximal enhancer is necessary to amplify and maintain its expression at the later developmental stage. Removal of both of these enhancers from the *ThPOK* locus abrogates expression of the *ThPOK* gene in CD4⁺ T cells, but not in B cells, revealing not only their redundant functions but also their T lymphocyte specific functions.

Further studies on the molecular control of these regulatory elements in the *ThPOK* gene will advance our understanding of how cellular signaling initiated by external stimuli are converted into genetic programs in the cell nucleus.

Role of Runx complexes in regulating function of FoxP3⁺ regulatory T cells.

FoxP3⁺ naturally occurring regulatory T (T_{reg}) cells play essential roles in the maintenance of immunological self-tolerance and immune homeostasis by actively suppressing aberrant or excessive immune responses. By using T_{reg} specific Cre transgenic mice, we show that inactivation of the *Runx1* or the *Cbfb* gene in FoxP3⁺ cells results in spontaneous development of autoimmune disease with an attenuated expression of FoxP3. Our chromatin immunoprecipitation assay revealed that the Runx1/Cbfb complex binds to regulatory regions in the *FoxP3* locus. Thus, the Runx1/Cbfb complexes are indispensable for *in vivo* suppressive function of T_{reg} cells in part via regulating expression of the *FoxP3* gene.

- Maekawa Y., Minato Y., Ishifune C., Kurihara T., Kitamura A., Kojima H., Yagita H., Satake-Yanagimoto M., Saito T., Taniuchi I., Chiba S., Sone S., Yasutomo K. Notch 2 integrates signaling by the transcription factors RBP-J and CREB1 to promote T cell cytotoxicity. *Nat. Immunol.* 9, 1140-47 (2008)
- Collins A., Littman D.R., Taniuchi I. RUNX proteins in transcription factor networks that regulate T-cell lineage choice. *Nat. Rev. Immunol.* 9, 106-15 (2009)
- Kitoh A., Ono M., Naoe Y., Ohkura N., Yamaguchi T., Yaguchi H., Kitabayashi I., Tsukada T., Nomura T., Miyachi Y., Taniuchi I., Sakaguchi S. Indispensable role of the Runx1-Cbfb transcription complex for *in vivo* suppressive function of FoxP3⁺ regulatory T cells. *Immunity.* 3, 609-20 (2009)

Group Director
Takashi Saito

Senior Scientist : **Tadashi Yokosuka**

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

Technical Staff : **Wakana Kobayashi,
Machie Sakuma, Masako Takamatsu,
Akiko Takumi, Midori Unno**

Administrative Staff : **Shihoko Kato, Hiroko Yamaguchi**

Visiting Scientists : **Hu Zeng, Ikuo Ishige,
Noriko M. Tsuji, Sho Yamasaki**

RCAI Central Facility FACS Laboratory :
Hanae Fujimoto, Yukiko Hachiman

RCAI Central Facility Confocal Laboratory :
Yasutaka Wakabayashi (Leica)

RCAI Central Facility mAb Laboratory :
Tomomi Aoyama, Mayuko Matsuda



Laboratory for Cell Signaling

The long-term goal of the Cell Signaling group is to determine the molecular mechanisms of activation, migration and homeostasis of T cells in order to be able to modulate T cell activation/function in immunological disorders. Therefore, our group is involved in a range of projects to analyze the basic mechanisms of antigen recognition, activation, differentiation, and functional regulation of T cells from the viewpoint of signal transduction. Particularly, the group has been using real-time imaging analysis, which has provided new insights into the dynamic regulation of the assembly of the TCR synapse and the immunological synapse, as well as related downstream signaling pathways upon antigen recognition. These include regulation of cytoskeleton and co-stimulation signals. The group also analyzes regulation at later phases of T cell activation for cell migration and establishment of peripheral antigen-specific effector cell functions.

Dynamic regulation of T cell activation and co-stimulation

We have 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 combination of imaging techniques, we could visualize the behavior of signaling molecules during the dynamic process of T cell activation. This visualization has provided a new insight that microclusters (MC) containing TCR, kinases and adaptors are generated upon stimulation and function as the minimal signaling unit responsible for the initial and sustained activation of T cells. Based upon the discovery of TCR-MCs, we had to revise the current models for the mechanism of T cell activation including co-stimulation.

T cell activation upon antigen recognition requires both antigen recognition signals through the TCR and co-stimulation signals through co-stimulatory receptors. The precise relationship between these two signals is still unclear. We have analyzed signal regulation through positive and negative co-stimulation receptors. For the major co-stimulation signals through CD28, we found that CD28 is accumulated

Recent publications

1. Yokosuka, T and Saito, T.: Dynamic regulation of T-cell costimulation through TCR-CD28 microclusters. *Immunol. Rev.* 229, 27-40 (2009)
2. Takeuchi A, Itoh Y, Takumi A, Ishihara C, Arase N, Yokosuka T, Koseki H, Yamasaki S, Takai Y, Miyoshi J, Ogasawara K, and Saito T.: CRTAM confers late-stage activation of CD8⁺ T cells to regulate retention within lymph node. *J. Immunol.* 183, 4220-4228 (2009)

Differential signaling function of TCR-MCs and c-SMACs

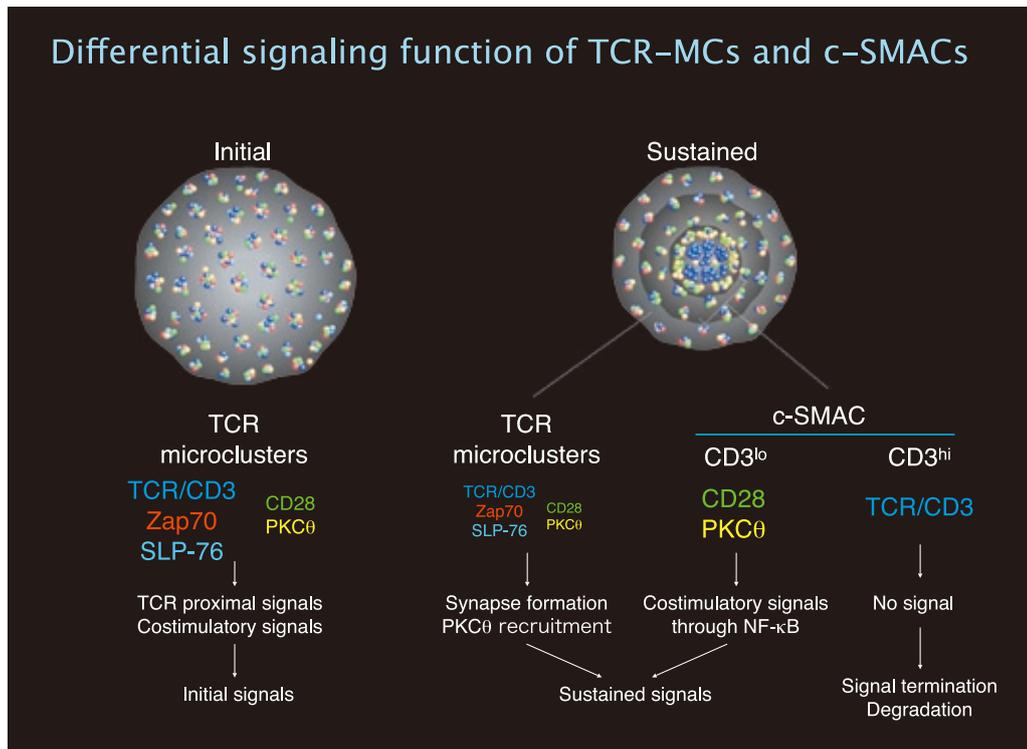


Figure : Spatially distinct regulation of T cell co-stimulation : CD28- and CTLA-4-mediated positive/negative costimulation signals through the “signaling cSMAC”. There are two regions within the cSMAC; CD3^{hi} and CD3^{lo}. Co-stimulatory receptors CD28 and CTLA-4 are accumulated and compete for the ligand binding in the CD3^{lo} area (signaling cSMAC). CD28 recruits and associates with PKCθ to this region to mediate costimulation signals while CTLA-4 inhibits CD28-PKCθ accumulation.

in a special co-stimulation regulatory region in the c-SMAC (signaling cSMAC), and recruits and associates with PKCθ in this region to induce co-stimulation signals. We also found that the negative co-stimulation receptor CTLA-4 is also accumulated and mediates its inhibitory function by competing for binding of the same ligands as CD28. Thus, we found the regulation mechanism of co-stimulation signals as “signal 2” through a spatially distinct region of the cSMAC from that of the TCR-signal mediating MCs. (Fig.)

Furthermore, to analyze mutual activation regulation between innate and acquired immunity, we have analyzed the function of TLRs expressed on T cells. We found that TLR-mediated signals function in T cell co-stimulation. In these analyses, we found that nucleic acids induce co-stimulation independently of TLRs, and therefore are attempting to identify the nucleic acid-recognizing sensors on T cells and the regulation of T cell activation through these receptors.

Regulation of T cell dynamics through adhesion molecules

We have identified CRTAM as a gene that is induced early during T cell activation, and Necl2 as the CRTAM ligand. CRTAM is specifically expressed on activated CD8⁺T cells and NK cells whereas Necl2 is expressed on epithelial cells and DCs. CRTAM binds and functions through heterotypic interaction with Necl2. Analysis of CRTAM-deficient mice revealed a reduction of effector T cell function in tumor rejection, DTH response, and virus infection. We found that CRTAM regulates the retention of activated T cells upon interaction with DCs within the lymph node where immune responses are induced. This system thus regulates maturation and translocation of effector T cells.

3. Yamasaki S., Matsumoto M., Takeuchi O., Matsuzawa T., Ishikawa E., Sakuma M., Tateno H., Uno J., Hirabayashi J., Mikami Y., Takeda K., Akira S., and Saito T. C-type lectin Mincle is an activating receptor for pathogenic fungus, *Malassezia*. *Proc. Natl. Acad. Sci. USA*. 10, 1897-1902 (2009)

4. Yokosuka T., Kobayashi W., Sakata-Sogawa K., Takamatsu M., Hashimoto-Tane A., Dustin M.L., Tokunaga M., Saito T. Spatiotemporal regulation of T cell costimulation by TCR-CD28 microclusters through protein kinase C θ translocation. *Immunity*. 29, 589-601 (2008)

5. Yamasaki S., Ishikawa E., Sakuma M., Ogata K., Saito T. Mincle is an ITAM-coupled activating receptor that senses damaged cells. *Nat. Immunol.* 9, 1179-1188 (2008)

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

Unit Leader

Kumiko Sakata-Sogawa

Technical Staff : **Yoshiyuki Sasaki**

Research Unit for Single Molecule Imaging

Single molecule approaches enable us to capture transient intermediates and heterogeneous behavior avoiding ensemble averaging. This ability is powerful in elucidating mechanisms of cellular functions: we can determine which molecule interacts with what, when, where, and how it works in living cells. Thus fluorescence imaging and quantitative analysis of single molecules are valuable methods to study the individual behavior of biological systems.

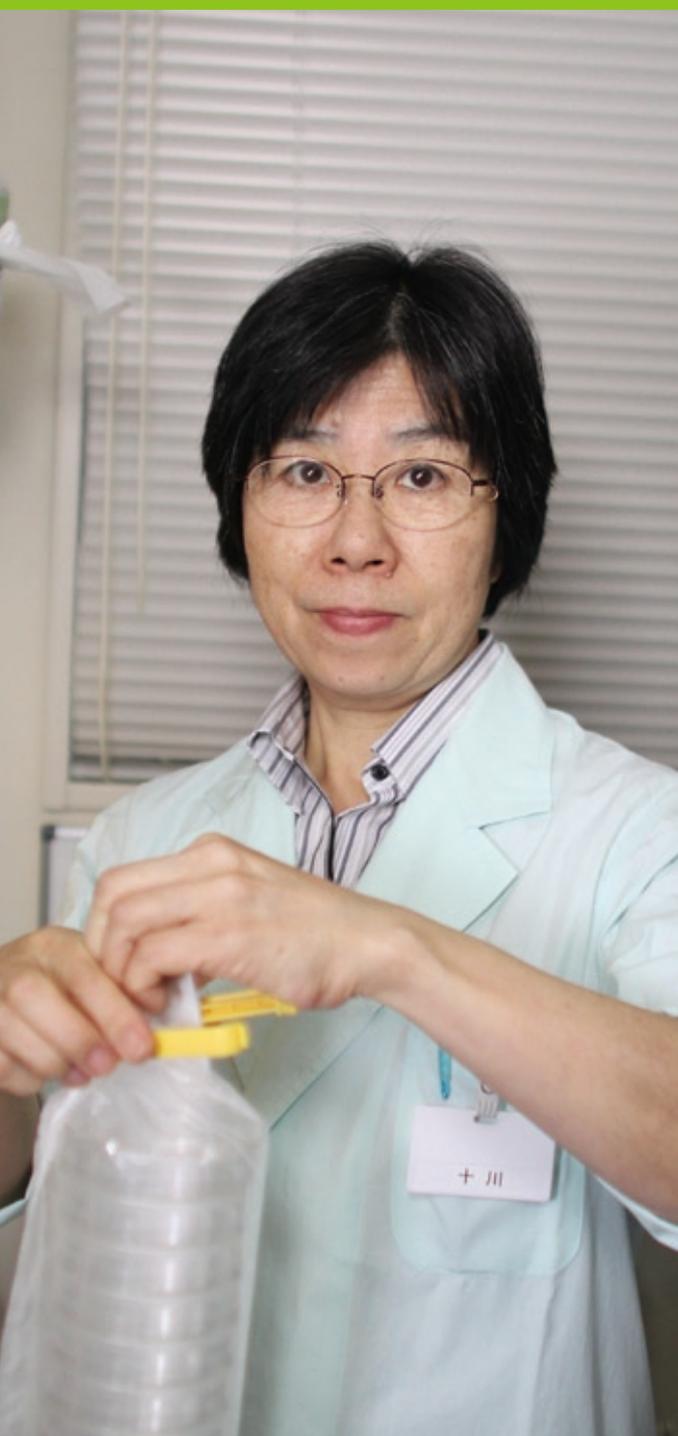
The main goal of the Research Unit for Single Molecule Imaging is to elucidate immunological responses and signaling processes with the technique of single molecule imaging and quantification. To this end we installed a microscope system and we have focused on the development of novel types of fluorescence microscopy (e.g., HILO) for use in single cell/single molecule studies. HILO has an advantage for making observation inside cells due to the high signal to noise ratio of 7.6. In addition, we are developing analysis software tools for imaging and quantitative analysis. We are also applying quantitative approaches to simulation of cell signaling. Our unit has a tight collaboration with the Research Unit for Molecular Systems Immunology for development of new microscopy techniques and analysis software.

Toward multi-color imaging of cell signaling

For quantitative analysis of the interaction of signaling proteins, it is important to observe multiple molecules simultaneously. To this end, we have established methods to obtain isogenetic cell lines expressing two fluorescence tagged proteins at homogenous and low level. Figure 1 shows examples of isogenetic CHO and Jurkat cell lines. An expression vector was designed to contain two sets of promoter and fluorescence-tagged protein sequences in tandem. To optimize the expression level of the fluorescence proteins for single molecule imaging, we selected suitable promoter sequences, and inserted insulator sequences if necessary. This method allows us to analyze interactions between two different molecules. We are expanding this technique to allow expression of three or more fluorescence-tagged proteins.

Recent publications

1. Miletic AV, Graham DB, Sakata-Sogawa K, Hiroshima M, Hamann MJ, Cemerski S, Kloeppe T, Billadeau DD, Kanagawa O., Tokunaga M. and Swat, W: Vav Links the T Cell Antigen Receptor to the Actin Cytoskeleton and T Cell Activation Independently of Intrinsic Guanine Nucleotide Exchange Activity. *PLoS One*. 4 (8), e6599 (2009)
2. Yokosuka T., Kobayashi W., Sakata-Sogawa K., Takamatsu M., Hashimoto-Tane A., Dustin ML., Tokunaga M., and Saito T.: Spatiotemporal Regulation of T Cell Costimulation by TCR-CD28 Microclusters and Protein Kinase C θ Translocation. *Immunity* 29, 589-601 (2008)



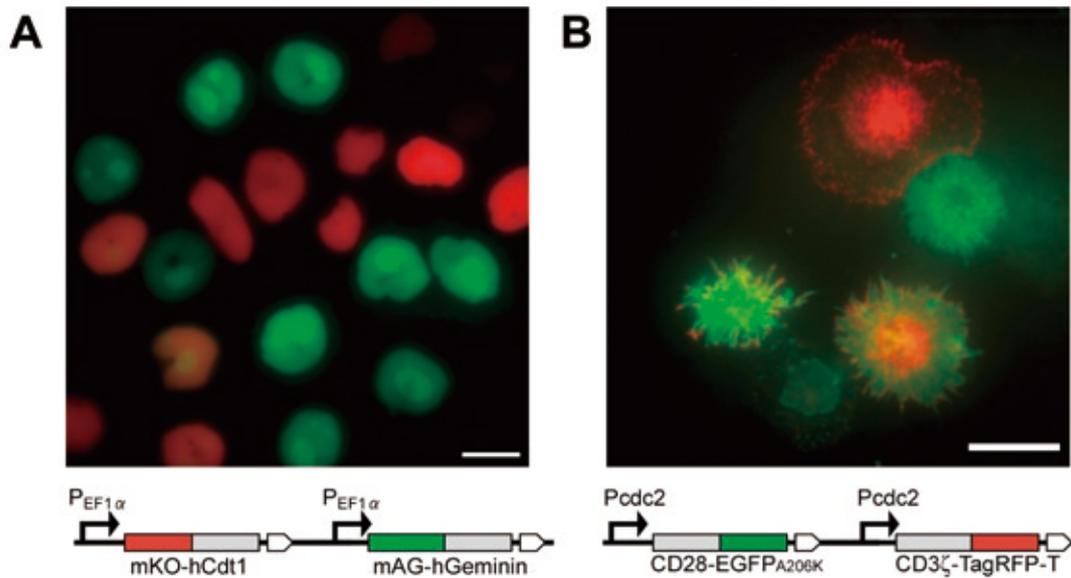


Figure: Control of protein expression levels by careful selection of promoter sequences. (A) CHO cell line expressing Fucci: fluorescent ubiquitination-based cell cycle indicator (combination of mKO2-Cdt1) and mAG1-Geminin) in the nucleus. (B) Jurkat cell line expressing CD28-EGFPA206K and CD3 ζ -TagRFP-T. The cells have been activated with immobilized anti-CD3 antibody. Bars: 10 μ m.

Dynamics of transcription factors in the nucleus

Transcription factors are known to be involved in regulation of target genes participating in specific signaling pathways. These protein factors bind to specific short sequences in DNA and associate with other activator proteins to regulate transcription. Although their association and regulatory function have been studied by biochemical approaches, many important features of their dynamics still remain to be elucidated.

Aiming at visualization of the dynamics of transcription factor proteins, we constructed Sp1-EGFP, a fusion protein of EGFP and the transcription factor Sp1, and prepared

CHO cell expressing low levels Sp1-EGFP. We confirmed that the Sp1 fusion protein was still function by testing its activity in a Luciferase assay. We next observed the cell nucleus using HILO microscopy, and obtained clear images of single Sp1-EGFP molecules. Trajectory analysis showed that there were several spots that single molecules visit repeatedly. Quantitative analysis revealed that there are two peaks in the distribution of Sp1 residence time, suggesting two modes of association, searching for specific sequences and binding. These results further our understanding of the mechanism of association of transcription factors to specific binding sites on DNA.

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Group Director

Tomohiro Kurosaki

Research Scientist : **Yuuich Aiba**
Hisaaki Shinohara
Kohei Kometani (SPDR)

Visiting Scientist : **Yoshihiro Baba**
Technical Staff : **Yoko Fujii**
Megumi Hamadate
Shiori Maeda

Administrative Staff : **Mari Kurosaki**



Laboratory for Lymphocyte Differentiation

Signals propagated through B cell receptors (BCRs), cytokine receptors, and chemokine receptors are crucial for the development of B lymphocytes and their subsequent antigen-triggered differentiation into memory B cells and antibody secreting plasma cells. The outcomes of these signaling events, for example, proliferation, differentiation, or cell migration, are dependent on the developmental stage of the cell and the quality of these signals. The latter is dictated by multiple factors including co-receptors, intracellular signaling/adaptor molecules, and transcription factors. Our laboratory has focused on understanding the molecular mechanisms of signaling pathways that lead to crucial cell fate decisions such as memory versus plasma cell differentiation.

Function of Erk kinases in B cell differentiation

B cell maturation can be divided into a series of developmental stages that are characterized by the differential expression of a variety of marker proteins and changes in the rearrangement status of the immunoglobulin heavy (*Igh*) and light (*Igl*) chain genes. *Igh* gene rearrangement is initiated during the pro-B cell stage, and the resulting μ heavy chain protein in association with $\lambda 5$ and VpreB forms a surface-expressed pre-B cell receptor (pre-BCR). Correct assembly of the pre-BCR triggers a signaling cascade that induces proliferation, downregulation of the pre-BCR complex, and differentiation into small pre-B cells. Previously, by using genetic deletion of both Erk1 and Erk2 kinases from early B cell developmental stages, we found that these kinases play a crucial role in regulating B cell development by initiating a transcription regulatory network and thereby pre-BCR-mediated cell expansion (Yasuda et al; *Immunity* 2008).

The above genetic strategy hindered the clarification of Erk's role in B cell immune responses, because in this settings peripheral B cells could not develop. Thus, by using inducible deletion of these kinases during immune responses, we found that Erk kinases are essential for generation of plasma cells. Introduction of a dominant-negative mutation in the Erk-mediated phosphorylation

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1. Hikida, M., Casola, S., Takahashi, N., Kaji, T., Takemori, T., Rajewsky, K. and Kurosaki, T. PLC- $\gamma 2$ is essential for formation and maintenance of memory B cells. *J. Exp. Med.* 206, 681-689 (2009).
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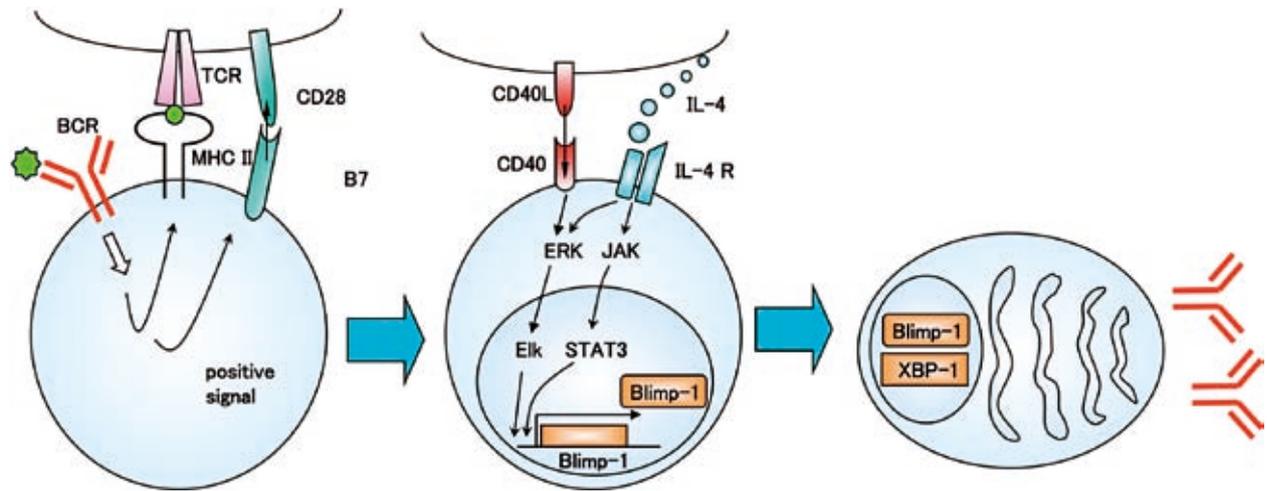


Figure : Follicular helper T cell-dependent activation of B cells and signaling pathways required for plasma cell differentiation. B cells expressing a B cell receptor (BCR) that binds antigen are activated, process the internalized antigen, and then present antigenic peptides in the context of MHC II proteins. Such activated B cells migrate to the interface between the B cell follicle and the T cell area of the secondary lymphoid organ in a chemokine-dependent manner. The interaction of antigen-specific B cells with its cognate follicular helper T cells triggers germinal center formation through the CD40L/CD40 interaction and the secretion of cytokines. Stimulation of B cells through CD40 and the interleukin-4 (IL-4) receptor strongly and immediately induces phosphorylation of Erk kinases. Phosphorylated Erk, then, translocates into the nucleus and promotes transcription of the *prdm1* gene, which encodes Blimp-1, through the activation of the Elk-1 transcription factor. JAK-mediated activation of Stat3 also positively regulates *prdm1* transcription. Blimp-1 is a transcriptional repressor that plays a crucial role, together with XBP-1, in the terminal differentiation of germinal center B cells into immunoglobulin-secreting plasma cells. Thus, Erk acts as a critical signal mediator for plasma cell differentiation through the regulation of Blimp-1 expression.

sites of Elk-1 suppressed IL-4/CD40-mediated Blimp-1 expression. Conversely, enforced expression of Blimp-1 in Erk-deficient B cells promoted plasma cell differentiation. Thus, in addition to a critical role of Erk kinases in early B cell development, these kinases participate in generation of plasma cells by connecting IL-4/CD40 and Blimp-1.

Localization of IgG type memory B cells

Humoral memory is characterized by recall immune responses that are more rapid than the primary response and by production of higher serum titers of antigen-specific antibodies, mostly of the IgG isotype. The prevailing view is that antigen-specific B cells are maintained as a pool of memory B cells after clonal expansion during the primary immune responses. Most memory B cells have been thought to originate from the germinal center (GC) reaction. In the GC, the combined processes of somatic hypermutation and selection based on the affinity of the BCR for the antigen, are responsible for the generation of high-affinity antibody variants which ultimately differentiate into long-lived plasma cells or long-lived memory B cells. In the GC reaction, de novo generated antigen-specific IgG memory B cells are

thought to acquire intrinsically different traits from their naive predecessors, accounting for faster and heightened secondary responses.

Although the importance of IgG memory B cells in long-term humoral memory has been well recognized, the lodging and activation sites of these cells remain elusive, in part, because of technical difficulties related to in situ detection of the rare IgG memory B cells specific for a given antigen. By using painstaking histochemical analysis and genetic approaches, we have shown that IgG type memory B cells are mainly located nearby the contracted germinal center (GC)-like structures and that these cells are indeed activated upon secondary antigen challenge. Based upon these observations, together with our evidence that CD4 T cells exist in close proximity with IgG memory B cells nearby the contracted GCs, we propose that this close proximity of IgG memory B cells and memory T cells can explain, at least partly, the rapid kinetics of memory responses. Unlike in the primary response, the movement of antigen-specific B cells and antigen-specific T cells toward the T-B border area and their subsequent interaction there would no longer be required.

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Team Leader
Ji-Yang Wang

Research Scientist : **Rika Ouchida**

Student Trainee : **Chie Kano**
Yingqian Li (IPA)

Technical Staff : **Hiromi Mori**

Administrative Staff : **Kanae Fukui**



Laboratory for Immune Diversity

Germinal center (GC) B cells represent a unique cell population that is induced during an immune response against foreign antigens. These rapidly dividing cells undergo dynamic genetic alterations including 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 our research is to understand how the activation-induced cytidine deaminase (AID) can induce a high frequency of A:T mutations in the Ig genes and to dissect the molecular mechanisms regulating B cell activation and terminal differentiation.

SHM is initiated by AID, which catalyzes the deamination of cytosine (C) to uracil (U) and generates a U:G lesion. One of the intriguing unanswered questions is how AID-triggered U:G lesions lead to a high proportion of mutations at non-damaged A:T base pairs. We have shown that GC B cells, but not other normal tissues/cell types, have an intrinsic property to generate A:T mutations. This property is dependent on the GC B cell environment but is independent of AID expression and the target gene location. We aim to isolate potential GC B-specific factors that are required to generate A:T mutations.

B cell activation and differentiation into antibody-producing plasma cells or memory B cells is a highly regulated complex process but the molecular mechanisms still remain poorly understood. We have identified a number of uncharacterized genes that are either differentially expressed during B cell activation or highly expressed in GC B cells, and have been analyzing their physiological roles by using gene-targeted mice. We have discovered a lysosomal protein that mediates the degradation of B cell antigen receptors (BCR) and negatively regulates B cell function. We have also identified a number of genes that appear to be involved in B cell activation and antibody production. Through further analyses of these gene-targeted mice, we hope to gain additional insights into the molecular mechanisms that control the survival, activation and terminal differentiation of B cells.

Recent publications

1. Ouchida, R., Kurosaki, T. and Wang, J.-Y. A role for LAPTM5 in the negative regulation of surface BCR levels and B cell activation. *J. Immunol.* 185: 294-301 (2010). (Featured in "In This Issue")
2. Shima, H. Takatsu, H., Fukuda, S., Ohmae, M., Hase, K., Kubagawa, H., Wang, J.-Y., Ohno, H. Identification of TOSO/FAIM3 as an Fc receptor for IgM. *Int. Immunol.*, 22: 149-156 (2010).

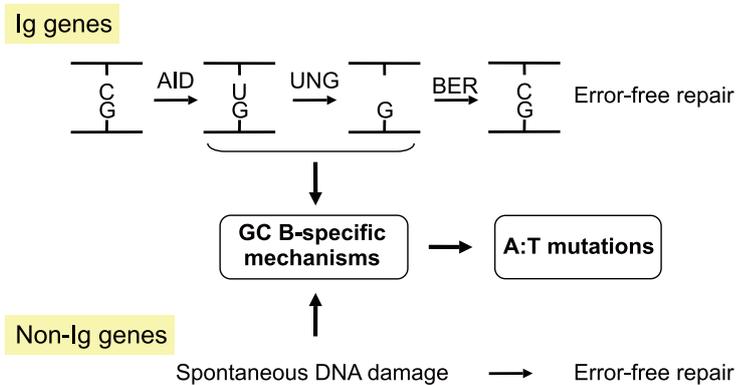


Figure 1: Pathways for A:T mutations in Ig and non-Ig genes in GC B cells. GC B cells have an intrinsic property to generate A:T mutations in Ig and non-Ig genes. In Ig genes, AID deaminates cytosine (C) to uracil (U) and generates a U:G lesion. Under normal situations, this U:G lesion is correctly repaired by the error-free base excision repair (BER) pathway. During Ig gene SHM, however, the U:G lesion can be resolved by a GC B-specific error-prone repair pathway, leading to mutations at non-damaged A:T base pairs. Using a lacZ-transgenic 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, likely utilizing a similar GC B-specific mechanism.

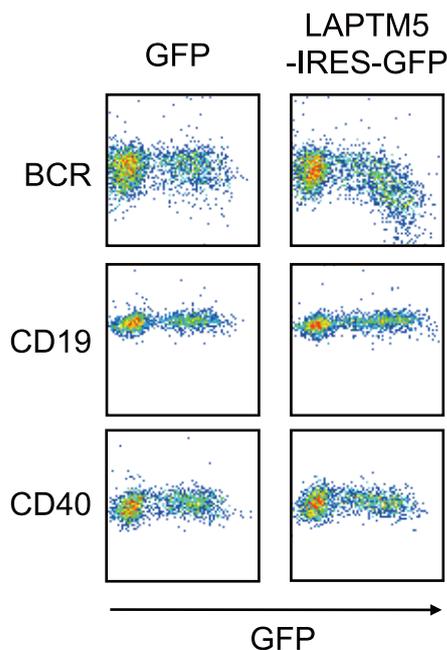


Figure 2: LAPT5 downmodulates cell surface BCR expression without affecting the levels of CD19 and CD40. Purified spleen B cells were stimulated with LPS (10 µg/ml) for 24 h and then transduced with retrovirus expressing GFP alone or LAPT5-IRES-GFP. The cells were further cultured for 2 days and then analyzed by FACS for the cell surface levels of BCR, CD19 and CD40.

Mechanism of A:T mutations in GC B cells

Using a sensitive *lacZ*-transgenic (*lacZ*-Tg) system to detect genome-wide mutations, we have found that GC B cells have an intrinsic propensity to mutate A:T pairs (Fig. 1). Further analysis revealed that the induction of A:T mutations is independent of AID and the target gene location. Experiments are now in progress to address the following three non-exclusive hypotheses for the high proportion of A:T mutations in GC B cells. 1) Rapid cell division of GC B cells interrupts the normal repair process and causes error-prone DNA synthesis. 2) GC B cells express a specific factor(s) that function to recruit POLH, a low-fidelity DNA polymerase essential for the induction of A:T mutations, instead of other high-fidelity polymerases during repair of DNA lesions. 3) POLH, mismatch repair (MMR) components, or AID itself undergo specific modifications in GC B cells, resulting in an atypical repair process not observed in other cell types.

Characterization of genes that are differentially expressed during B cell activation or highly expressed in GC B cells

We have thus far generated mice deficient in each of these genes and are now analyzing B cell abnormalities in these mice. One such gene, LAPT5, is a lysosomal protein specifically expressed in hematopoietic cells and transiently down-modulated during B cell activation. We found that LAPT5 negatively regulated cell surface BCR levels and B cell activation. Ectopic expression of LAPT5 in spleen B cells downmodulated BCR levels in a dose-dependent fashion, without affecting the levels of CD19 and CD40 (Fig. 2). We aim to clarify how LAPT5 specifically targets BCR but not other cell surface molecules for degradation.

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5. Masuda, K., Ouchida, R., Li, Y., Gao, X., Mori, H. and Wang, J.-Y. A critical role for REV1 in regulating the induction of C:G transitions and A:T mutations during immunoglobulin gene hypermutation. *J. Immunol.* 183: 1846-1850 (2009).

Group Director

Toshitada Takemori

Research Scientist : **Tomohiro Kaji**

Technical staff : **Akiko Sugimoto
Jyunko Taka
Tomoko Nishio
Natsumi Yoneda**

Visiting Scientists : **Yoshimasa Takahashi
Masaki Hikida**

Administrative Staff : **Satomi Law**

Laboratory for Immunological Memory

Immunological memory is the hallmark of adaptive immunity and is characterized by the prompt and effective response to antigen upon re-exposure, even a long time after initial stimulation. Memory provides a highly selective mechanism to provide quick protection upon re-exposure to recurrent pathogens. Therefore, an important question is how immunological memory is maintained for such long periods in the absence of repeated antigenic stimulation. An additional question is how memory and naïve B cells might differ in their functions, such as their longevity and their prompt differentiation into plasma cells that secrete large amounts of antibodies upon antigen re-exposure. Finally, another important question is how and when memory B cells develop after initial antigen exposure.

Our goals are to answer these questions, initially by utilizing a murine system, which is advantageous for assessing cellular and molecular mechanisms by multiple approaches. Furthermore, based on the information developed in the mouse, we intend to define the mechanisms underlying memory B cell maintenance in human.

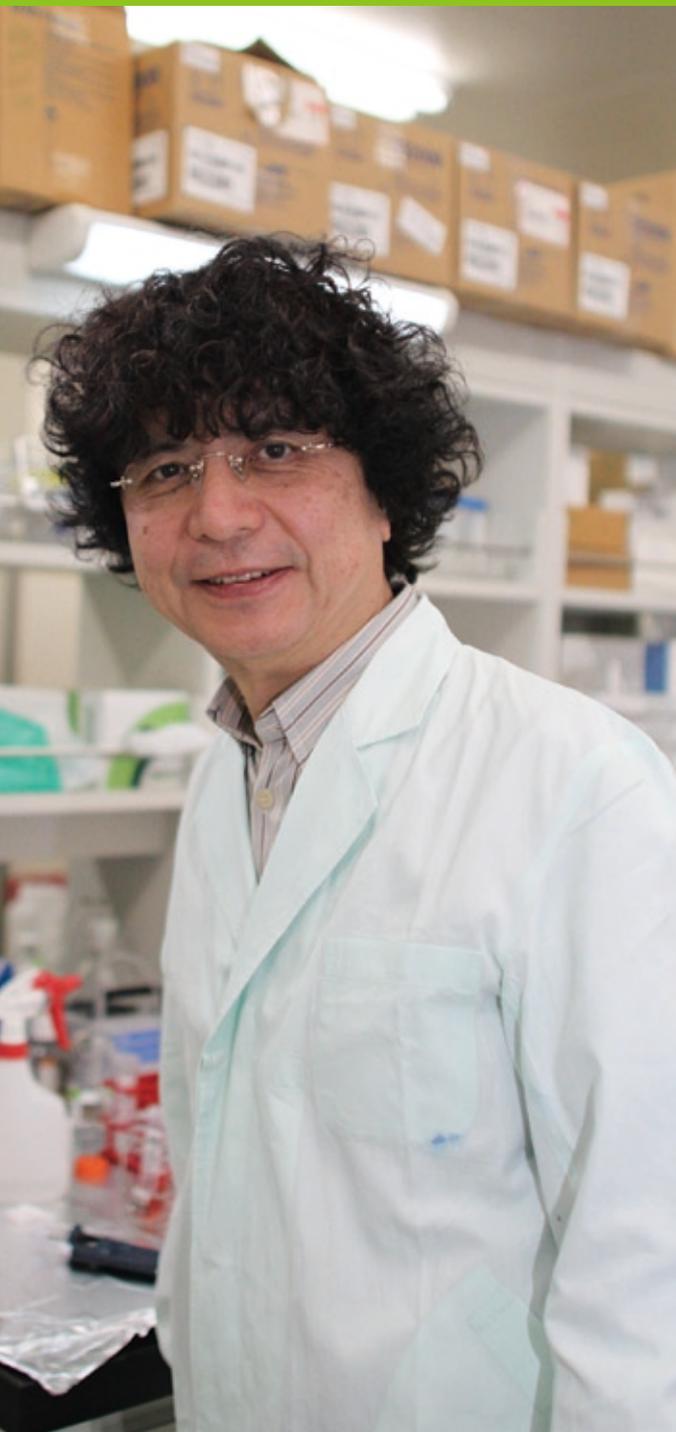
Class-switched non-mutated memory B cells develop independently of the GC reaction.

In T-cell dependent response, antigen-activated B cells follow two spatially distinct pathways of differentiation, either in extrafollicular regions of the secondary lymphoid organs or in B cell follicles, where antigen-activated B cells form GCs. While it has been widely accepted that mutated, high-affinity memory B cells originate from the GC response, we suggested that non-mutated, low affinity memory B cells develop independently of the GC reaction. Recently, three different laboratories have confirmed the development of non-mutated memory B cells in the early primary response, however, little is known of the cell population dynamics underlying development of GC-independent memory B cells. To address this issue, we established conditional *Bcl6* deficient mice, a strategy that avoids the influence of *Bcl6* deletion in immune cells other than B cells.

Our results demonstrated that conditional *Bcl6*-deletion in B cells impaired the development of NP-specific IgG1⁺

Recent publications

1. M. Hikida M., Casola. S., Takahashi N., Kaji T., Takemori T., Rajewsky K., Kurosaki T. PLC-gamma2 is essential for formation and maintenance of memory B cells. *J.Exp.Med.* 206 681-689 (2009)
2. Ato M., Ikebe T., Kawabata H., Takemori T., Watanabe H. Incompetence of neutrophils to invasive group A streptococcus is attributed to induction of plural virulence factors by dysfunction of a regulator. *PLoS One* 3[10].e3455 (2008)



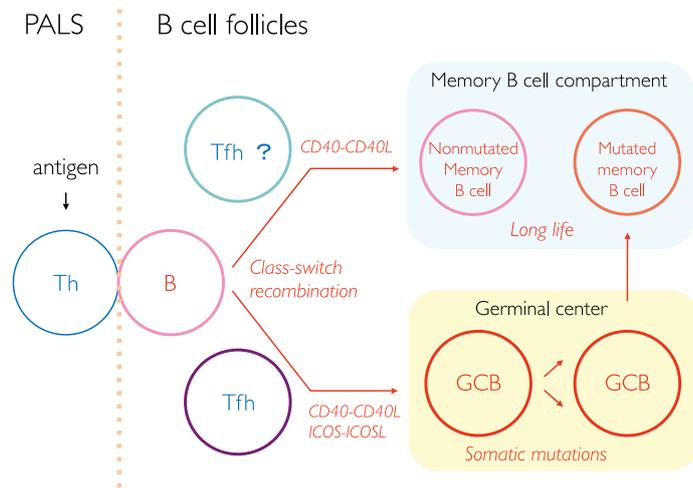


Figure: **Memory B cell development early after immunization.** A model for the generation and selection of B cells in the primary immune response. Following T-cell dependent antigen stimulation, a cognate interaction between T and B cells causes B cell proliferation and isotype class-switching from μ to γ 1 at the border of the B-cell/T-cell areas in the spleen and lymph nodes. Subsequently, B cells either migrate to extrafollicular sites, where they differentiate into plasma cells, or migrate into follicles, where they can follow two distinct pathways of differentiation, either towards GC B cells under control of T follicular helper cells (T_{FH}) or toward non-mutated memory B cells under T cell help by cognate interaction with CD40 and CD40L.

GC B cells in response to NP-CG, whereas IgG1⁺ non-mutated memory B cells developed independently of GCs within the first week post-immunization. Thus, it appears that following T-cell dependent antigen stimulation, class-switched B cells either migrate to extrafollicular sites, where they differentiate into plasma cells or migrate into follicles, where they can follow two distinct pathways of differentiation, either towards GC B cells or non-mutated memory B cells. We previously showed that inhibition of the ICOS-ICOSL interaction impaired GC B cell development, but not non-mutated memory B cell development, findings compatible with the notion that development of non-mutated memory B cells is helped by a distinct subset of T cells different from the T follicular helper cells (TFH), which play a crucial role in GC B cell development.

The memory B cell pool sustains its size under homeostatic regulation and attains functional maturation after its generation early in the immune response

The IgG1⁺ non-mutated memory B cells that develop in the absence of GCs establish a long-lived IgG1 memory compartment. On the other hand, mutated memory B cells are generated in the GCs and recruited into the non-mutated memory compartment as the immune response progresses. Because the splenic environment has a limited capacity to sustain memory B cells, the non-mutated cells are gradually replaced over time by mutated GC B cell progeny. However, how such a turnover is regulated remains obscure.

We observed that the early (day 7) memory B cells could not elicit a significant IgG1 antibody response in the secondary response, however, non-mutated memory cells that develop independently of the GC could elicit a low-affinity adoptive secondary response at day 40 after immunization,

comparable in the number of antibody secreting cells (ASCs) to wild type day 40 memory B cells. Co-stimulatory molecules needed for T-cell interactions were almost equally expressed on both day 7 and day 40 memory B cells, thereby, leading us to speculate that the absence of a response by day 7 memory B cells may reflect a deficit in a cell-intrinsic activity needed for this response. There were differences in the expression of certain genes between the day 7 and day 40 memory B cell populations that develop in the absence of GCs, leading us to propose that some external signals alter the transcriptional profile of memory B cells, which promotes their functional maturation. We are attempting to pinpoint the genes responsible for the functional maturation of memory B cells as the response progresses.

Analysis of memory B cells features in common variable immunodeficiency (CVID)

Among the humoral primary immunodeficiencies, CVID is characterized by antibody deficiencies in childhood or early to mid adulthood. Because it has been suggested that 50–75% of patients with CVID had reduced numbers of class-switched memory B cells in peripheral bloods (PB), we intended to confirm these findings using multicolor FACS analysis, enabling us to dissect B cell subsets by new criteria. We observed that all patients tested have a reduction in the numbers of both class-switched memory B cells and plasma blasts, together with or without an increase in the number of transitional B cell fraction 3. These findings may suggest that CVID is not solely linked to defects in memory B cell development. We have begun to analyze gene expression profiles in human memory B cells and other subsets of B cells, information that should provide further insight into the B cell defects in CVID.

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Team Leader

Hiroshi Ohno

Research Scientists : **Koji Hase, Shunsuke Kimura, Shinji Fukuda (SPDR), Takashi Kanaya (SPDR)**

Technical Staff : **Yumi Chiba, Yumiko Fujimura, Chie Nishigaki, Yoko Ohara, Masumi Ohmae, Sayuri Sakakibara, Ayako Yamashita**

Student Trainees: **Yasuhiro Date (JRA), Yumiko Nakanishi (JRA), Gaku Nakato (JRA), Daisuke Takahashi (JRA), Masashi Ebisawa (JRA), Toshi Jinnohara (JRA), Kazunori Kadokura (JRA), Tamotsu Kato (JRA), Masumi Shinbori, Kaoru Aizawa, Konomi Ishimaru, Junnosuke Onozato, Ryo Yamazaki, Chikako Uetake, Hideaki Shima, Misaho Hanazato**

Administrative Staff : **Yuuki Yamada**



Laboratory for Epithelial Immunobiology

The mucosal epithelium that lines the inner surfaces of the body, especially within the intestine, is exposed to a wide range of antigens, including food-borne macromolecules and microorganisms as well as numerous commensal microbiota, and efficient uptake of these antigens 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 absorptive epithelial cells of the villi and are termed follicle-associated epithelium (FAE). The FAE contains a specialized subset of epithelial cells, the M cells, which are thought to play a pivotal role in immune surveillance by delivering luminal microorganisms to the underlying lymphoid cells via transcytosis. 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.

Uptake by Glycoprotein 2 on M cells of FimH⁺ bacteria is essential for an efficient mucosal immune response to these microbes

Toward understanding the molecular mechanisms of M-cell function and differentiation, we took advantage of a microarray-based approach for a genome-wide survey of M-cell specific molecules. We reported last year that glycoprotein-2 (GP-2) was exclusively expressed by Peyer's patch M cells, but not by villous epithelial cells, in collaboration with Dr. Kiyono's group in the University of Tokyo. We have extended our finding to show that GP-2 serves as a bacte-

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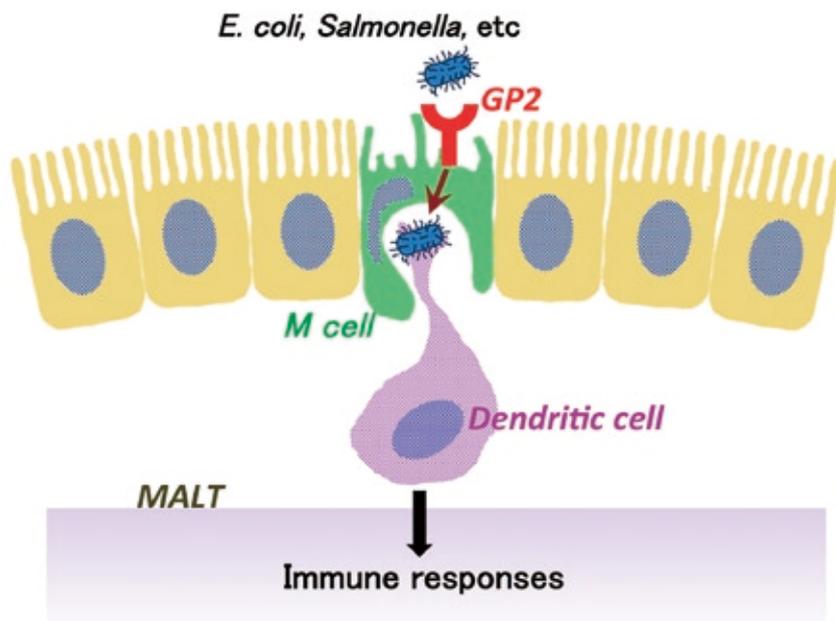


Figure: Schematic representation of the role of GP2 in mucosal immune response. FimH⁺ enteric bacteria such as *Escherichia coli* and *Salmonella* are captured by GP2 on the M-cell surface and subjected to transcytosis to be eventually transferred to dendritic cells closely associated with M cells. Subsequently, dendritic cells process and present bacterial antigens to T cells to initiate mucosal immune response against these bacteria, which ultimately lead to production of bacterial antigen-specific IgA.

rial uptake receptor on M cells. Recombinant GP2 protein selectively bound a subset of commensal and pathogenic enterobacteria, including *Escherichia coli* and *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*), by recognizing FimH, a component of type I pili on the bacterial outer membrane. Consistent with these findings, the same bacteria were colocalized with endogenous GP2 on the apical plasma membrane as well as within cytoplasmic vesicles of M cells. Moreover, deficiency of bacterial FimH or host GP2 led to defects in transcytosis of type-I-piliated bacteria through M cells, resulting in an attenuation of antigen-specific immune responses in Peyer's patches. GP2 is therefore a previously unrecognized transcytotic receptor on M cells for type-I-piliated bacteria and is a prerequisite for the mucosal immune response to these bacteria. Given that M cells are considered a promising target for oral vaccination against various infectious diseases, the GP2-dependent transcytotic pathway could provide a new target for the development of M-cell-targeted mucosal vaccines.

M-Sec promotes tunneling nanotube formation in association with Ral and the exocyst complex

Cell–cell communication is essential for the development and homeostasis of multicellular organisms. Recently, a new type of cell–cell communication was discovered that is based on the formation of thin membranous nanotubes between remote cells. These long membrane tethers,

termed tunneling nanotubes (TNTs), form an intercellular conduit and have been shown to enable the transport of various cellular components and signals. However, the molecular basis for TNT formation remains to be elucidated. We have recently shown that a mammalian protein, M-Sec, induces *de novo* formation of numerous membrane protrusions extending from the plasma membrane, some of which tether onto adjacent cells and subsequently form TNT-like structures. Depletion of M-Sec by RNA interference (RNAi) greatly reduced endogenous TNT formation as well as intercellular propagation of a calcium flux in a macrophage cell line. Furthermore, blockage of the interaction of M-Sec with Ral and the exocyst complex, which serves as a downstream effector of Ral, attenuated the formation of membrane nanotubes. Our results reveal that M-Sec functions as a key regulator of membrane nanotube formation through interaction with the Ral–exocyst pathway. The discovery of M-Sec as a marker for TNTs and a promoter of TNT formation will help clarify the mechanisms of formation of these structures as well as their structural and functional properties. We have recently established M-Sec knockout mice, which should be a useful model to uncover the physiological role of TNTs. In addition, the identification of M-Sec-targeted drugs may provide a new strategy for containment of viral infections such as HIV, given the recent report of the involvement of TNT in intercellular viral propagation and viral pathogenesis.

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3. Shima H., Takatsu H., Fukuda S., Ohmae M., Hase K., Kubagawa H., Wang J.-Y., Ohno H. Identification of TOSO/FAIM3 as an Fc receptor for IgM. *Int. Immunol.* 22, 149-156 (2010)

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5. Hase K., Takahashi D., Ebisawa M., Kawano S., Itoh K., Ohno H. Activation-induced cytidine deaminase deficiency causes organ-specific autoimmune disease. *PLoS ONE* 3, e3033 (2008)

Team Leader

Sidonia Fagarasan

Research Scientists : **Keiichiro Suzuki**
Tran Huy Thinh

Research Associate : **Mikako Maruya**

Technical Staff : **Yasuko Doi**
Yumi Tsutsui

Student Trainee : **Shimpei Kawamoto**

Administrative Staff : **Mari Kurosaki**



Laboratory for Mucosal Immunity

Our long-term goal is to understand the cellular and molecular basis of gut immune regulation, a front line of body defense, and to apply such knowledge for health and disease of the mucosal immune system. More specifically, given its safety, low cost and potential efficacy, oral vaccination is considered to be a desirable route for immunization against mucosal as well as systemic infections.

To achieve this goal we have to define and functionally characterize the main players in mucosal immunity, namely: the bacterial community living in our intestinal tract; all immune cells residing in lymphoid tissues closely associated with bacteria; and the stromal cells that constitute the natural backbone for the immune cells. Furthermore, a comprehensive view of an immune response in natural settings requires not only the individual characterization of these compartments, but also a full understanding of their continuous interactions and cross-talk, which inevitably lead to dynamic phenotypic and functional changes for all players.

T cell-independent and T-cell dependent immunoglobulin A generation in the gut

Immunoglobulin A (IgA) is generated in the gut by both T cell-dependent and T cell-independent processes. We recently showed that lymphoid gut structures called isolated lymphoid follicles (ILF), which develop in a ROR γ t⁺LTi-dependent manner after colonization of the intestine with bacteria, serve as sites for IgA synthesis. We found not only that ILFs serve as sites where B cells undergo activation, induction of activation-induced cytidine deaminase (AID) and IgA class switching, but also that this process apparently does not require the help of T cells.

Nevertheless, the number and the quality of IgA plasma cells present in the intestine of mice having only ILFs differed from those found in mice with Peyer's patches (PPs) and ILFs. Unlike in the ILFs, the IgAs generated in PPs require germinal center formation and the presence of CD4⁺ follicular B helper T (T_{FH}) cells. Although T_{FH} cells have been known for more than a decade, the nature of the T_{FH} cells present in peripheral lymphoid organs or in the Peyer's patches has been elusive. We demonstrated that suppressive Foxp3⁺CD4⁺ T cells have the potential to differentiate

Recent publications

1. Tran TH, Nakata M, Suzuki K, Begum NA, Shinkura R, Fagarasan S, Honjo T, Nagaoka H. B cell-specific and stimulation-responsive enhancers derepress *Aicda* by overcoming the effects of silencers. *Nat Immunol.* 2010 Feb;11(2):148-54
2. Tsuji M, Komatsu N, Kawamoto S, Suzuki K, Kanagawa O, Honjo T, Hori S, Fagarasan S. Preferential generation of follicular B helper T (T_{FH}) cells from Foxp3⁺ T cells in gut Peyer's patches. *Science*, 323, 1488-1492.

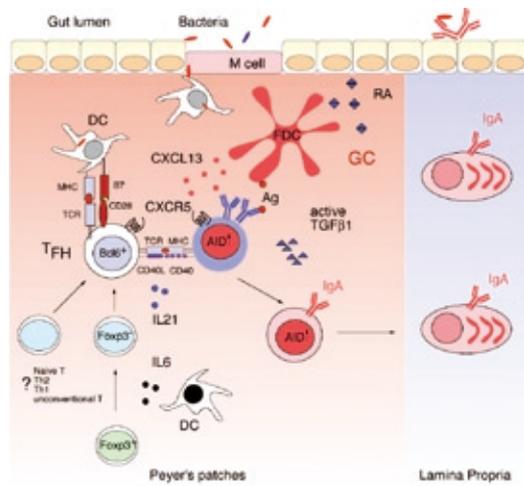


Figure 1: T cell-dependent induction of IgA⁺ B cells in the germinal center of Peyer's patches. Dendritic cells (DCs) located beneath specialized M cells take up bacteria, and then migrate to the T-cell zone where they activate T cells. Upon induction of the transcription factor Bcl-6, activated CD4⁺ T cells, express CXCR5 and migrate toward CXCL13 into the FDC network, where they provide help for activated B cells (T_{FH} cells). Some T_{FH} cells are derived from Foxp3⁺ T cell precursors that lose Foxp3-expression in the presence of IL-6 (produced by DCs) and IL-21 (produced by T cells). Conventional B-T interactions through MHC-TCR and CD40-CD40L are critical for activation of B cells, induction of AID and class switch recombination (CSR)/somatic hypermutation (SHM) in GCs of PPs. Preferential generation of IgA⁺ B cells in PPs is due to the abundant production of activated TGF-β1, IL-21 and retinoic acid (RA) in this environment. The IgA⁺ B cells generated in GC of PPs home to the lamina propria where they further differentiate into IgA plasma cells.

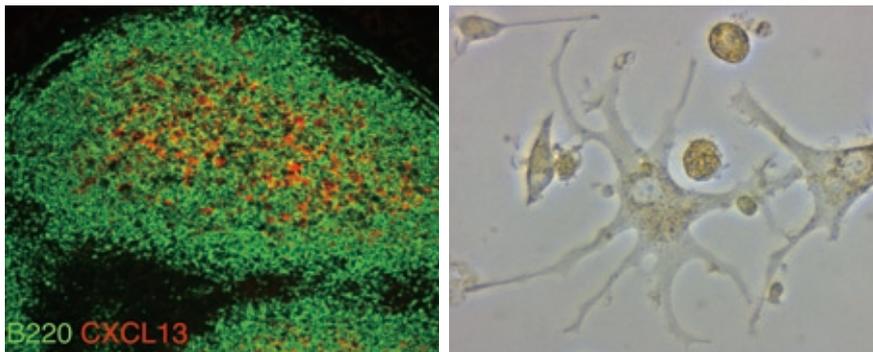


Figure 2: In vivo and in vitro images of FDCs. (Left panel) A horizontal section of Peyer's patches showing a B cell follicle (B220 in green) and a B-cell chemoattractant produced by FDCs (CXCL13 in red). (Right panel) A phase-contrast micrograph of FDCs cultured for two weeks on a collagen-coated plate in the presence of an LT-βR agonist antibody and TNF-α.

into T_{FH} cells in mouse Peyer's patches. The conversion of Foxp3⁺ T cells into T_{FH} cells requires the loss of Foxp3 expression and subsequent interaction with B cells through CD40-CD40L. Thus, we demonstrated a rather unexpected plasticity of the CD4⁺ T cells expressing Foxp3, which under gut environmental conditions (antigens and cytokines) convert to distinct helper T cells such as T_{FH} cells.

The ILF formation/T-cell independent IgA synthesis project was in collaboration with Dr. Dan Littman, Skirball Institute of Biomolecular Medicine, NYU. The T cell-dependent IgA synthesis project was in collaboration with the Research Unit for Immune Homeostasis led by Dr. Shohei Hori, the Laboratory for Autoimmune Regulation led by Dr. Osami Kanagawa, and Professor Tasuku Honjo from Kyoto University.

Follicular dendritic cells and their role in regulation of mucosal immune responses

Follicular dendritic cells (FDCs) are radio-resistant cells that were identified by their ability to retain immune complexes. More recently it was found that FDCs secrete several cytokines and chemokines involved in the migration and aggregation of lymphocytes, especially B cells into follicles. However,

most of the FDC studies are based on either histological characterization or generation of cell lines with properties resembling FDCs. We aimed to characterize FDCs *ex vivo* in order to understand their functional compartmentalization and to determine if FDCs play roles not only for SHM required for affinity maturation but also for CSR of B cells. Thus, we set up to evaluate the functional differences between the FDCs located in organized gut lymphoid tissues (GALT) and the FDC from peripheral lymph nodes. Our experiments revealed that GALT FDCs express surface, cytoplasmic and nuclear receptors that can signal the presence of bacterial and metabolic products such as RA, the active metabolite of vitamin A in the gut. Synergistic stimulation of FDCs with bacteria and RA enhances the expression of molecules by the FDCs, among them many proteins involved in activation of TGF-β1. Our results indicate that gut FDCs, are conditioned by environmental stimuli to express key factors for B cell migration, survival and preferential generation of IgA in gut. (Suzuki et al., *Immunity*, in press.)

This project is in collaboration with the Laboratory for Immunogenomics led by Dr. Osamu Ohara and with Professor William Agace, from Lund University, Sweden.

3. Suzuki K, Fagarasan S. Diverse regulatory pathways for IgA synthesis in the gut. *Mucosal Immunol.* 2009 Nov;2(6):468-71.

4. Tsuji M, Suzuki K, Kitamura H, Maruya M, Kinoshita K, Ivaylo II, Itoh K, Littman DR, Fagarasan S. Requirement for Lymphoid tissue inducer cells in isolated follicle formation and T cell-independent immunoglobulin A generation in the gut. *Immunity.* 2008, Aug, 15.

5. Suzuki K, Fagarasan S. How host-bacterial interactions lead to IgA synthesis in the gut. *Trends Immunol.*, 2008, 29(11), 523-531.

Team Leader

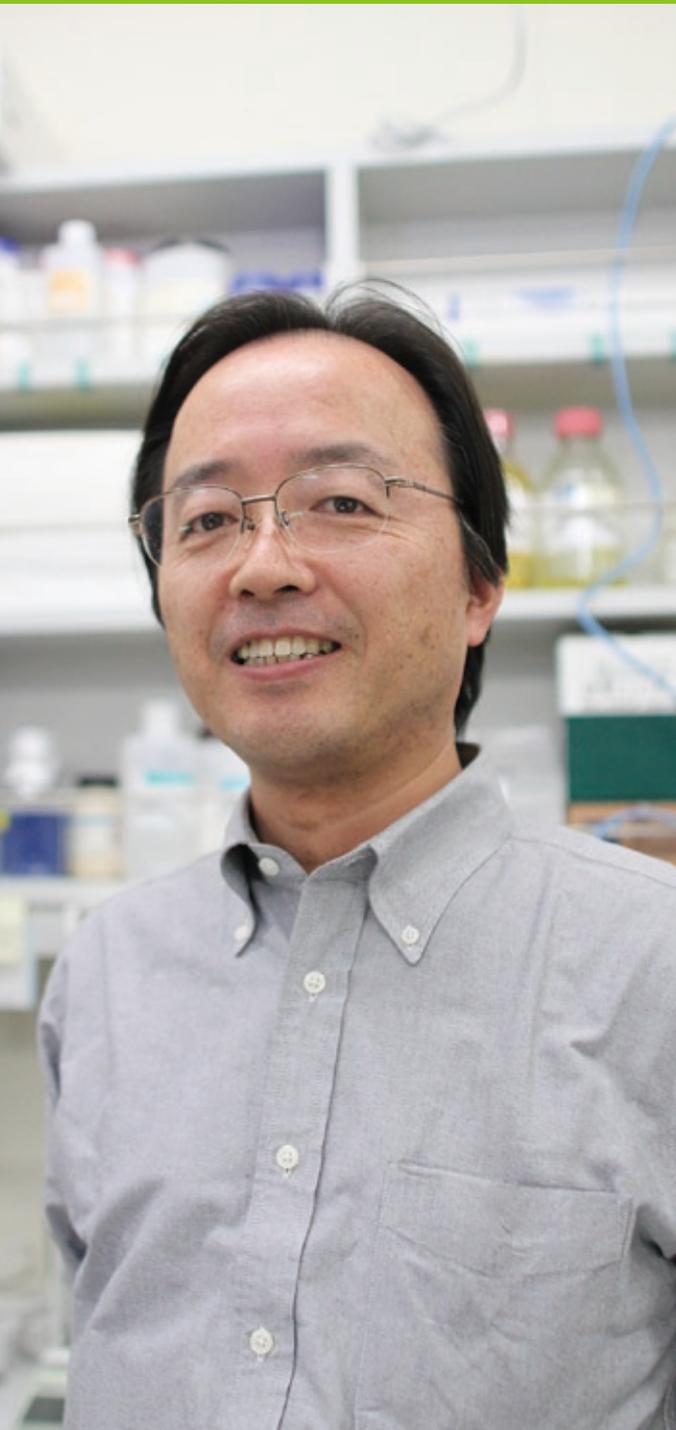
Tsuneyasu Kaisho

Research Scientist : **Katsuaki Hoshino**

Technical Staff : **Yuri Fukuda**
Nana Iwami
Akiko Kato

Student Trainees : **Izumi Sasaki**
Chihiro Yamazaki
Takeshi Hirashima
Akihiko Iizuka
Eri Hamada

Administrative Staff : **Sachiko Haraguchi**



Laboratory for Host Defense

Host defense in mammals consists of innate and adaptive immunity. Innate immunity functions as a pathogen sensor and is involved in their eradication. Furthermore, innate immunity also contributes to the establishment of adaptive immunity. Dendritic cells (DCs) are antigen presenting cells critically involved in the sequence of these immune responses. DCs sense various pathogen-derived molecular components and exert their immunostimulatory functions by producing inflammatory cytokines or upregulating expression of costimulatory molecules. Those components are termed immune adjuvants based on their ability to activate DC. Immune adjuvants are recognized by various types of pattern recognition receptors including Toll-like receptors (TLRs). Identification of new types of immune adjuvants and characterization of the mechanisms by which they activate DCs should contribute to the development of novel immunoregulatory maneuvers. We are attempting to clarify how DCs are activated through pattern recognition receptors and to obtain critical information for effectively manipulating the immune response. Various immune adjuvants, including TLR ligands, and gene targeting mice are important tools for our studies.

DC subset-specific mechanisms in TLR7/9-induced type I IFN gene induction

DCs sense nucleic acid adjuvants and produce type I interferon (IFN) in a subset-dependent manner. Among nucleic acid sensors, TLR7 and TLR9 are peculiar in that they recognize not only pathogen- but also host-derived nucleic acids. In fact, accumulating evidence suggests that TLR7/9-induced type I IFN production plays important roles in the pathogenesis of autoimmune disorders such as SLE. Therefore, clarifying the TLR7/9 signaling mechanisms should contribute to the development of therapeutic intervention for such diseases.

Plasmacytoid DCs (PDC) are a unique DC subset expressing nucleic acid sensors, TLR7 and TLR9. PDC are featured by the ability to produce type I IFN, especially IFN- α , in response to TLR7/9. TLR7 and TLR9 are quite similar in their amino acid sequence and can activate similar sig-

Recent publications

1. T. Tanaka, M. J. Grusby, T. Kaisho. PDLIM2-mediated termination of transcription factor NF- κ B activation by intranuclear sequestration and degradation of the p65 subunit. *Nature Immunol.* 8, 584-591 (2007).
2. S. K. Pathak, S. Basu, K. K. Basu, A. Banerjee, S. Pathak, A. Bhattacharyya, T. Kaisho, M. Kundu, J. Basu. Direct extracellular interaction between the early secreted antigen ESAT-6 of mycobacterium tuberculosis and TLR2 inhibits TLR signaling in macrophages. *Nature Immunol.* 8, 610-618 (2007).

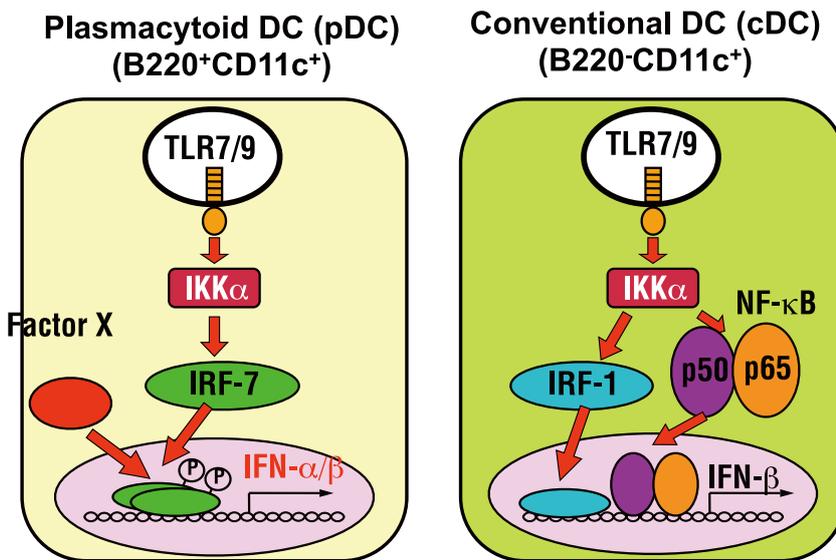


Figure: DC subset-specific mechanisms in TLR7/9-induced type I IFN gene induction. In response to TLR7/9 signaling, pDC can produce vast amounts of type I IFNs including IFN- α and IFN- β , while cDC can produce only IFN- β . IKK α is required for this response by both DC subsets. Notably, the underlying mechanism depends on the DC subset. IRF-7, which is abundantly expressed in pDC, is critical for pDC function. However, it is still possible that other pDC-specific factors also contribute to pDC function.

naling pathways, which depend on the cytoplasmic adapter molecule, MyD88. Downstream of MyD88, signaling pathways bifurcate into NF- κ B and IRF-7 activation pathways, leading to the induction of inflammatory cytokines and type I IFNs, respectively. We have discovered that a serine threonine kinase, I κ B kinase- α (IKK α), is critically involved in this type I IFN induction pathway in pDC (Hoshino et al. *Nature*, 2006). IKK α associates with and phosphorylates IRF-7, which is critical for type I IFN gene expression in response to TLR7/9 signaling. In response to TLR7/9 signaling, conventional DC (cDC) can also produce IFN- β , but not IFN- α . This IFN- β production requires molecular mechanisms that differ from those involved in type I IFN production by pDC. However, we have found that IKK α is also required for the IFN- β production by TLR7/9-stimulated cDC. In IKK α -deficient cDC, IRF-7 activation was not impaired, but activation of IRF-1 and NF- κ B was decreased. These results indicate that IKK α is involved in TLR7/9-mediated type I IFN induction in a DC subset-specific manner. Thus, IKK α should be a unique target not only for manipulating antiviral immunity but also for treating autoimmune diseases in which type I IFN production is elevated. IRF-7 is a critical molecule for pDC function and is constitutively highly expressed in pDC. In addition to our studies of the IKK α -IRF-7 axis, we are further planning to clarify the underlying molecular mechanisms for pDC function and development.

Critical roles of TLR3 and TLR3-expressing cDC

Double stranded RNA (dsRNA) is also a nucleic acid adjuvant that can activate DCs. and a synthetic dsRNA, poly(I:C), is recognized by an endosomal sensor, TLR3, and a cytosolic sensor, MDA5. Each sensor exhibits its own function. Poly(I:C) induces IL-12p40 production through TLR3 signaling, while it leads to type I IFN production through MDA5 signaling. We have analyzed the immune adjuvant effects of another dsRNA, poly(A:U), and found that it is a ligand for TLR3 and TLR7, but not for the cytosolic sensor. By using poly(A:U) and TLR3- and/or TLR7-deficient mice, we have discovered that TLR3 is involved in mediating the signal for CD8 T cell responses *in vivo*, i.e. cross presentation. Crosspresentation is a unique function of DCs and is critical for effective immune responses against tumors or viral infections. cDCs can be further divided into 3 subsets, CD4⁺8⁻, CD4⁺8⁺ and CD4⁻8⁻. Notably, among these cDC subsets, CD4⁺8⁺ cDCs are characterized by their high crosspresenting activity and are the only DC subset that expresses TLR3 and responds to TLR3 signaling. The other cDCs as well as pDC fail to respond to TLR3 signaling. We are planning to clarify how CD8⁺ cDCs are generated and acquire their function and how the function is regulated by TLR3 signaling. For this purpose, we characterize gene expression profiles of various DC subsets and focus on some CD8⁺ cDC-specific genes.

3. T. Sugiyama, K. Hoshino, M. Saito, T. Yano, I. Sasaki, C. Yamazaki, S. Akira, T. Kaisho. Immunoadjuvant effects of polyadenylic:polyuridylic acids through TLR3 and TLR7. *Int. Immunol.* 20, 1-9 (2008).

4. K. Hoshino, T. Kaisho. Nucleic acid sensing Toll-like receptors in dendritic cells. *Curr. Opin. Immunol.* 20:408-413 (2008).

5. T. Kaisho, T. Tanaka. Turning NF- κ B and IRFs on and off in DC. *Trends Immunol.* 29, 329-336 (2008).

Team Leader

Satoshi Ishido

Research Scientists : **Mari Ohmura-Hoshino**

Eiji Goto

Yohei Matsuki

Mizuho Kajikawa

Student Trainee : **Eiji Uota**

Technical Staff : **Masami Aoki-Kawasumi**

Mari Mito-Yoshida

Administrative Staff : **Sachiko Dohi**



Laboratory for Infectious Immunity

We have demonstrated that the expression level of MHC class II (MHC II) is regulated through ubiquitination of the MHC II β chain. We also found that MARCH-I, an E3 ubiquitin ligase, is critical for this process. At present, however, the physiological importance of MARCH-I-mediated MHC II regulation *in vivo* is still unknown, and resolving this issue is the goal of one of our major projects. Given that MHC II is a critical molecule for initiation of immune responses, this project will provide new insight into the molecular basis of how antigen presentation regulates immune responses *in vivo*.

We are also exploring the molecular mechanism of ubiquitin-regulated trafficking of MHC molecules by using a new experimental tool, a Tet-on expression system for MIR E3 ligases. With this new experimental system, we are now able to analyze the detailed relationship between the status of ubiquitination and the trafficking of MHC molecules. Since ubiquitination-mediated regulation of transmembrane proteins (e.g. cytokine receptors) contributes to critical cell signaling pathways, we will be able to provide important insight into fields even beyond immunology.

To understand the physiological function of MARCH-I, we and other groups have examined the regulation of MARCH-I expression at the transcriptional and post-transcriptional levels. We demonstrated that TLR signals inhibit the expression of MARCH-I mRNA, and inhibit MHC II ubiquitination in dendritic cells. Also, inhibition of DC maturation by IL-10 was shown to rescue the down-regulation of MARCH-I expression and MHC II ubiquitination.

Given that MARCH-I is a physiological E3 for MHC II, we hypothesize that down-regulation of MARCH-1 mRNA is an important initiation signal for immune responses. However, the question remains of why constitutive MHC II ubiquitination by MARCH-I is necessary at the steady state *in vivo*. To address this question, we examined T-dependent immune responses in conventional MARCH-I KO mice. So far, we have not observed any evidence of enhanced MHC II function in these mice. Instead, to our surprise, we observed DC abnormalities *in vivo*. MARCH-I-deficient splenic DCs showed high expression of MHC II as expected.

Recent publications

1. Ohmura-Hoshino, M., Matsuki, Y., Mito-Yoshida, M., Goto, E., Aoki-Kawasumi, M., Nakayama, M., Ohara, O., and Ishido, S. Cutting edge: Requirement of MARCH-I-mediated MHC II ubiquitination for the maintenance of conventional dendritic cell. *J Immunol.*, 2009;183(11):6893-7.
2. Ishido S., Goto, E., Matsuki, Y. and Ohmura-Hoshino, M: E3 ubiquitin ligases for MHC molecules, *Curr Opin Immunol*, 2009;21(1):78-83.

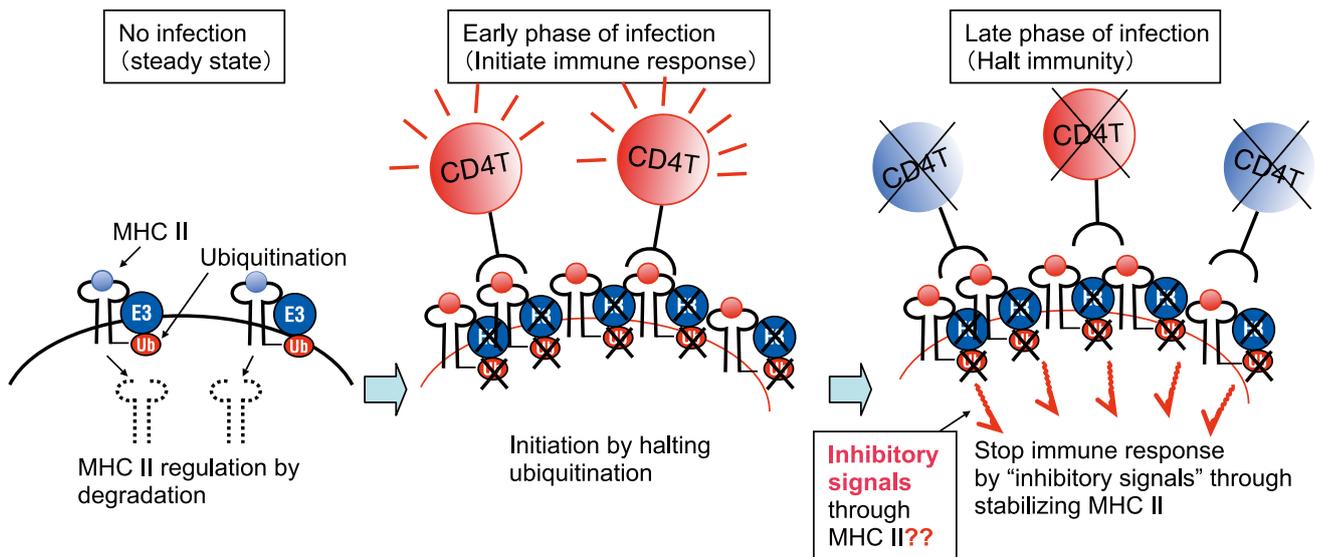


Figure: Model for the role of MARCH-I in immune responses. In the steady state, MARCH-I (shown as "E3") is constitutively expressed in APCs and regulates their expression of MHC II through ubiquitination-mediated lysosomal degradation (*left panel*). Once APCs are activated and matured by infection, MARCH-I expression is down-regulated and pathogen-derived peptides (shown in red) are presented by MHC II stabilized due to loss of ubiquitination (*middle panel*). This stabilization results in sustained MHC II expression that eventually delivers as yet to be identified "inhibitory signals" to APCs, directly or indirectly, halting the immune response.

Interestingly, the co-stimulatory molecule B7-2/CD86 was also highly expressed on the KO DCs. Since over-expression of MARCH-I inhibited B7-2 expression, it seems to be another physiological MARCH-I substrate. Surprisingly, even though the molecules required for antigen presentation were highly expressed, the ability of splenic DCs to present the antigens was impaired in the MARCH-I KO, as was LPS-induced cytokine production. Consistent with these observations, T-dependent immune responses were impaired in the MARCH-I KO mice.

Why are splenic DCs abnormal in MARCH-I KO mice? Examination of MARCH-I deficient DCs generated *in vitro* (bone marrow-derived DCs) and the results of transplantation experiments using these cells strongly suggested that the DC abnormality is due to an *in vivo* environmental effect. Since MHC II and B7-2 are both highly expressed on DCs and potentially interact with the microenvironment (e.g. T cells), the contribution of these molecules to the DC abnormality was examined. Each molecule was deleted from MARCH-I KO mice by crossing them with MHC II or B7-2 KOs. Interestingly, deletion of MHC II completely rescued

the abnormality of MARCH-I-deficient DCs, even though B7-2 was still highly expressed. Consistent with the lack of a role for B7-2, its deletion had no effect on the abnormality. These results demonstrated the requirement of MHC II for the DC abnormality induced by MARCH-I deletion.

It has been demonstrated that stimuli that induce DC maturation stabilize surface MHC II molecules by inhibiting MHC II ubiquitination. Furthermore, loss of MHC II ubiquitination is accompanied by the downregulation of MARCH-I expression. As these events are similar to those that occur in MARCH-I KO mice, we now hypothesize that matured/activated DCs receive signals that cause the DC abnormalities indirectly, via stabilized MHC II. If this is the case, such an inhibitory signal may prevent excessive immune reactions, which may prevent detrimental immune responses such as occur in autoimmune diseases. Consistent with this idea, several groups have reported on LPS-induced DC malfunction *in vivo*. Taken together, our results and those of others lead us to propose a new model for the physiological role of MARCH-I-mediated MHC II ubiquitination in the immune system (see Fig.).

3. Goto, E., M. Mito-Yoshida, M. Uematsu, M. Aoki, Y. Matsuki, M. Ohmura-Hoshino, H. Hotta, M. Miyagishi, and S. Ishido. An excellent monitoring system for surface ubiquitination-induced internalization in mammals. *PLoS ONE*, 2008;3(1):e1490.

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Team Leader

Masato Tanaka

Research Scientists : **Kenichi Asano**
Qiu Chunhong
Gen Nishitai

Technical Staff : **Ai Kurita**
Ami Nabeyama
Kumiko Ito
Minako Aihara

Administrative Staff : **Mizuki Shimura**

Laboratory for Innate Cellular Immunity

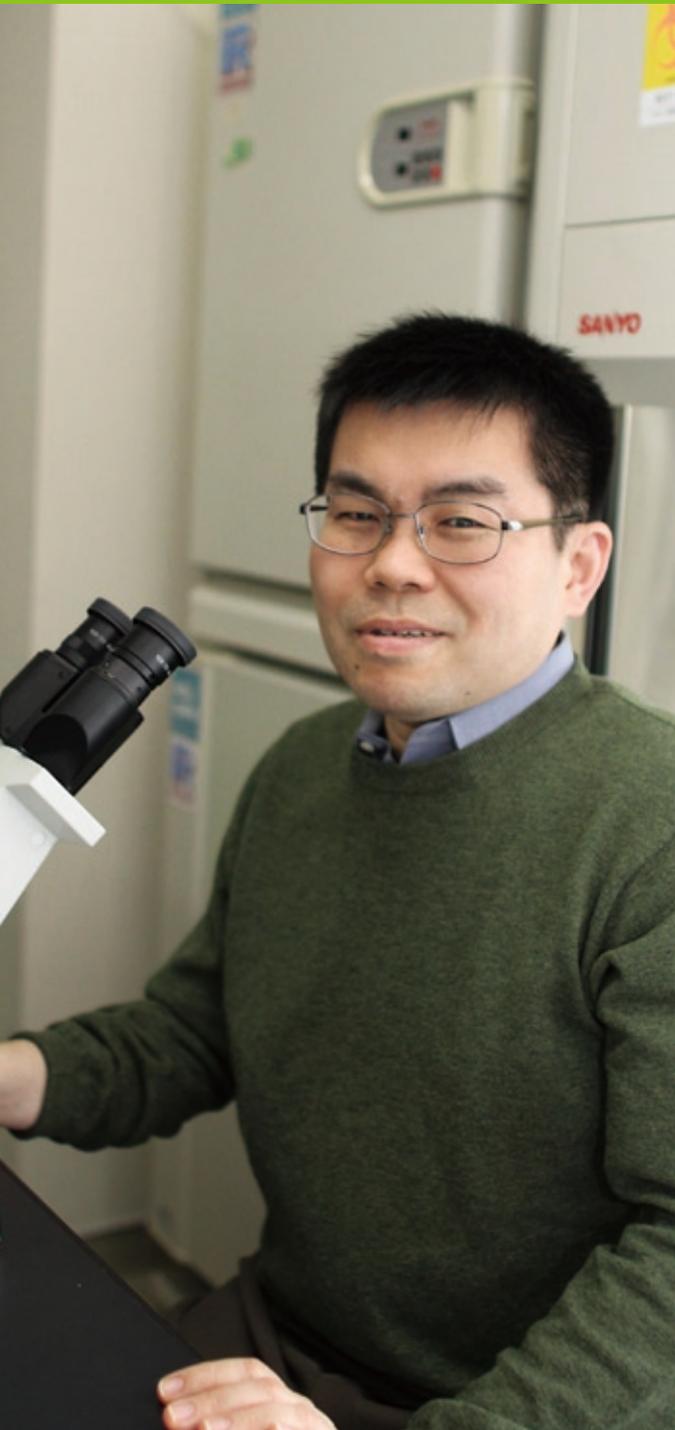
Macrophages are key players in the initiation and regulation of inflammation. At the inflammatory site, macrophages infiltrating in response to an acute injury or infection recognize invading microorganisms or endogenous adjuvants released by injured cells, such as High Mobility Group Box Chromosomal Protein 1 (HMGB1) and uric acid, through pattern-recognition receptors. In response to this recognition, macrophages produce inflammatory cytokines and chemokines. Macrophages are also required for the resolution of inflammation, which is critical for successful tissue repair. During the late course of inflammation, infiltrated neutrophils and cell debris from tissue injury are cleared by macrophages. A defect of this clearance has been shown to cause unremitting inflammation in acute injury mouse models. In addition to the resolution of inflammation, apoptotic cell clearance by phagocytes also plays a crucial role for maintenance of self tolerance. In the periphery, tissue-resident dendritic cells (DCs) constantly phagocytose apoptotic cells generated during normal tissue turnover, and migrate to draining lymph nodes where they present antigens derived from the apoptotic cells. This presentation of self-antigens leads to deletion or anergy of any self-reactive T cells, thereby providing another mechanism to maintain T cell tolerance. Failure of apoptotic cell clearance results in autoimmune disorders. The Laboratory for Innate Cellular Immunity 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.

Immune regulation by apoptotic cell clearance

Apoptotic cell clearance by phagocytes is essential for the maintenance of self-tolerance under physiological conditions. Consistent with these findings, the intravenous injection of apoptotic cells can induce specific immunosuppression or tolerance to the cell associated antigens. We previously found that this mechanism of tolerance induction requires the contributions of two populations of cells in the splenic marginal zone (MZ). The MZ contains two types of mac-

Recent publications

1. Nabeyama A, Kurita A, Asano K, Miyake Y, Yasuda T, Miura I, Nishitai G, Arakawa S, Shimizu S, Wakana S, Yoshida H, Tanaka M. xCT deficiency accelerates chemically induced tumorigenesis. *Proc Natl Acad Sci USA*. 107, 6436-41 (2010)
2. Qiu, C. H., Miyake, Y., Kaise, H., Kitamura, H., Ohara, O., and Tanaka, M. Novel subset of CD8 α + dendritic cells localized in the marginal zone is responsible for tolerance to cell-associated antigens. *J. Immunol.* 182, 4127-4136 (2009)



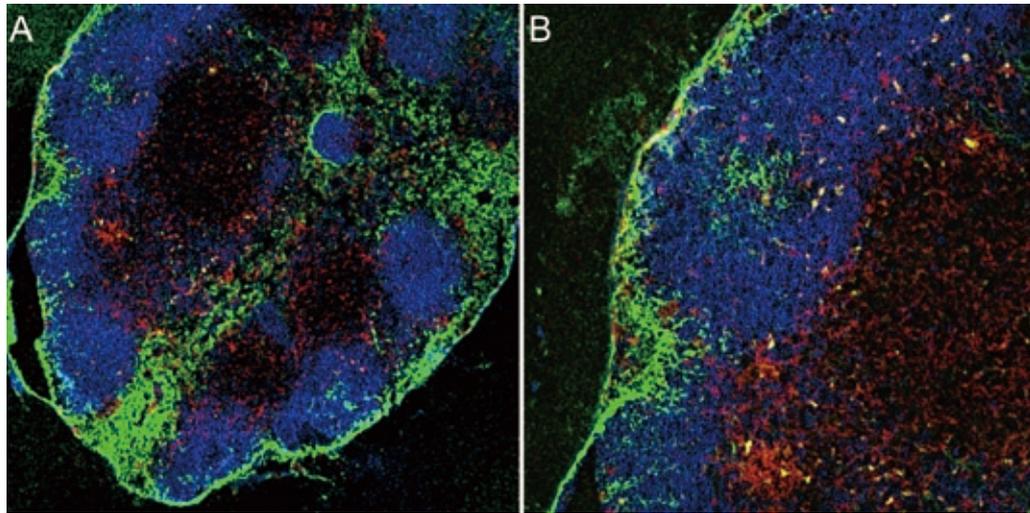


Figure: Brachial lymph node from wild type C57BL/6 was snap frozen in OCT. 5 μm -thick cryosection was stained for CD169 (Green), CD11c (Red) and B220 (Blue) and observed under confocal microscope. Original magnification; x40 (A), x200 (B).

rophages, marginal metallophilic macrophages (MMM) and marginal zone macrophages (MZM). These macrophages contribute to rapid clearance of cellular corpses in the blood flow. In addition, we found that $\text{CD8}\alpha^+$, CD103^+ DCs localized in the MZ selectively phagocytose blood-borne dead cells and subsequently present dead cell-associated antigen to induce antigen-specific immunosuppression or tolerance.

In contrast to the outcome following intravenous injection, subcutaneously injected apoptotic cells are often immunogenic and many investigators have taken advantage of immunogenic tumor corpses for tumor vaccination. These findings suggest that apoptotic cells in the periphery are cleared and processed in a very different way from blood-borne apoptotic cells in spleen. Antigen presentation in peripheral lymph nodes (LNs) is thought to be a process that is coordinately performed by migratory DCs from peripheral tissues and LN-resident APCs. However, little is known about the role of different APCs in the clearance of dead cells and the presentation of dead cell-associated antigens in peripheral tissue or LNs. We are attempting to understand the molecular and cellular mechanisms of anti-tumor immunity by subcutaneous tumor vaccination. We previously established transgenic mice in which CD169-positive cells could be transiently depleted by diphtheria toxin (DT) administration (CD169-DTR mice). We found that DT treatment selectively depleted sinus macrophages in

LNs of these mice. We are now using these mice to examine the role of sinus macrophages in the presentation of dead-cell associated antigens in LNs.

Role of the anti-oxidant system in survival of activated macrophages

During the course of inflammation and its resolution, macrophages are exposed to various cytotoxic compounds including reactive oxygen species (ROS). In the active phase of inflammation, neutrophils are recruited to the inflammation site, where they produce ROS to kill the invading organisms. Macrophages themselves also produce ROS in response to lipopolysaccharide (LPS). Thus, macrophages require a protective mechanism against oxidative stress in order to survive and accomplish the task of resolving inflammation. We recently established a mutant mouse line induced by *N*-ethyl-*N*-nitrosourea mutagenesis, in which *in vitro* generation of macrophages and dendritic cells from hematopoietic progenitor cells was impaired. Genetic mapping revealed that the mutation existed in a gene related to the intracellular anti-oxidant system. We found that this gene was expressed in infiltrating macrophages and neutrophils at the inflammatory site, and that macrophages from the mutant mice showed impaired survival in response to LPS stimulation. We are investigating the role of the anti-oxidant system in activated macrophages *in vivo* by analyzing the mutant mice.

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4. Miyake Y., Asano K., Kaise, H., Uemura, M., Nakayama, M. and Tanaka, M. Critical role of macrophages in the marginal zone in the suppression of immune responses to apoptotic cell-associated antigens. *J. Clin. Invest.* 117, 2268-2278 (2007)

5. Miyake Y., Kaise H., Isono K., Koseki H., Kohno K., and Tanaka M. Protective role of macrophages in non-inflammatory lung injury caused by selective ablation of alveolar epithelial type II cells. *J. Immunol.* 178, 5001-5009 (2007)

Unit Leader

Takashi Tanaka

Technical Staff : **Emiri Haga**

Administrative Staff : **Sachiko Haraguchi**



Research Unit for Inflammatory Regulation

There are two sequential phases in the host defense response. First is the inflammatory response, which is triggered by inflammatory cytokines produced by dendritic cells and is critical for eliminating invading pathogens. Inflammatory responses also direct T-helper (Th) lymphocyte differentiation into distinct subsets, such as Th1, Th2 and Th17, to deal with different varieties of microorganisms. The second phase is the tissue repair process during which fibrogenesis and vascularization are induced to repair the damaged tissues. It is well known that inflammatory responses can be induced by Toll-like receptor (TLR)-mediated activation of the NF- κ B transcription factor, while TGF β -mediated activation of the Smad transcription factor is essential for tissue repair. It is important that these responses be tightly controlled, otherwise, exaggerated inflammation can lead to inflammatory diseases, including rheumatoid arthritis and bronchial asthma. On the other hand, excess tissue repair activity causes fibrotic disorders, such as pulmonary fibrosis and scleroderma. Our research goal is to identify key regulators of signal transduction pathways in inflammatory and tissue repair responses, and to clarify the molecular mechanisms to regulate these responses. These studies should contribute to the development of new therapeutic tools to control human inflammatory and fibrotic disorders. Our research now focuses on the role of PDLIM2 (PDZ and LIM-domain containing protein-2) in the negative regulation of these responses.

PDLIM2 is a negative regulator of NF- κ B-mediated signaling.

PDLIM2, also known as SLIM (STAT-interacting protein), was originally isolated as a molecule that interacts with the STAT4 transcription factor. We found that PDLIM2 is a nuclear ubiquitin E3 ligase that promotes ubiquitination and subsequent proteasome-mediated degradation of STAT4, thereby negatively regulating Th1 cell differentiation (Tanaka, T. et al, *Immunity*, 22, 729-736, 2005). We have recently demonstrated that PDLIM2 negatively regulates NF- κ B activity, acting as a nuclear ubiquitin E3 ligase targeting the p65 subunit of NF- κ B (Tanaka et al, *Nat. Immunol.* 8, 584-

Recent publications

1. Yan, P., Fu, J., Qu, Z., Li, S., Tanaka, T., Grusby, M.J., Xiao, G. PDLIM2 suppresses HTLV-I Tax-mediated tumorigenesis by targeting Tax into the nuclear matrix for proteasomal degradation. *Blood*, 113, 4370-4380 (2009)
2. Kaisho, T. and Tanaka, T. Turning NF- κ B and IRFs on and off in DC. *Trends Immunol.*, 29, 329-336 (2008)

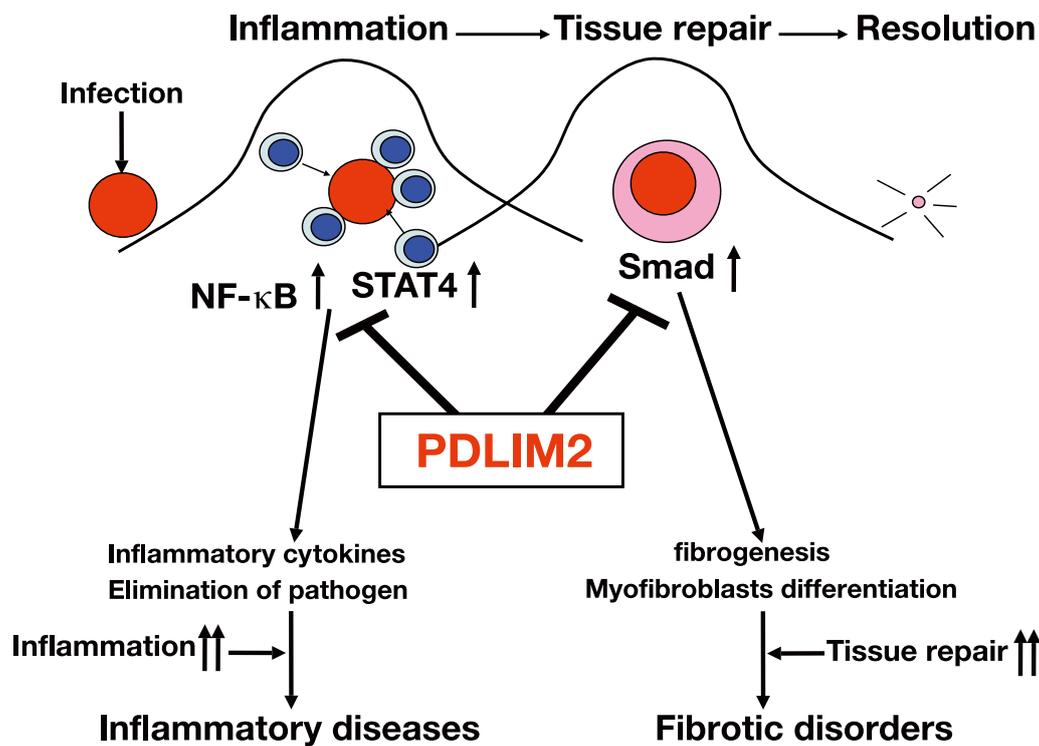


Figure : PDLIM2, a nuclear ubiquitin E3 ligase, negatively regulates both inflammatory and tissue repair responses.

591, 2007). PDLIM2 binds to p65 and promotes its polyubiquitination and targeting to discrete intranuclear compartments, called PML nuclear bodies, where the p65 is degraded by the proteasome. Consistent with these findings, PDLIM2 deficiency resulted in larger amounts of nuclear p65, defective p65 ubiquitination and augmented production of proinflammatory cytokines in response to innate stimuli. Thus, PDLIM2 can inhibit inflammatory responses by terminating NF-κB activation through its intranuclear sequestration and subsequent degradation. These findings suggest that upregulating PDLIM2 function can be applied for treating various inflammatory diseases. We therefore investigated how the activity of PDLIM2 itself is controlled. We attempted to identify the molecules that interact with PDLIM2 and modify its activity, and have recently found that heat shock protein 70 (HSP70) binds to PDLIM2 and promotes the degradation of NF-κB p65 synergistically with PDLIM2.

PDLIM2-mediated regulation of *in vivo* inflammatory responses

Several *in vivo* models are useful for further clarifying the

biological roles of the PDLIM2-mediated regulatory system, and for identification of potential PDLIM2-directed therapies. For example, granuloma formation is a type of host defense response against intracellular bacteria. We found that bacteria-induced development of granulomatous lesions is enhanced in PDLIM2-deficient mice. Moreover, we recently demonstrated that PDLIM2 also controls the tissue repair process. Skin wound healing is a typical model of inflammation-related tissue repair responses. We found that wound healing is significantly accelerated in PDLIM2-deficient mice. Moreover, TGFβ-mediated differentiation of myofibroblasts, the special fibroblasts essential for fibrogenesis and wound healing, is enhanced in fibroblasts derived from PDLIM2-deficient mice, which may account for the accelerated wound healing observed in these mice. Notably, PDLIM2 bound to Smad2/3, promoting their polyubiquitination and degradation, thus terminating TGFβ-mediated signaling.

These studies demonstrate that PDLIM2 is essential for the negative regulation of both inflammatory and tissue repair responses. PDLIM2 may be a new useful molecular target for the treatment of human inflammatory and fibrotic diseases.

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5. Hoshino, K., Sugiyama, T., Matsumoto, M., Tanaka, T., Saito, M., Hemmi, H., Ohara, O., Akira, S., and Kaisho, T. IκB kinase-α is critical for interferon-α production induced by Toll-like receptors 7 and 9. *Nature* 440, 949-953 (2006)

Unit Leader
Kanako Shimizu

Technical staff : **Miki Asakura**

Administrative Staff : **Chikako Nishimura**

Research Unit for Therapeutic Model

The goal of our laboratory is to develop immunotherapeutic models for cancer. We have been focusing on the role of *in vivo* DC maturation as a link between innate and adaptive immunity. In particular, we have studied the biological interactions between dendritic cells (DCs) and NKT cells. NKT cells can be stimulated with the glycolipid, alpha-galactosylceramide (α -GalCer)-loaded on APCs. NKT cells have unique immunoregulatory features that include the ability to rapidly produce large quantities of cytokines. We have been attempting to generate an approach for inducing adaptive immunity based on the adjuvant effect of α -GalCer and using *in vivo* DC maturation, which we have found to be more effective than *ex vivo* manipulation of DCs. Recently, we have developed an immunotherapeutic strategy using α -GalCer-loaded tumor cells for enhancing both innate (NKT and NK cells) as well as adaptive immunity (T cells). We further have been elucidating the mechanism of DC functions in detail and continue to analyze memory T cells. In addition, we developed this approach and established a cellular vector, composed of glycolipid-loaded, mRNA-transfected allogeneic fibroblasts to provide iNKT cell activation, leading to DC maturation and T cell immunity.

Analyses of critical role of host DCs in immunized mice with tumor cells loaded with α -GalCer.

We have demonstrated that tumor cells loaded with α -GalCer (tumor/Gal) are effective APCs for driving innate and adaptive immune responses, which are able to resist the establishment of metastases *in vivo*. With the strategy of using NKT cell ligand-loaded tumor cells, we initially found that tumor/Gal cells were killed by innate lymphocytes, mainly NKT and NK cells *in vivo* as above. Subsequently, we have continued to study the mechanisms of activation of adaptive immunity through *in vivo* maturation of DCs and to optimize tumor antigen delivery systems to DCs. When DCs *in situ* were analyzed, neighboring DCs captured dying tumor/Gal cells and then matured by activated NKT cells via CD40-CD40L interactions (Figure). Importantly, when we analyzed the crucial characteristics

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1. Fujii S, Goto A, Shimizu K. Antigen mRNA-transfected, allogeneic fibroblasts loaded with NKT-cell ligand confer antitumor immunity. *Blood* 113, 4262-72 (2009)
2. Shimizu K, Fujii S. DC therapy induces long-term NK reactivity to tumors via host DC. *Eur J Immunol.* 39, 457-68 (2009)



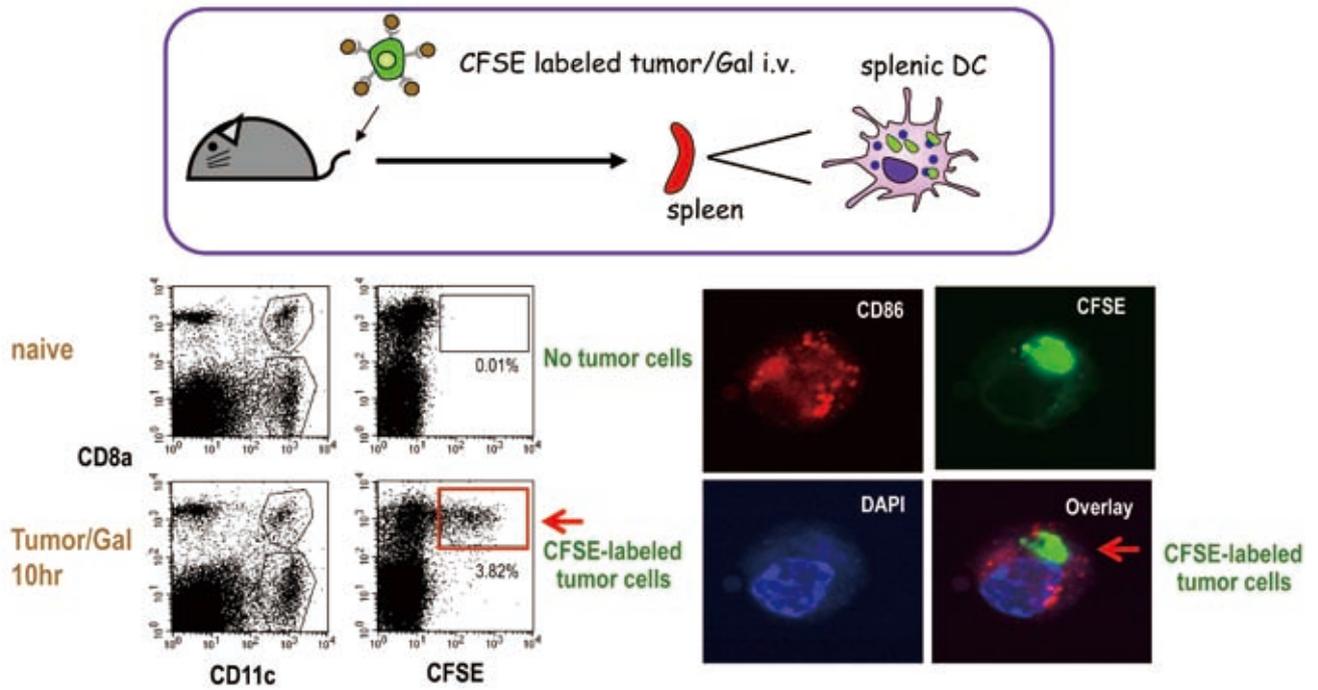


Figure: Maturing DCs capture tumor cells for cross-presentation *in situ*. Activated iNKT cells are responsible for DC maturation and cross presentation to T cells in tumor/Gal immunized mice. To understand the mechanism, *ex vivo* DCs were analyzed by flow cytometry and confocal microscopy. The maturing DCs captured CFSE-labeled tumor debris for at least 24 h after tumor/Gal administration. Confocal image of CD8a⁺DCs that have taken up the injected CFSE-labeled tumor cells.

of DCs *in situ* in the immunized mice, we have found that these cells can play a role in cross presentation of tumor antigen and glycolipid to T cells and NKT cells, respectively (two types of cross-presentation). After tumor regression occurred, a variety of T cells responding to a variety of specific tumor antigens, such as Trp2, Tyrp, Dct and gp100, persisted as memory T cells.

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

In the above studies, we demonstrated that the activation of invariant (i)NKT cells by administration of tumor/Gal cells can act as a cellular adjuvant through the maturation of DCs. In the current study, instead of tumor cells, we used allogeneic fibroblast cells loaded with α -GalCer and transfected with antigen-encoding mRNA, thus combining the

adjuvant effects of iNKT cell activation with delivery of antigen to DCs *in vivo*. We found that these cells produce antigen protein and activate NK and iNKT cells. When injected into MHC mismatched mice, they elicited antigen-specific T cell responses and provided tumor protection, suggesting that these immune responses depend on host DCs. Also, antigen expressing fibroblasts loaded with α -GalCer lead to a more potent T cell response than those expressing NK cell ligands. Thus, glycolipid-loaded, mRNA-transfected allogeneic fibroblasts act as cellular vectors to promote iNKT cell activation, leading to DC maturation and T cell immunity. By harnessing the innate immune system and generating an adaptive immune response to a variety of antigens, this unique tool could prove clinically beneficial in the development of immunotherapies for malignant and infectious diseases.

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4. Fujii S, Shimizu K, Hemmi H, Steinman RM. Innate V α 14⁺ natural killer T cells mature dendritic cells, leading to strong adaptive immunity. *Immunol Rev.* 220, 183-98 (2007)

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Group Director
Masaru Taniguchi

Senior Research Scientist : **Hiroshi Watarai**

Research Scientists : **Takuya Tashiro**
Michishige Harada

Visiting Scientist : **Nyambayar Dashtsoodol**

Technical Staff : **Sakura Sakata**
Etsuko Sekine-Kondo
Yuko Nagata
Sayo Inoue
Tomokuni Shigeura
Natsuki Kobayashi

Student Trainees : **Naomi Hongo**
Yohei Yoshiga

Administrative Staff : **Norie Takeuchi**



Laboratory for Immune Regulation

CD1d-restricted invariant natural killer T (NKT) cells bridge innate and acquired immunity and play an important role in both protective and regulatory responses. The nature of the response is dictated by the initial cytokine environment: interaction with IL-10-producing cells induces negative regulatory Th2 T cell-type NKT cells, while that with IL-12-producing cells results in pro-inflammatory Th1-type responses. Particularly in the anti-tumor response, NKT cells mediate adjuvant activity by their production of IFN- γ , which in turn activates both innate and acquired immune systems. Thus, upon activation of NKT cells, both MHC⁻ and MHC⁺ tumor cells can be efficiently eliminated. On the basis of these mechanisms, NKT cell-targeted adjuvant cell therapies have been developed and have shown great promise in initial clinical trials on cancer patients.

Identification of CD4⁺CD8⁻ double-negative natural killer T cell precursors in the thymus.

It is well known that CD1d-restricted V α 14 NKT cells are derived from cells in the CD4⁺CD8⁺ double-positive (DP) population in the thymus. However, the developmental progression of NKT cells during the earlier stages remains unclear, and the possible existence of NKT cell precursors in stages earlier than the DP stage had not been tested. We were able to demonstrate that NKT cell precursors expressing invariant V α 14-J α 18 transcripts but devoid of surface expression of the V α 14 receptor are present in the late CD4⁺CD8⁻ double-negative (DN)4 stage and have the potential to generate mature NKT cells in both *in vivo* and *in vitro* experimental conditions. Moreover, the DN4 population with NKT cell potential in CD1dKO mice was similar to that in wild-type C57BL/6 mice, but failed to develop into NKT cells *in vitro*. However, these precursors could develop into NKT cells when co-cultured with normal thymocytes or in an *in vivo* experimental setting, indicating that functional NKT cell precursors are present in CD1dKO mice. Together, these results demonstrate that thymic DN4 fraction contains NKT cell precursors. Our findings provide new insights into the early development of NKT cells prior to surface expression of the invariant V α 14 antigen receptor and suggest a

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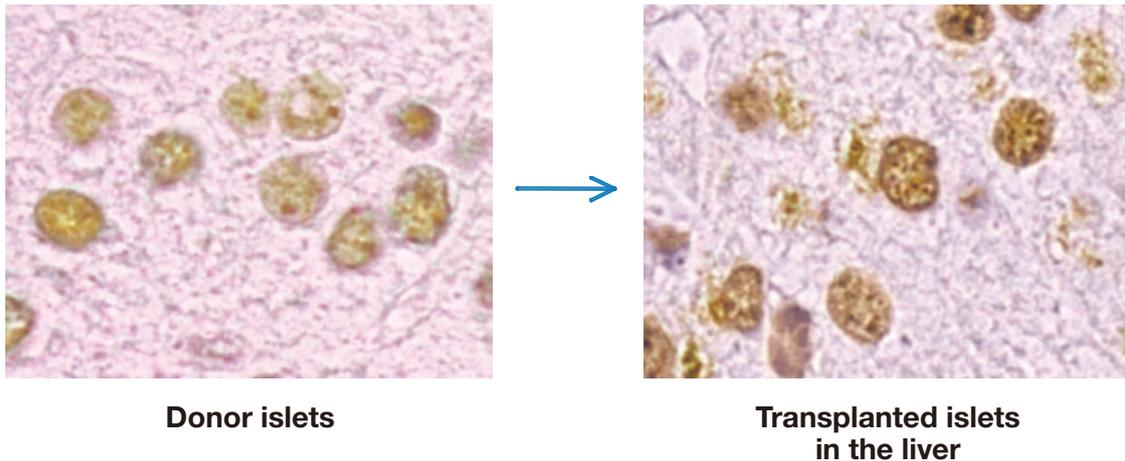


Figure: HMGB1 shuttled from nucleus to cytoplasm of islets after transplantation.

possible alternative developmental pathway for NKT cells.

Generation of functional NKT cells *in vitro* from embryonic stem cells bearing a rearranged invariant V α 14-J α 18 TCR α gene.

Establishment of a system for efficient generation of NKT cells from embryonic stem (ES) cells would enable us to identify the cells with NKT-cell potential and obtain NKT cells with desired function. Using cloned ES (NKT-ES) cells generated by the transfer of nuclei from mature NKT cells, we were able to establish a culture system that preferentially developed functional NKT cells. We also identified early NKT progenitors, which first appeared on day 11 as a c-kit^{lo} population in cocultures on OP9 cells expressing Notch ligand, delta-like1 (OP9/DII-1). The progenitor cells became c-kit^{lo/-} on day 14. Interestingly, in the presence of Notch signals, NKT-ES cells differentiated only into thymic-type CD44^{lo} CD24^{hi} NKT cells producing mainly interleukin-4 (IL-4). By contrast, NKT cells resembling CD44^{hi} CD24^{lo} liver NKT cells producing mainly interferon gamma (IFN- γ) and exhibiting strong adjuvant activity *in vivo* developed in switch cultures starting at day 14 in the absence of Notch. The cloned ES culture system offers a new opportunity for the elucidation of the molecular events during NKT-cell development and for the establishment of NKT-cell therapy.

High-mobility group box 1 is involved in the initial events of early loss of transplanted islets in mice.

Islet transplantation for the treatment of type 1 diabetes mellitus is limited in its clinical application mainly due to early loss of the transplanted islets, resulting in low transplantation efficiency. NKT cell-dependent IFN- γ production by Gr-1⁺CD11b⁺ cells is essential for this loss, but the upstream events in the process remain undetermined. We have demonstrated that high-mobility group box 1 (HMGB1) plays a crucial role in the initial events of early loss of transplanted islets in a mouse model of diabetes. Pancreatic islets contained abundant HMGB1, which was released into the circulation soon after islet transplantation into the liver (Fig.). Treatment with an HMGB1-specific antibody prevented the early islet graft loss and inhibited IFN- γ production by NKT cells and Gr-1⁺CD11b⁺ cells. Moreover, mice lacking two of the known HMGB1 receptors, TLR2 and receptor for advanced glycation end products (RAGE), but not the third HMGB1 receptor TLR4, failed to exhibit early islet graft loss. Mechanistically, HMGB1 stimulated hepatic mononuclear cells (MNCs) *in vivo* and *in vitro*; in particular, it upregulated CD40 expression and enhanced IL-12 production by DCs, leading to NKT cell activation, and subsequent NKT cell-dependent augmented IFN- γ production by Gr-1⁺CD11b⁺ cells. Thus, treatment with either IL-12- or CD40L-specific antibody prevented the early islet graft loss. These findings indicate that the HMGB1-mediated pathway eliciting early islet loss is a potential target for intervention to improve the efficiency of islet transplantation.

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5. Burrows PD, Kronenberg M, Taniguchi M. NKT cells turn ten. *Nat. Immunol.* 10(7):669-671(2009).

Team Leader
Katsuaki Sato

Technical Staff : **Kaori Sato**
Yumiko Sato
Kawori Eizumi

Student Trainee : **Tomohiro Fukaya (JRA)**
Hideaki Takagi (JRA)
Honami Taya

Administrative Staff : **Mai Yamamoto**
Naomi Uchimura



Laboratory for Dendritic Cell Immunobiology

Dendritic cells (DCs) are essential antigen-presenting cells (APCs) that initiate primary immune responses. DCs consist of heterogeneous subsets, including conventional DCs (cDCs) and plasmacytoid DCs (pDCs), distinguishable by surface and intracellular phenotypic markers, immunologic function, and anatomic distribution. Immature DCs (iDCs) serve as sentinels, recognizing the presence of invading pathogens through various pattern-recognition receptors. Subsequently, they become mature DCs (mDCs) with up-regulated expression of MHC and costimulatory molecules under inflammatory conditions. The mDCs then move via the afferent lymphatics into the T-cell areas of secondary lymphoid tissues, where they prime rare antigen-specific naive T cells for differentiation into effector T cells (T_{eff}), including T helper type (T_{H})1 cells, $T_{\text{H}}2$ cells, and $T_{\text{H}}17$ cells, depending on environmental cues. DCs thereby play a crucial role in the link between innate and adaptive immunity. Conversely, accumulating evidence suggests that systemic iDCs are also crucial for the induction of immunological tolerance under steady-state conditions. The tolerogenic mechanisms include recessive tolerance mediated by clonal deletion and anergy as well as dominant tolerance involving active immune suppression by $CD4^+Foxp3^+$ regulatory T cells (T_{regs}) in the periphery, a function of likely importance in self-tolerance as well as immune disorders and transplant rejection. However, the precise functional role of each DC subset in immune responses remains unclear. Our goal is to characterize the specific DC subsets involved in immune regulation.

Role of the B7 family of costimulatory molecules expressed on systemic and mesenteric lymph node dendritic cells in the differentiation of $Foxp3^+$ regulatory T cells and $T_{\text{H}}17$ cells

In this study, we examined the role of the B7 family of costimulatory molecules in the function of dendritic cells (DCs) in circulation and in mesenteric lymph nodes (MLNs) to induce differentiation of antigen-specific naive $CD4^+Foxp3^-$ T cells into $CD4^+Foxp3^+$ inducible regulatory T cells (iT_{regs}) and interleukin (IL)-17-producing $CD4^+$ T cells ($T_{\text{H}}17$ cells).

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2. Candolfi M., Yagiz K., Foulad D., Alzadeh G.E., Tesarfreund M., Muhammad A.K., Puntel M., Kroeger K.M., Liu C., Lee S., Curtin J.F., King G.D., Lerner J., Sato K., Mineharu Y., Xiong W., Lowenstein P.R., and Castro M.G. Release of HMGB1 in response to proapoptotic glioma killing strategies: efficacy

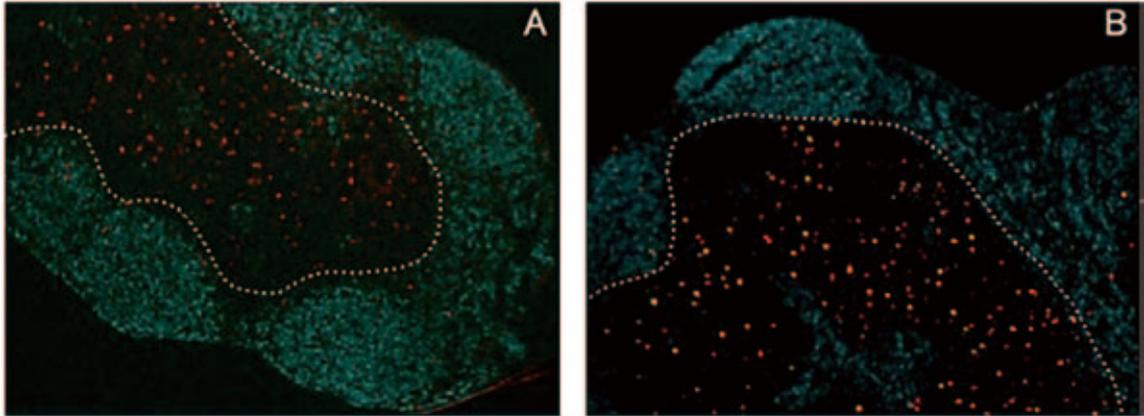


Figure: Antigen-specific *de novo* conversion of CD4⁺Foxp3⁺T cells into CD4⁺Foxp3⁺iT_{regs} in mesenteric lymph nodes during oral tolerance. KJ1-26⁺Foxp3^{EGFP}T cells were transferred into BALB/c mice, and the animals were subsequently fed PBS or OVA protein one day later. Cryosections from mesenteric lymph nodes on day 11 after the adoptive transfer were stained for KJ1-26 (red) and B220 (blue). Foxp3 was detected by green fluorescence from the Foxp3^{EGFP} knock-in allele.

In wild-type (WT) mice, MLN DCs displayed significantly higher levels of B7-H1 and B7-DC as well as I-A/I-E and CD103 than systemic DCs, whereas they showed similar levels of CD80, CD86, and B7-H2. MLN DCs markedly enhanced the generation of CD4⁺Foxp3⁺iT_{regs} from CD4⁺Foxp3⁺T cells, whereas, relative to systemic DCs, they significantly reduced the conversion of CD4⁺Foxp3⁺T cells into T_H17 cells. Analysis of mice deficient in B7 family costimulatory molecules (B7^{-/-}mice) suggested that B7-H1 and B7-DC expressed on systemic and MLN DCs are required for the conversion of CD4⁺Foxp3⁺T cells into CD4⁺Foxp3⁺iT_{regs}, whereas costimulation with CD80/CD86 through CD28 abrogated this conversion. In contrast, the induced differentiation of T_H17 cells by these DCs was enhanced in B7h1^{-/-}mice and B7dc^{-/-}mice or decreased in Cd80/Cd86^{-/-}mice and B7h2^{-/-}mice compared with WT mice, suggesting that B7-H1 and B7-DC suppress T_H17 cell differentiation, whereas CD80/CD86 and B7-H2 participate in this development. Collectively, the expression of B7-H1 and B7-DC could be essential for the ability of MLN DCs to induce the preferential differentiation of iT_{regs} rather than T_H17 cells. Thus, our findings suggest that the fine-tuning of iT_{regs}/T_H17 cell equilibrium by MLN DCs through the B7 family is instrumental in the maintenance of intestinal immune homeostasis.

Essential roles of B7-H1 and B7-DC in the establishment of oral tolerance mediated through the *de novo* generation of antigen-specific CD4⁺Foxp3⁺inducible regulatory T cells

Oral tolerance is a key feature of intestinal immunity, generating systemic tolerance to ingested antigens, however, the molecular mechanisms mediating oral tolerance remain unclear. In this study, we examined the role of the B7 family members of costimulatory molecules in the establishment of oral tolerance. Deficiencies of B7-H1 and B7-DC abrogated oral tolerance; this was accompanied by enhanced antigen-specific CD4⁺T cell-response and IgG1 production. Furthermore, the antigen-specific conversion of CD4⁺Foxp3⁺T cells into CD4⁺Foxp3⁺inducible regulatory T cells (iT_{regs}) occurred in MLNs to a greater extent than in peripheral organs during oral tolerance, and this conversion required B7-H1 and B7-DC, whereas it was severely impaired under inflammatory conditions. Together, B7-H1 and B7-DC appear to be necessary for inducing active immune suppression mediated through the *de novo* generation of antigen-specific CD4⁺Foxp3⁺iT_{regs} under steady-state conditions, thereby leading to the establishment of oral tolerance.

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Team Leader

Heiichirô Uono

Research Scientists : **Shusaku Mizukami**
Takashi Imai

Technical Staff : **Chiaki Kajiwara**
Hidetoshi Sugahara

Administrative Staff : **Akiko Imaizumi**



Laboratory for Immuno-chaperones

CD8⁺ T cells recognize naturally processed peptides in the context of MHC class I molecules. Peptides presented by MHC class I molecules are products of cellular proteins degraded by the proteasome. Ribosomal *de novo* synthesized, misfolded proteins (defective ribosomal products; DRiPs) are recognized by molecular chaperones (heat shock proteins:HSPs), hsc70/hsp40 and hsp90, and are believed to be polyubiquitinated by the E3 ubiquitin ligase, CHIP (carboxyl terminus of hsc70 interacting protein). These ubiquitinated DRiPs are degraded by the proteasome and have been implicated as the main source of antigen peptides presented by MHC class I molecules.

During viral infection or malignant transformation, intracellular changes in non-APC (antigen presenting cells) must be reported to CD8⁺ T cells, a process that is indispensable to fighting virus-infected cells and cancer cells. However, the cancer cells cannot act by themselves as APC to prime T cells, although they express tumor antigens on the cell surface. Moreover, APC such as DCs (dendritic cells) do not become infected by every virus, and thus, cannot present all viral antigens directly to CD8⁺ T cells. Therefore, DCs must internalize nearby tumor or infected cells, digest them, and then present antigen peptides to CD8⁺ T cells in the context of MHC class I molecules. This pathway is called cross-presentation and is believed to be specific to DCs, not other APC types such as macrophages or B cells.

We hypothesize that molecular chaperones such as hsp90 also play important roles in this antigen cross-presentation. Our research goal is to identify the mechanisms of proteasome-mediated epitope production and cross-presentation, especially focusing on the role of the molecular chaperone hsp90.

The role of hsp90 in endogenous MHC I antigen processing

Heat shock protein 90 (hsp90) and the proteasome activator, PA28, stimulate MHC class I antigen processing. However, whether hsp90 influences the proteasome activity to produce T cell epitopes was unknown, although association of PA28 with the 20S proteasome stimulates its enzyme activity.

Recent publications

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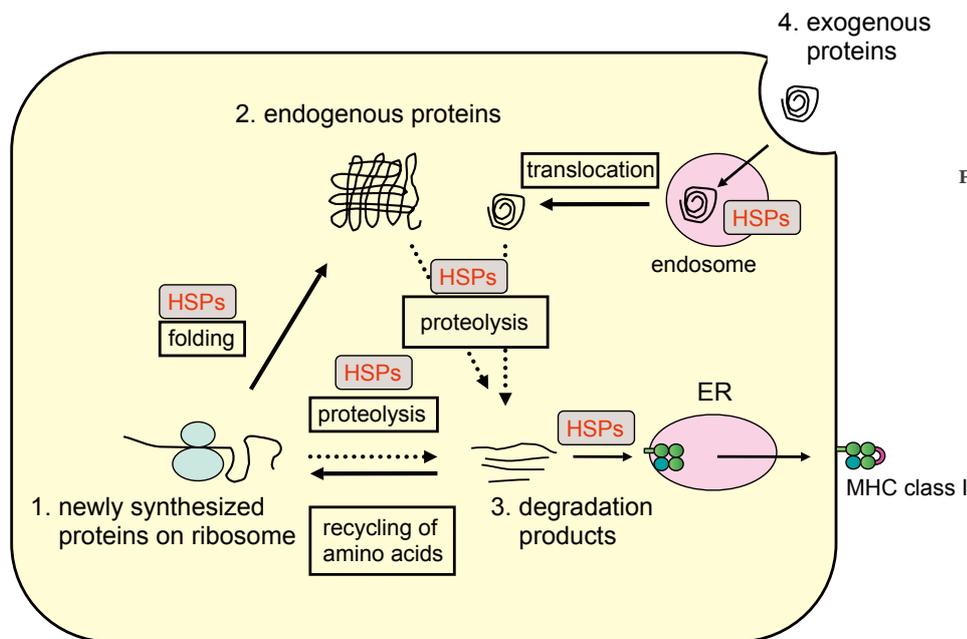


Figure: HSPs mediate degradation of a few percent of newly synthesized proteins as well as mature proteins. In dendritic cells (DC), HSPs seem to facilitate the degradation of even internalized exogenous proteins following their translocation to the cytoplasm. The proteolysis step depends on the ubiquitin-proteasome system to produce peptides presentable by MHC class I molecules.

We demonstrated that hsp90 is essential for assembly of the 26S proteasome and, as a result, is involved in epitope production. Addition of recombinant hsp90 α to cell lysates enhanced chymotrypsin-like activity of the 26S proteasome in an ATP dependent manner. We pulled down histidine-tagged hsp90 α - and PA28 α -induced, newly assembled 26S proteasomes from cell extracts for *in vitro* epitope production assays, and found that these structures are sensitive to geldanamycin, an hsp90 inhibitor. We found that a cleaved epitope unique to the proteasome was pulled down by both hsp90 α and PA28 α , while two different epitopes were identified in the hsp90 α and PA28 α pulldowns, respectively. Processing of these respective peptides *in vivo* was enhanced faithfully by the protein combinations used for the proteasome pulldowns. Inhibition of hsp90 *in vivo* by geldanamycin partly disrupted the 26S proteasome structure, consistent with downregulated MHC class I expression. Thus, hsp90 facilitates MHC class I antigen processing through epitope production in a complex of the 26S proteasome.

The role of hsp90 in antigen cross-presentation

In general, the main functions of HSPs are (i) facilitate protein folding/refolding and prevent aggregation of newly synthesized proteins on the ribosome; (ii) maintain the normal function of mature proteins; (iii) target misfolded and/or

damaged mature proteins for degradation by the proteasome; (iv) transport proteins between distinct microorganisms. All proteins denoted here ultimately undergo degradation by the proteasome in order to recycle amino acids and their by-products (degradation products, peptides) are simultaneously utilized as antigen peptides mainly presented by MHC class I molecules. Thus, the use of proteasomal degradation products as ligands of MHC molecules makes perfect sense in the immune system to alert the T cell immune system to any antigenic alterations, as well as life cycle of all proteins with no waste.

Each step towards proteolysis by the proteasome is, at least in part, regulated by HSPs. To consider how HSPs are involved in cross-presentation (in terms of translocation of endosomal antigen to cytosol), there could be two distinct mechanisms. First is that HSPs direct CHIP mediated polyubiquitinylation of retro-translocated unfolded proteins for proteasomal degradation. Second is that HSPs direct retro-translocation of proteins from endosome to cytosol, a model supported by the findings that Bip and cytosolic hsp70/hsp90 drive polypeptide into the ER and the mitochondria, respectively. Our recent observations clearly indicate that HSPs play a pivotal role in antigen trafficking, an activity that links them to antigen cross-presentation as well as endogenous antigen processing.

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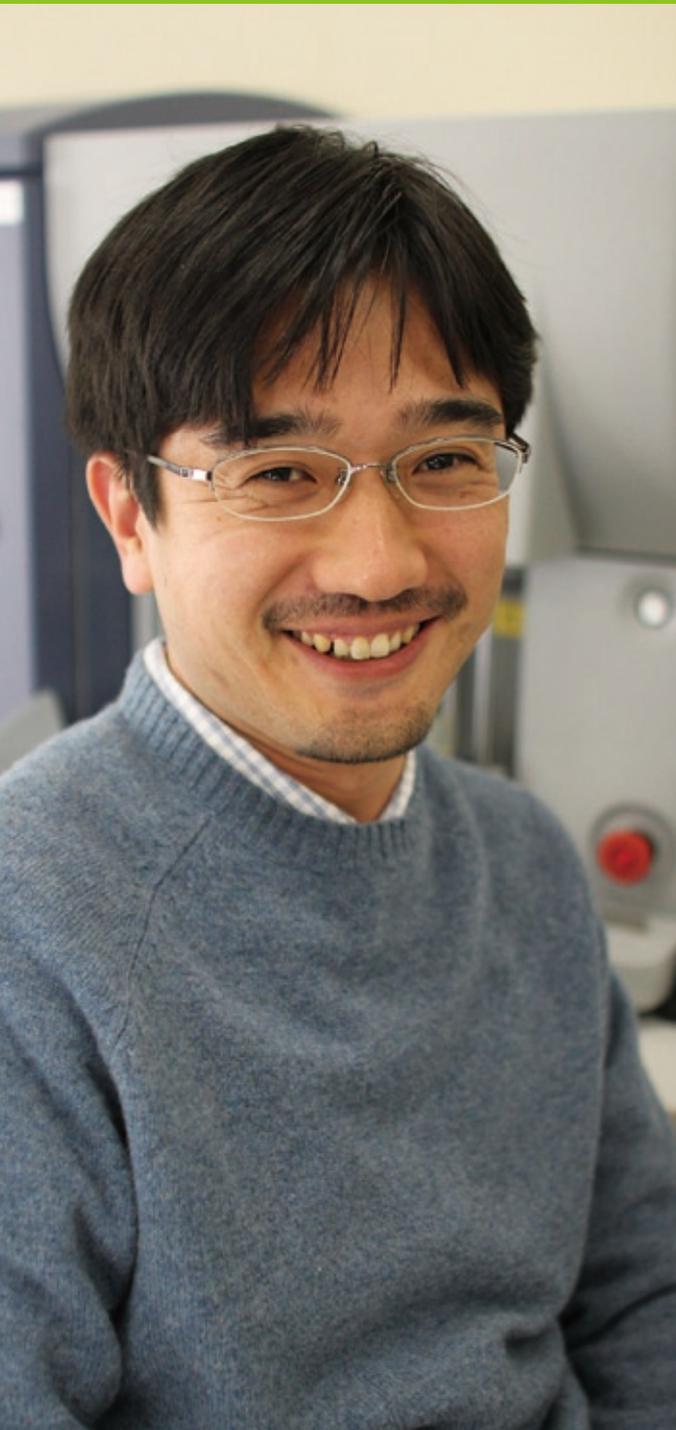
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Unit Leader
Shohei Hori

Research Scientists : **Masashi Tachibana**
Maria Encarnita Mariotti-Ferrandiz
(JSPS Foreign Post-Doctoral Fellow)

Technical Staff : **Takahisa Miyao**
Nana Shimizu
Erina Furuhata
Guanying Wang



Research Unit for Immune Homeostasis

A small subpopulation of T lymphocytes known as regulatory T (T_{reg}) cells play a central role in preventing pathological immune responses including autoimmunity, inflammation and allergy, and thus ensure dominant tolerance to self and innocuous environmental antigens. This has been well illustrated by the findings that the development and function of T_{reg} cells is controlled by the X-linked transcription factor Foxp3 and that defective generation of functional T_{reg} cells underlies the catastrophic autoimmune pathology that develops in Foxp3-mutant *scurfy* mice and human patients with the IPEX syndrome.

The identification of Foxp3 as a central regulator of T_{reg} cell differentiation and function has provided a key to a number of outstanding unresolved questions concerning their role 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.

Stability and plasticity in regulatory T cell differentiation

Early studies of organ-specific autoimmunity provoked by neonatal thymectomy, and of transplantation tolerance induced by previous transplantation of xenogeneic or allogeneic thymic epithelium have suggested that natural Foxp3⁺ T_{reg} cells represent a distinct thymus-committed lineage of T lymphocytes, although it has also become evident that additional Foxp3⁺ T_{reg} cells can be generated from peripheral naïve CD4⁺ T cells upon “tolerogenic” antigen presentation. Recent findings have challenged, however, this notion of a stable lineage and suggested that T_{reg} cells may be a flexible population exhibiting developmental plasticity. To reconcile these apparently contradictory views, we examined the stability of Foxp3 expression and T_{reg} phenotype in natural Foxp3⁺ T cells isolated from un-manipulated Foxp3-reporter mice. Our results showed that, whereas the majority of natural CD4⁺Foxp3⁺ T cells maintain stable Foxp3 expression after adoptive transfer to lymphopenic or lymphoreplete recipients, a minor fraction enriched within the CD25⁻ subset actually lose expression. Some of those Foxp3⁻ T cells adopt effector helper T (T_H) cell functions. Of

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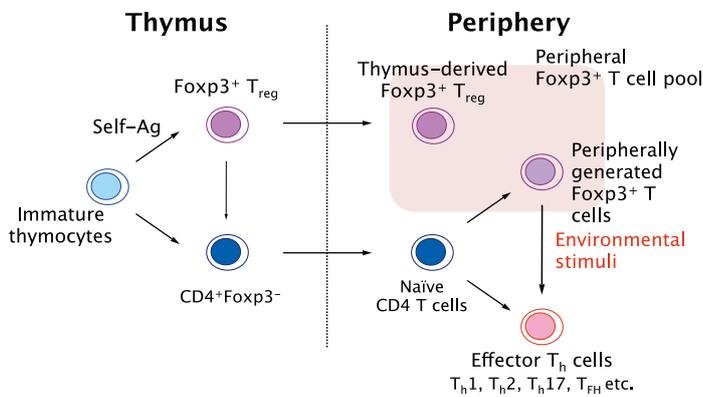


Figure: A model for stability and plasticity in regulatory T cell differentiation. Immature thymocytes up-regulate Foxp3 upon recognition of self-antigens presented by thymic stromal cells. Although some of Foxp3⁺ thymocytes may fail to stabilize Foxp3 expression and thus undergo abortive T_{reg} differentiation, thymus-generated Foxp3⁺ T cells that are stably incorporated into the peripheral Foxp3⁺ T cell pool exhibit stable Foxp3 expression and are thus irreversibly committed, thereby ensuring tolerance to self-antigens. In contrast, Foxp3⁺ T cells generated from peripheral naive Foxp3⁺ CD4⁺ T cells show unstable Foxp3 expression and can be converted into other effector T_h cells upon suitable environmental stimuli, thereby contributing a delicate balance between immune responsiveness and tolerance to environmental (non-self) antigens.

note, they preferentially differentiated into follicular B helper T (T_{FH}) cells that promoted IgA production in the Peyer's patches. This minority "plastic" population exhibits flexible responses to cytokine signals, relying on TGF- β to maintain Foxp3 expression, and responding to others by differentiating into effector T_h cells *in vitro*. In contrast, CD4⁺Foxp3⁺CD25^{high} T cells are resistant to such conversion to effector T_h cells even after many rounds of cell division. These results demonstrate that natural Foxp3⁺ T cells are a heterogeneous population consisting of a committed T_{reg} lineage and an uncommitted subpopulation with developmental plasticity.

To address the origin, nature, and functions of these committed and plastic populations under physiological and pathological conditions, we have recently established a mouse model that allows us to track previous history of Foxp3 expression as well as ongoing Foxp3 transcription. Our recent analyses revealed that plastic Foxp3⁺ T cells are contained predominantly, if not exclusively, within peripherally generated Foxp3⁺ T cells, whereas thymus-derived Foxp3⁺ T cells that are stably incorporated into the peripheral pool exhibit remarkably stable Foxp3 expression (Fig.). We suggest that the plasticity of Foxp3⁺ T cells contributes to the maintenance of a delicate balance between immune responsiveness and tolerance to non-self antigens in the face of environmental perturbations, whereas the stability of the T_{reg} lineage ensures robust tolerance to self-antigens even under inflammatory environments. The molecular and epigenetic basis of T_{reg} lineage commitment in the thymus is one of the next focus areas for our research.

Dissecting the Foxp3-dependent molecular program of T_{reg} cell development and function

In order to understand the molecular mechanisms by which Foxp3 controls T_{reg} cell differentiation and function, we have been addressing whether and how Foxp3 mutations that had been identified in IPEX patients impair T_{reg} cell develop-

ment and function. We previously showed that all the missense mutations examined were amorphic or hypomorphic in that, upon retroviral transduction, these mutants fail to convert conventional T cells into fully functional T_{reg} cells. To determine the impact of these mutations *in vivo*, we have established three independent knock-in mouse strains harboring *foxp3* mutations that mirror those in human IPEX. We found that hemizygous mutant males developed a *scurfy*-like lymphoproliferative autoimmune disease, although mice harboring hypomorphic mutations developed a milder disease than mice with *scurfy* or amorphic mutations. Furthermore, by analyzing healthy heterozygous female mice, in which mutant and wild-type Foxp3⁺ T_{reg} cells co-exist (due to random X-chromosome inactivation), we found that two of the three mutations compromised thymic differentiation, peripheral maintenance, and suppressive effector functions of Foxp3⁺ T_{reg} and affected overall transcriptional profiles. In contrast, T_{reg} cells expressing one of the mutants displayed normal thymic differentiation, normal *in vitro* suppressor function and a transcriptional profile similar to that of wild-type T_{reg} cells. Interestingly, however, the mutant T_{reg} cells were less competitive than wild-type T_{reg} cells in the periphery and showed altered expression of a small subset of Foxp3 target genes, suggesting that impaired peripheral homeostasis of T_{reg} cells underlies the autoimmune pathology in this mutant strain. We are currently investigating which Foxp3 target gene(s) are responsible for this homeostasis defect and dissecting the molecular mechanisms that control T_{reg} cell homeostasis.

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

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

4. Hori, S. Rethinking the molecular definition of regulatory T cells. *Eur. J. Immunol.* 38, 928-930 (2008)

5. Komatsu, N. and Hori, S. Full restoration of peripheral Foxp3⁺ regulatory T cell pool by radioresistant host cells in *scurfy* bone marrow chimeras. *PNAS* 104, 8959-8964 (2007)

Group Director
Osami Kanagawa

Senior Research Scientist : **Michio Tomura**

Research Scientists : **Naoki Ogawa**
Yosiko Mori

Technical Staff : **Hidemi Uchiyama**
Mitsuyo Takase
Takako Kato
Edith Kanagawa
Gyobai Yu
Saori Misawa

Administrative Staff : **Izumi Inoue**
Chiyo Takahashi



Laboratory for Autoimmune Regulation

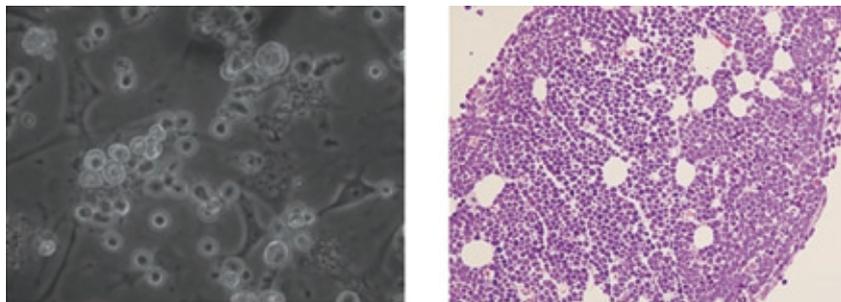
In my laboratory, we are developing new technologies for the analysis of immune responses *in vivo* and applying these technologies for the understanding of the autoimmune process. We have established, at present, four new *in vivo* imaging technologies: 3D visualization of target organ and cells, monitoring cellular migration/emigration using the photoconvertible fluorescence protein “Kaede”, *in vivo* visualization of cell death with the caspase 3 indicator SCAT3.1 fluorescence protein, and *in vivo* visualization of lymphocytes / hematopoietic cell cycle using Fucci fluorescence protein. These technologies can be used to identify lymphocytes *in vivo* based on their location, movement and functions. Since we have the technology to establish mouse lines derived from a single lymphocyte using nuclear transfer, it is now possible to isolate lymphocytes directly from animals using these imaging technologies and establish a mouse line from a single lymphocyte. These technologies will be used in all aspects of immunology research. Our focus is to apply these technologies to understanding the development of autoimmune type I diabetes in the NOD mouse model.

Although KAEDE, SCAT3.1 and Fucci imaging systems are very useful for *in vivo* imaging, these transgenic mice express fluorescence protein in all cell types due to the use of viral promoter. This ubiquitous expression of fluorescence protein often caused a problem with very high background. Thus, we made knockin (KI) mouse lines in which expression of these proteins can be induced with tissue specific Cre transgenes. These mouse lines will provide better *in vivo* imaging systems for cells and tissues of interest.

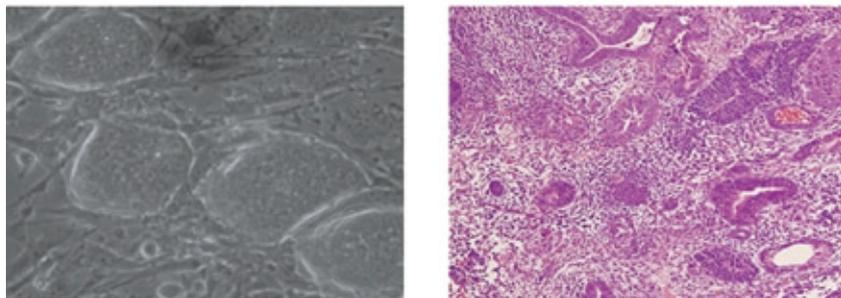
Monitoring Molecular Movement using Diffracted X-ray tracking (DXT) has been undertaken in collaboration with Drs. Kozono (Tokyo Science Univ.) and Sasaki (Spring8). DXT can detect sub-micro second level movements of proteins in solution and has been shown to be a useful tool to visualize molecular movement in physiological conditions. We used this technology to monitor the movement of the MHC/peptide complex and found that the movement of the peptide in the groove is relatively constant among peptides with different affinity to the MHC. However, movement of

Recent publications

1. Tomura M, Itoh K, Kanagawa O. Naive CD4⁺ T Lymphocytes Circulate through Lymphoid Organs To Interact with Endogenous Antigens and Upregulate Their Function. *J Immunol.* 184, 4646-53, 2010
2. Fagarasan S, Kawamoto S, Kanagawa O, Suzuki K. Adaptive immune regulation in the gut: T cell-dependent and T cell-independent IgA synthesis. *Annu Rev Immunol.* 28:243-73, 2010



B Lymphoma



Lymphoma derived ES cell

Figure : Comparison of the original B lymphoma and the lymphoma-derived ES cells. In vitro cultured cells (*left panel*) and in vivo tumor/teratoma formation (*right panel*)

MHC differs significantly depending on the peptide in the groove. This is the first demonstration that peptides bound to the MHC influence the structural stability of the MHC/peptide complex. Moreover, analysis of the relationship between T cell recognition of MHC/peptide complex and movement of MHC/peptide complex revealed the following new information. 1) T cell recognition of the complex is greatly affected, either positively or negatively, by the movement of whole complex. Some T cells prefer a stable complex but others respond exclusively to unstable complexes. 2) comparison of the movement of two low affinity peptides in the groove revealed distinct differences. This change can also influence T cell reactivity by more than 100 fold. These results indicate that the TCR is extremely sensitive to the molecular movement of the MHC/peptide complex. Since activation of T cells by unstable MHC/peptide is a unique feature of diabetes related MHC class II (I-Ag7 in mouse and DQ2 and DQ8 in human), our observations here should provide new insights into the TCR/MHC/peptide interactions that control pathogenic T cell activation for the development of autoimmune diabetes.

Pluripotent stem cells (PSCs) provide a promising source of cells for the treatment of a wide range of diseases, particularly in tissue regeneration and repair. To date, PSCs have been successfully derived from differentiated cells, in the normal or diseased state, using either a nuclear transfer or a gene expression protocol. While tumor-derived stem

cells may have significant potential in advancing cancer research and drug development, there were reported limitations with the reprogramming of various tumor cells to the stem cell state. In collaboration with Dr. Wakayama's group at CDB Kobe RIKEN, we demonstrate that PSCs could indeed be established from a B- and a T-lymphomas at frequencies comparable to those with normal lymphocytes. The PSCs generated from a Myc-induced B lymphoma exhibited gene expression patterns and pluripotency characteristics similar to those of normal ES cells (A comparison between the original tumor and ES cells can be seen in the Fig.). The PSCs can differentiate into tumor cells *in vitro* that grow autonomously and form tumors *in vivo*. Unexpectedly, while the generation of the parental B lymphoma cells is Myc-dependent, the progeny tumor cells have evolved to be Myc-independent. These results show that the genetic factors that promote tumor initiation are distinguished from those required to maintain tumorigenicity at later stages. Thus, understanding the mechanism of oncogenesis requires a more comprehensive approach with additional emphases placed at the later stages of tumor development. Our study also shows that PSCs derived from tumors could be a unique and powerful tool for cancer research. We are currently attempting to establish cancer derived iPSC in human system in collaboration with Dr. Yamanaka's group at Kyoto University.

3. Tomura M, Honda T, Tanizaki H, Otsuka A, Egawa G, Tokura Y, Waldmann H, Hori S, Cyster JG, Watanabe T, Miyachi Y, Kanagawa O, Kabashima K. Activated regulatory T cells are the major T cell type emigrating from the skin during a cutaneous immune response in mice. *J Clin Invest.* 120(3):883-93, 2010

4. Tomura M, Mori YS, Watanabe R, Tanaka M, Miyawaki A, Kanagawa O. Time-lapse observation of cellular function with fluorescent probe reveals novel CTL-target cell interactions. *Int Immunol.* (10):1145-50, 2009

5. Hong H, Takahashi K, Ichisaka T, Aoi T, Kanagawa O, Nakagawa M, Okita K, Yamanaka S. Suppression of induced pluripotent stem cell generation by the p53-p21 pathway. *Nature.* 460(7259):1132-5, 2009

Group Director
Toshio Hirano

Senior Research Scientist : **Toshiyuki Fukada**
Keigo Nishida

Research Scientists : **Satoru Yamasaki**
Wakana Ohashi
(Research Associate)

Technical Staff : **Ayumi Ito**
Masami Kawamura
Mayumi Hara

Student Trainee : **Shintaro Hojyo (JRA)**
Byn Beum-Ho (JRA)
Aiko Hasegawa

Administrative Staff : **Ryoko Masuda**
Mizuki Shimura



Laboratory for Cytokine Signaling

Despite their increasing prevalence in developed countries, the molecular mechanisms leading to autoimmune diseases and allergies remain poorly understood. The ultimate 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.

Role of zinc and its transporter in mast cell-mediated allergic reactions

We are investigating the role of Zn of mast cells in allergy and immune responses. Previously, we have used the Zn chelator TPEN to show that Zn is required for Fc epsilon RI-induced granule translocation to the plasma membrane, mast cell degranulation, and cytokine production (Kabu et al., *J Immunology*. 2006). In addition, we found that Zn 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. These reports suggested that Zn is involved in Fc epsilon RI-mediated mast cell function. Based on this finding, we are analyzing in role of zinc transporters in mast cell-mediated allergic reactions. We reported that a Zn transporter, Znt5/Slc30a5 is required for Fc epsilon RI-mediated cytokine production, but not degranulation. As a result, *Znt5*^{-/-} mice had defects in mast cell-mediated delayed-type allergic reactions but not in the immediate-type reaction. Znt5 was required for Fc epsilon RI-induced translocation of PKC to the plasma membrane and the nuclear translocation of NF-

Recent publications

1. Nishida K*, A. Hasegawa*, S. Nakae, K. Oboki, H. Saito, S. Yamasaki, and T. Hirano. (*equal contribution) Zinc transporter Znt5/Slc30a5 is required for the mast cell-mediated delayed-type allergic reaction but not the immediate-type reaction. *J. Exp. Med.* 206, 1351-1364, (2009)
2. Sawa Y., Y. Arima, H. Ogura, C. Kitabayashi, J-J Jiang, T. Fukushima, D. Kamimura, T. Hirano, and M. Murakami. Hepatic Interleukin-7 Expression Regulates T Cell Responses. *Immunity* 30, 447-457, (2009)

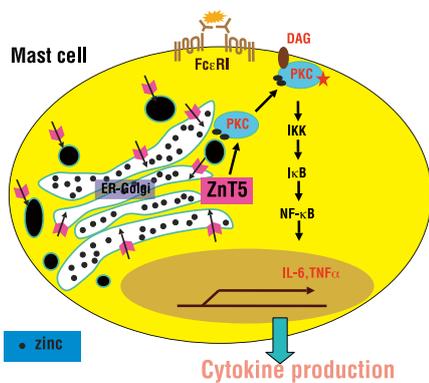


Figure 1: Role of the zinc transporter, Znt5/Slc30a5 in Fc epsilon RI-mediated signaling pathway

Znt5 is selectively required for Fc epsilon RI-mediated cytokine production, and is a novel player in PKC signaling.

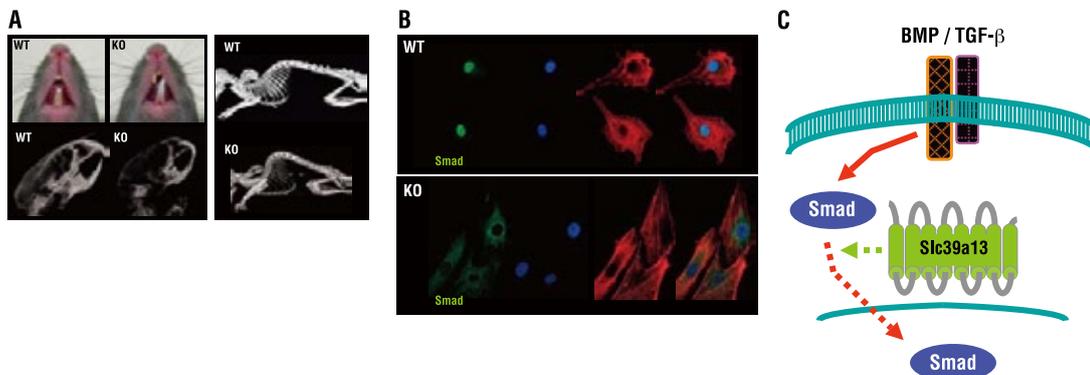


Figure 2: Significant role of the zinc transporter Slc39a13/Zip13 in connective tissue development.

A. Left: *Slc39a13*-KO mice show abnormal tooth development and reduction of bone mass. Right: *Slc39a13*-KO mice develop kyphosis, and show impairment of cartilage development.

B. Nuclear localization of Smad proteins (green) mediated by BMP4 stimulation is impaired in *Slc39a13*-KO osteoblasts. Nuclei (blue), actin (red)

C. Involvement of *Slc39a13*/Zip13 in BMP/TGF- β signaling pathways. *Slc39a13* protein, which is expressed in osteoblasts, odontoblasts, chondrocytes, and fibroblasts, controls nuclear translocation of Smad transcription factors.

kappa B. Thus, Znt5 is selectively required for mast cell-mediated delayed-type allergic responses, and is a novel player in PKC signaling (Fig. 1, Nishida et al., *J. Exp. Med.* 2009).

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

We found that the Zn transporter *Slc39a6*/Zip6/Liv1 is a STAT3 target gene and showed that it has a role in cell migration during early zebrafish development. (Yamashita et al., *Nature*. 2004). We also found that LPS-induced maturation of DCs is partly mediated through lowering the intracellular concentration of free Zn by down regulating Zn transporters, including *Slc39a6* (Kitamura et al., *Nature Immunology*. 2006). These observations suggest that intracellular Zn is involved in MHC class II cell surface expression through regulating endocytosis and membrane trafficking. In order to understand the role and function of Zn transporters *in vivo*, we have generated mice deficient in the *Slc39*/Zip family of zinc transporters. Recently, we found that *Slc39a13*/Zip13 deficient mice show changes in con-

nective tissues reminiscent of the human disease, Ehlers-Danlos syndrome (EDS). The *Slc39a13*-KO mice have defects in the maturation of osteoblasts, chondrocytes, odontoblasts, and fibroblasts. Impairment of bone morphogenic protein (BMP) and TGF- β signaling was observed in the corresponding tissues and cells. Homozygosity for a *SLC39A13* loss of function mutation was identified in sibs affected with a unique variant of EDS that recapitulates the phenotype observed in *Slc39a13*-KO mice. Hence, our results have revealed a crucial role of *SLC39A13* in connective tissue development at least in part due to its involvement in BMP/TGF- β signaling pathways (Fig. 2, Fukada et al, *PLoS ONE*, 2008). Together these results support the idea that Zn transporters have roles not only in maintaining Zn homeostasis, but also for mediating intracellular signaling events. We propose the term “Late Zn signaling” for this type of Zn signaling, in contrast to “Early Zn signaling”. The former is dependent on changes in the transcription of Zn transporter genes, whereas the latter is not (Hirano et al, *Adv. Immunol.*, 2008).

3. Fukada, T*, N. Civic*, T. Furuichi*, S. Shimoda, K. Mishima, H. Higashiyama, Y. Idaira, Y. Asada, H. Kitamura, S. Yamasaki, S. Hojyo, M. Nakayama, O. Ohara, H. Koseki, H.G. dos Santos, L. Bonafe, R. Ha-Vinh, A. Zankl, S. Unger, M.E. Kraenzlin, J.S. Beckmann, I. Saito, C. Rivolta, S. Ikegawa, A. Superti-Furga, and

T. Hirano. (*equal contribution). The Zinc Transporter *SLC39A13*/ZIP13 is Required for Connective Tissue Development; Its Involvement in BMP/TGF- β Signaling Pathways. *PLoS ONE* 3 (11), e3642, (2008)

4. Hirano, T., M. Murakami, T. Fukada, K. Nishida, S. Yamasaki, and T. Suzuki. Roles of Zinc and Zinc signaling in immunity: Zinc is an intracellular signaling molecule. *Advances in Immunology* 97, 149-176, (2008)

5. Ogura, H.*, M. Murakami*, Y. Okuyama, M. Tsuruoka, C. Kitabayashi, M. Kanamoto, M. Nishihara, Y. Iwakura and T. Hirano. (*equal contribution) Interleukin-17 Promotes Autoimmunity by Triggering a Positive-Feedback Loop via Interleukin-6 Induction. *Immunity* 29, 628-636, (2008)

Team Leader

Masato Kubo

Visiting Scientist : **Shin-ichiro Ohno**

Student Trainees : **Yasutaka Motomura (JRA)**
Minoru Sawaguchi
Emi Ohkubo
Tatsuki Ogura

Technical Staff : **Yoshie Suzuki**

Administrative Staff : **Eiko Hayashi**



Laboratory for Signal Network

T cells play a central role in the effector and regulatory functions in immune surveillance, and aberrations in these functions can lead to various immunological disorders. T helper 1 (Th1) cells secrete interleukin-2 (IL-2), IFN- γ and TNF- α in cellular immune responses against intracellular pathogens and viruses. Th2 cells produce IL-4, IL-5, IL-6, IL-10 and IL-13, mainly against extracellular pathogens, and account for allergic immune responses. Th17 cells make IL-17 and IL-22 in response to certain bacterial infections, but are mainly known for their association with autoimmune tissue inflammation. These 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.

MINA53, an *IL4* co-repressor controlling Th2-bias

Th2-bias, the propensity of naïve CD4 T cells to differentiate into high-level IL-4 secreting Th2 cells, is a genetic trait impacting infectious, autoimmune and allergic disease susceptibility. When the immune system encounters a potential threat, antigen-presenting cells deliver peptide from the invading pathogen to naïve T cells. These naïve cells respond by differentiating into mature helper T cells. IL-4 drives Th2 differentiation into high-level IL-4 secreting Th2 cells, resulting in a positive feedback loop that fuels Th2 production while suppressing Th1 production through the IL4R/STAT6/GATA3 pathway. The degree of initial IL-4 production resulting in Th2 bias can have serious clinical implications because Th2 bias is tightly associated with the pathology of allergy. It was known that the BALB/c strain of mice have a strong Th2 bias, producing large quantities of IL-4 following T cell activation, while B10.D2 mice have a strong Th1 bias. However, a hybrid BALB/c strain that contains chromosome 16 from the B10.D2 strain also exhibited Th1 bias, suggesting that chromosome 16 includes a gene pertinent to this phenotype. Closer analysis highlighted *Mina53*, a JmjC family member, as a genetic determinant of Th2-bias. *Mina53* specifically binds to and represses the *IL4* promoter. Its over-expression in transgenic mice impairs *IL4*

Recent publications

1. Kubo, M., Notch: filling a hole in T helper 2 cell differentiation. *Immunity* 27, 3-5, 2007
2. 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 the transcription factor IRF1 to the IFN- γ -IL-12 axis of signaling networks in CD4⁺ T cells. *Nature Immunology* 9 (1):34 - 41, 2008

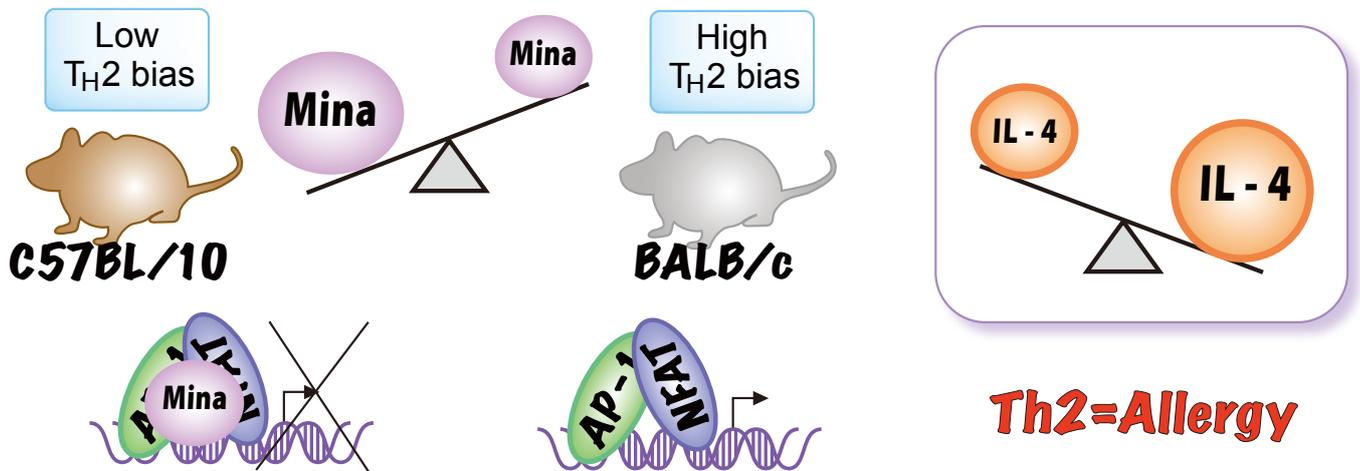


Figure: Th1/Th2 bias and Mina expression. Th1 bias strain C57BL/10 (including B10.D2) has high expression of Mina, while Th2 bias strain BALB/c has low expression of Mina. Mina protein binds to the NFAT binding site on the *Il4* promoter and impairs *Il4* transcription, leading to repression of *Il4* and inhibition of allergic response.

expression, while its knockdown in primary CD4 T cells leads to *Il4* derepression. Together, these findings provide mechanistic insight into an *Il4* regulatory pathway controlling Th differentiation and genetic variation in Th2-bias.

Natural occurring IL-17 producing T cells regulate the airway responses.

Effector Th17 cells are a major source of IL-17, a critical inflammatory cytokine in autoimmune diseases and in host defenses during bacterial infections. Recently, splenic lymphoid tissue inducer (LTi)-like cells have been reported to be a source of T cell independent IL-17 (Takatori, H., J. Exp. Med. 206:35-41, 2008). Here, we report that the immune system contains a unique set of naturally occurring IL-17 producing cells, "natural" Th17 (nTh17), which are a memory-like T cell subset. The nTh17 cells can develop in the

absence of the IL-6/STAT3 signaling axis required by inducible Th17 (iTh17) cells. The nTh17 cell population is distinct from conventional iTh17 cells, since nTh17 cells express substantial amounts of IL-17A, but not IL-17F, under the control of the master regulator, ROR γ t. The nTh17 cells simultaneously produce IFN- γ . DO11.10 transgenic (Tg) mice on a Rag $^{-/-}$ background (DO11.10 Rag $^{-/-}$) lack nTh17 cells, and, following intranasal administration of OVA, IL-17 dependent-neutrophil infiltration occurs in DO11.10 Tg mice, but not in DO11.10 Rag $^{-/-}$ mice. This impaired neutrophil-dependent airway response is restored by adoptive transfer of nTh17 cells into the DO11.10 Rag $^{-/-}$ mice. These results demonstrate that a novel T cell subset, nTh17, facilitates the early phase of antigen-induced airway responses and host defenses against pathogen invasion prior to the establishment of acquired immunity.

3. Lu, L-F, Thai, T-H, Dinis Pedro Calado, D.P., Chaudhry, A., Kubo, M., Kentaro Tanaka, K. Gabriel, B. G., Lee, H., Yoshimura, A., Klaus Rajewsky, R., Alexander Y. Rudensky Foxp3-dependent microRNA155 confers competitive fitness to regulatory T cells through targeting SOCS1. *Immunity* 30:80-91, 2009.

4. Okamoto, M., Van Stry, M., Chung, L., Koyanagi, M., Sun, X., Suzuki, Y., Ohara, O., Kitamura, H., Hijikata, A., Kubo, M.*and M., Bix,*; MINA53, an *Il4* co-repressor controlling Th2-bias *Nature Immunology* 10, 8, 872-879, 2009.*co-corresponding authors.

5. Tanaka, S., Yoshimoto, T., Naka, T., Nakae S., Iwakura Y., Cua, D. and Kubo, M.; Natural Occurring IL-17 producing T cells regulate the initial phase of neutrophil mediated airway responses. *J. Immunol.* 183(11), 7523-7530, 2009

Team Leader
Hisahiro Yoshida

Research Scientist : **Takuwa Yasuda**

Technical Staff : **Hitomi Fukiage**
Ayako Kobayashi
Mikiko Sato
Akimi Banno
Yuko Dokiya

Student Trainee : **Machiko Sugiyama**

Administrative Staff : **Aiko Iyama**



Laboratory for Immunogenetics

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 have screened 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

Recent publications

1. Wada H., Yasuda T., Miura I., Watabe K., Sawa C., Kamijuku H., Kojo S., Taniguchi M., Nishino I., Wakana S., Yoshida H., Seino K. Establishment of an improved mouse model for infantile neuroaxonal dystrophy that shows early disease onset and bears a point mutation in Pla2g6. *Am J Pathol.* 175(6), 2257-63 (2009)
2. Kakugawa K., Yasuda T., Miura I., Kobayashi A., Fukiage H., Satoh R., Matsuda M., Koseki H., Wakana S., Kawamoto H., Yoshida H. A novel gene essential for the development of single positive thymocytes. *Mol Cell Biol.* 29(18), 5128-35. (2009)

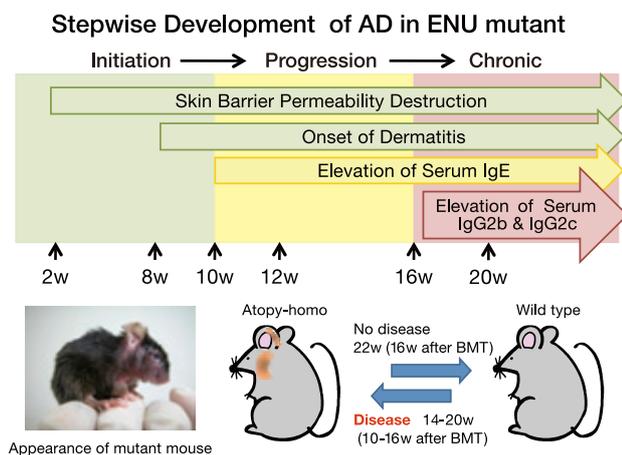


Figure1: An atopic dermatitis-like mutant mouse was established from the ENU screen. The scheme indicates the stepwise progression of AD disease in this mutant animal. The study of the precise mechanism(s) of the responsible mutation in this disease is now underway. Photo shows the dermatitis in some of the heterozygotes.

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

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 at 3 weeks after the dermatitis onset. Pathologically, the epidermal layer was hypertrophic and many inflammatory cells were found in the dermis of the lesion. Finally, they developed chronic inflammation in their face and ear skin and not only Th2 serum immunoglobulin but also Th1 serum immunoglobulin level increased. These stepwise symptoms and findings are compatible with the criteria for human infantile atopic dermatitis (AD). We have mapped the phenotype responsible point mutation to a distinct gene and confirmed the phenotype is induced by the mutation by genetic manipulation of the wild type animal. Bone marrow transplantation study clarified the disease is induced by mutant skin tissue but not by mutant bone marrow. We are now analyzing the mechanism, which initiates the AD like disease in this mutant animal to prevent

ENU mutant phenotype screen; 80 pedigrees, 4 years

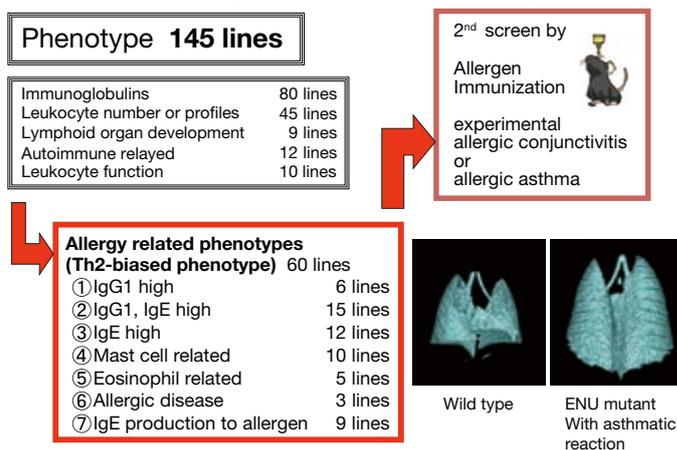


Figure2: Summary of four years of ENU mutant screening. In total, approximately 8,000 mutant mice from 80 genomes have been screened and 145 phenotypes have been identified in mutant lines. Figures in right bottom show the 3 dimensional image of lungs after OVA immunization and challenge.

or cure the AD in this animal model.

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

We are now backcrossing some of the mice to the C3H/HeJ strain in order to map the responsible loci. As of December 2009, we have mapped twenty eight independent mutant-responsible loci to distinct regions, and nine of them have been identified to be point mutations of independent genes. Along with allergic disease model mutant screening, we have identified and characterized a few other interesting phenotype mutant line with/without allergic defects. One of the interesting mutant animal had a defect in thymocyte development resulting in peripheral T cell number reduction. The responsible point mutation was mapped to a gene coding an unknown molecule participating in single positive thymocyte development in thymus. Although the mutation of the human orthologue is not reported yet, this gene would be a good candidate for searching the cause of the immunodeficiency condition in human patients.

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Team Leader
Yasuyuki Ishii

Visiting Scientists : **Hidetoshi Akimoto**
Satoshi Komaniwa
Omar Duramad
Kenichi Masuda

Technical Staff : **Haruka Katagiri**
Risa Nozawa

Laboratory for Vaccine Design

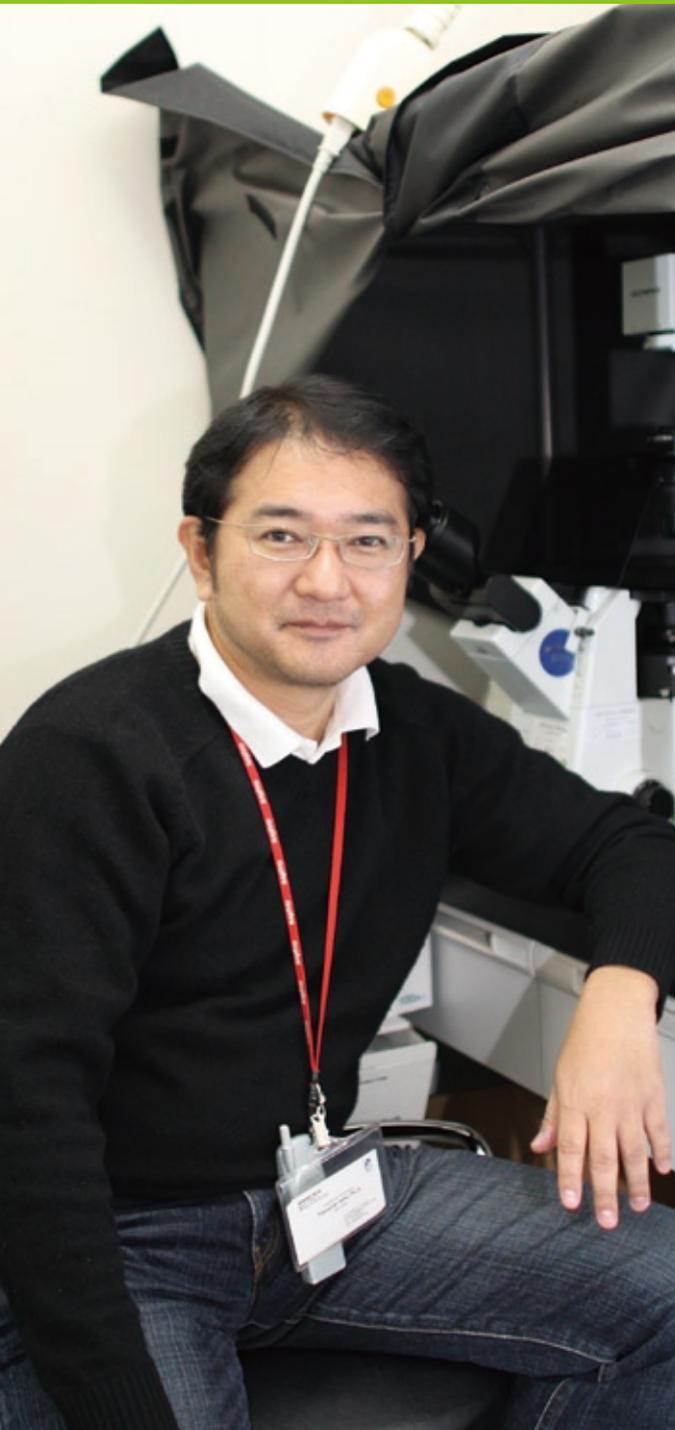
Allergen-specific immunotherapy (ASIT) for various pollinosis is globally utilized in the treatment of allergies. However, in Japan, the only ASIT is subcutaneous immunotherapy (SCIT) for Japanese cedar pollinosis and it is not so popular due to the length of the treatment and an unclear mechanism of action. We have been studying two types of vaccine technologies with the goal of clinical application of ASIT for treatment of Japanese cedar pollinosis. The first candidate vaccine is a PEGylated recombinant fusion protein of Cry j 1 with Cry j 2, which has been designed to have much less anaphylactic risk than natural allergen extracts, and could be utilized not only for SCIT but also for sub-lingual immunotherapy (SLIT). The second candidate vaccine is a liposomal α -GalCer, a ligand for invariant natural killer T (iNKT) cells, encapsulating the Cry j 1/2 fusion protein. These liposomes showed not only cause reduction of on-going IgE antibody formation but also suppress tertiary antibody responses. Moreover, oral administration of this vaccine also showed efficacy in suppressing the IgE antibodies. Experiments have been carried out to elucidate the detailed mechanisms of both vaccines.

PEGylated recombinant Cry j 1/2 fusion protein

Our recombinant Cry j1/2 fusion protein is composed of full length mature regions of the Cry j 1 and Cry j 2 proteins containing all potential human and mouse T-cell epitopes to overcome the problems associated with all the other recombinant polypeptide vaccines so far developed. For the purpose of drug development, we have to develop a method to prepare homogenous samples. However, there are 23 target cysteine residues for PEGylation in the recombinant fusion protein, indicating that PEGylated proteins would be very heterogeneous. To produce a uniform product, we generated a mutant fusion protein in which all but one of the cysteine residues was replaced by serine. The fusion protein does not retain any IgE-binding epitopes because the native tertiary structure of Cry j 1 and Cry j 2 regions are destroyed by the fusion of these two proteins. In fact, the binding of IgE antibodies derived from 100-cedar pollinosis patients to the fusion protein was completely lost, although

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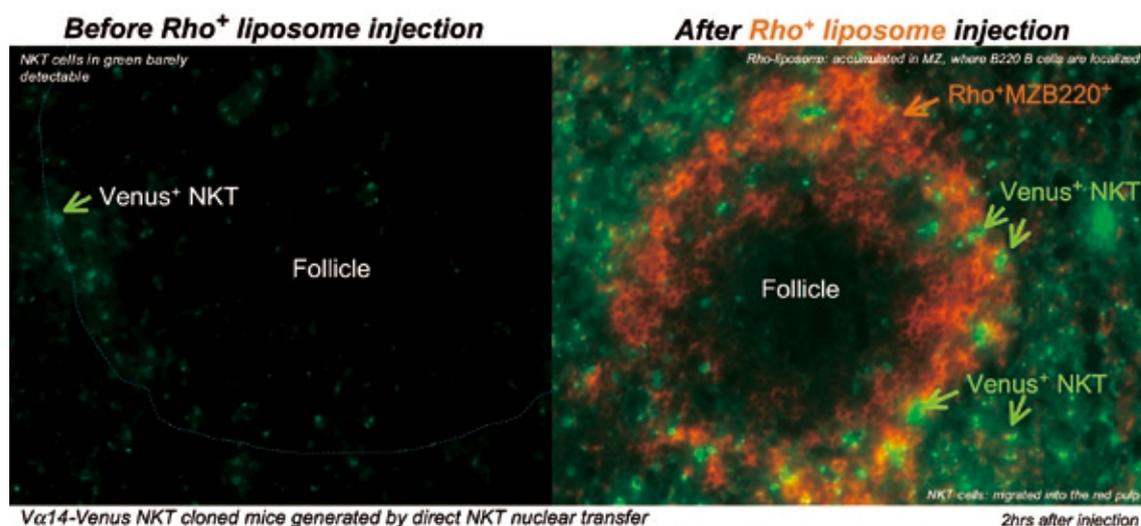


Figure: Migration of venus-NKT cells (green) and accumulation of Rho-liposome (red) *in vivo*

most of the patients were strongly reactive to native Cry j1 protein. This result suggests that PEGylated recombinant fusion protein will not bind to IgE/FcεRI on the surface of mast cells and basophils, so there should be no risk of anaphylaxis shock. Next, to test whether the recombinant protein could enhance antibody responses against native cedar pollen antigens, mice were immunized with alum-adsorbed PEGylated recombinant protein and boosted with native Cry j 1 protein. Anti-native Cry j 1 IgE antibody formation was not induced by priming with the PEGylated recombinant protein. On the other hand, anti-native Cry j 1-IgG1 antibody formation was observed in this situation, although the titer was remarkably low compared to native Cry j 1-priming. Although anti-recombinant IgG1 antibody was detected in the immunized mice, IgE antibody was not. These results collectively indicate that the PEGylated recombinant fusion protein is safe because it does not bind to IgE antibodies of patients and there is no induction of cedar antigen-specific IgE antibody formation.

Liposomal α -GalCer -Cry j 1/2 fusion protein

The original idea of the liposome vaccine was born from the observation that α -GalCer could lead to IFN- γ production by iNKT cells, Th1 differentiation and the suppression of IgE antibody responses. However, the injection of liposomal α -GalCer-recombinant Cry j 1/2 fusion protein into native Cry j-primed mice resulted in the suppression of IgE anti-

body as well as IgG1 and IgG2a antibody responses.

To elucidate the detailed mechanism of liposome vaccine-mediated antigen specific suppression of antibody responses, the liposome vaccine target cells were first analyzed by using rhodamine-labeled liposomes. The results of flow cytometric analysis indicated that the major target cells of the liposome vaccine are splenic marginal zone B220⁺CD1d^{high} cells but not B220⁺CD1d^{low} cells. It has been reported that the chemokine receptor CXCR6 is preferentially expressed on iNKT cells. Flow cytometric analysis showed that a ligand for CXCR6, CXCL16, was highly expressed on the surface of rhodamine-positive B220⁺CD1d^{high} cells but not on the rhodamine-negative cells, suggesting that iNKT cells could potentially be attracted to the B220⁺CD1d^{high} cells in the spleen. Histopathological analysis clearly indicated that iNKT cells colocalized with the cells that had taken up the liposomes (Fig.). The significance of the interaction of iNKT cells with marginal zone B220⁺CD1d^{high} cells was supported by the result that co-administration of neutralizing antibody against CXCL16 with liposomal α -GalCer -Cry j 1/2 fusion protein in the primed mice diminished the immunosuppressive effects of the liposome vaccine. Taken together, these results suggest that the interaction of iNKT cells with marginal zone B220⁺CD1d^{high} cells is essential for the immunosuppressive activity of the liposomal α -GalCer -Cry j 1/2 fusion protein.

Unit Leader
Fumihiko Ishikawa

Research Scientists : **Yoriko Saito**
Yuho Najima

Technical Scientist : **Hidetoshi Ozawa**

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

Student Trainees : **Shinsuke Takagi**
Yuki Aoki

Administrative Staff : **Toshiko Nakamura**



Research Unit for Human Disease Models

Current immunology and biomedical research has largely been driven by investigations of murine biology. Although mouse research will continue to provide important biological insights in the 21st century, we would also like to better understand human immunology and try to overcome human disorders that cannot be cured with current therapeutics.

To accomplish these goals, we developed an *in vivo* model enabling us to investigate normal human hematopoiesis and immunity as well as disorders involving these systems. In the FY2009, we attempted to address the following three major issues using this *in vivo* model.

- 1: Creation of a novel humanized mouse system to evaluate HLA-restricted human immune response
- 2: Identification of a mechanism underlying human AML relapse and development of therapeutic interventions against AML leukemic stem cells
- 3: Understanding of the developmental pathway from human iPS/ES cells to the hematopoietic lineage

These three issues are considered to be essential to translate research findings into medicine based on human immunology and humanized mouse research. We here report progress made on two major projects in our laboratory during FY2009.

Creation of a novel humanized mouse with HLA-restricted human CTL response

Especially since the IL2 γ -null mutated immune-compromised newborn transplantation model has been established, humanized mice have contributed significantly to human immunology research. However, we encounter a major constraint in that human T cells mature and are selected through their interaction with mouse thymic epithelial cells. Therefore human T cells developing in a mouse thymic environment fail to demonstrate HLA-restricted function. To overcome the lack of HLA-restricted human immune response, we evaluated human T cell development and its function in a novel immune-compromised NOD/SCID/IL2 γ null mouse expressing covalently bound HLA-class I heavy chain and light chain (NSG-HLA-A2). Transplantation

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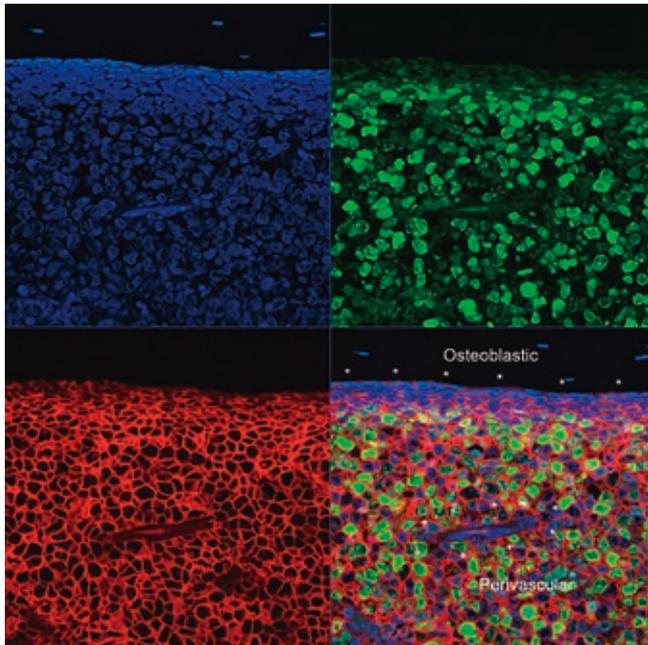


Figure: Preferential location of LSCs in the endosteal niche. (Upper left) Nuclear staining with DAPI (Upper right) Staining for Ki67 (Lower left) Staining for hCD45 (Lower right) Merged image

of purified Lin-CD34⁺CD38⁻ human hematopoietic stem cells into NSG-HLA-A2 newborns resulted in the development of human CD4⁺ and CD8⁺ TCR $\alpha\beta$ ⁺ T cells and CD4⁺CD8⁻ and CD8⁺ TCR $\gamma\delta$ ⁺ cells in the recipient BM and spleen. Human CTLs become functionally mature in these mice, as evidenced by the production of granzyme, corresponding to the phenotypic transition from naïve to effector memory CTLs. EBV infection in the humanized NSG-HLA-A2 recipients resulted in the formation of lymphoproliferative lesions consisting mainly of human B cells with scattered human T cells. Human CTLs developing in the recipients recognized EBV-derived peptides in an HLA-restricted manner and exerted HLA-restricted cytotoxicity against autologous and HLA-matched EBV-infected human B cells. The HLA-expressing humanized mouse with functional HLA-restricted T cells has overcome a major constraint in human immunology research using the humanized mouse model, and will serve as a model for investigation of human immune responses against pathogens and development of therapeutic strategies against human diseases.

Understanding AML relapse and therapeutic interventions eliminating LSCs

Although the concept of cancer stem cell has yet to be completely clarified, we previously demonstrated that human AML consists of heterogeneous cell fractions and that the CD34⁺CD38⁻ cells satisfy all the criteria for leukemic stem cells proposed by the American Association for Cancer

Research. We further identified the specific location of chemotherapy resistant LSCs as the osteoblastic niche rather than the perivascular niche. Now we demonstrate that the cells in the central region of the BM are actively cycling, while the LSCs in the endosteal region are cell cycle quiescent. We further explored the therapeutic strategies for eliminating LSCs to improve patient outcome and to overcome relapse. One of the strategies we have taken is to modify the cell cycle status of the human AML LSCs. Treatment of AML engrafted mice with G-CSF resulted in the cell cycle induction of LSCs in the niche. After cell cycle induction, chemotherapy eradicated LSCs to a substantial extent. The other strategy we take is to compare global gene expression profiles of normal long-term hematopoietic stem cells and leukemic stem cells. Both types of stem cells can generate all the cells in their respective system and are characterized by the CD34⁺CD38⁻ phenotype. Among various gene categories, we focused on those with characteristics favorable for drug development. We identified specific genes expressed in LSCs but not in HSCs at the RNA level and the protein level. Among the candidates, we confirmed the expression of several genes specifically in AML stem cells residing in the osteoblastic niche that keep AML LSCs quiescent and chemotherapy resistant. We are currently developing an shRNA lentiviral infection system to elucidate whether down-regulation of identified candidate molecules results in anti-leukemic activity *in vivo*.

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marrow endosteal region. *Nature Biotechnology* 25(11):1315-21,2007.

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Unit Leader

Shin-ichiro Fujii

Technical staff : **Yohei Shozaki**

Student Trainee : **Asako Kikuchi**

Administrative Staff : **Chikako Nishimura**

Research Unit for Cellular Immunotherapy

Human $V\alpha 24^+$ NKT cells bearing an invariant $V\alpha 24J\alpha 18$ antigen receptor are activated by a specific ligand, α -GalCer, in a CD1d-dependent manner. We previously showed that circulating $V\alpha 24$ NKT cells present in lung cancer patients were functional. We 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/IIa study we detected an increase in the number of NKT cells in the high dose DC administration group, and then continued to use that cell number in a subsequent Phase IIa trial. Based on our initial analyses, we have focused on a careful immunological analysis of lung pre- and post- treatment with this therapy in phase I/IIa trials (A collaboration with RCAI Director Dr. Taniguchi and Drs. Nakayama and Motohashi in Chiba University).

We have also studied the role 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.

Phase I/IIa immunotherapy for advanced non-small cell lung cancer (NSCL) patients (A collaboration with Chiba University and RCAI Director Dr. Taniguchi)

We have been developing a Phase I/IIa clinical study of the application of NKT cell therapy for advanced non-small cell lung cancer (NSCLC) patients (stage IIIB, IV or recurrence). α -GalCer-pulsed APCs ($1 \times 10^9/m^2$ PBMC-DCs) were intravenously administered four times. Twenty-three patients were enrolled and 17 patients (73.9%) completed the study. When we compared the responder group, which had an increased number of IFN- γ producing NKT cells and the poor responder group, the MST (median survival time) was

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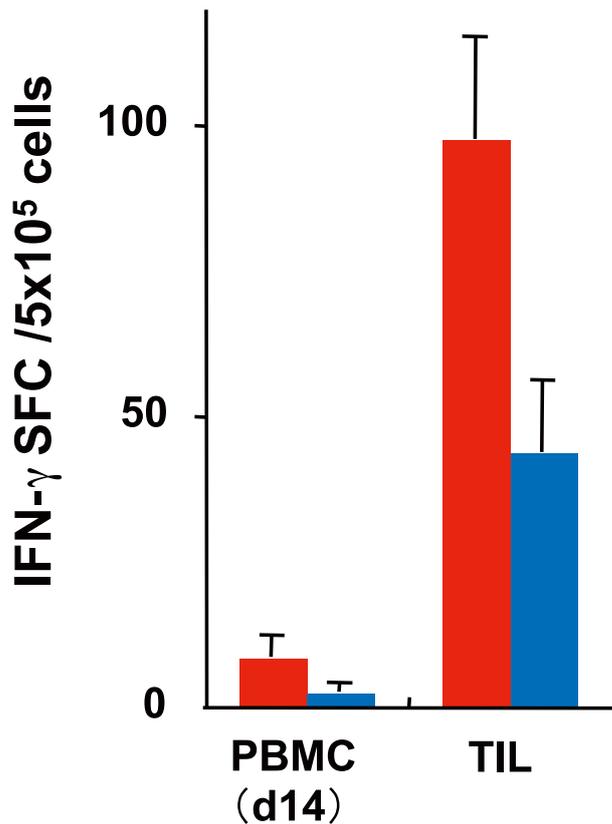


Figure : The IFN- γ producing NKT cells increased in the lung of NSLC patients treated with DC/Gal. Prophylactic therapy by administration of DC/Gal can prevent recurrence in operative lung cancer patients (stage IIB and IIIA) in a phase I/II trial. NKT cells in tumor infiltrating lymphocytes (TILs) in DC/Gal treated patients were analyzed at surgery. IFN- γ ELISPOT assays were performed using mononuclear cells (MNCs) derived from peripheral blood or lung after culture in the presence (red) or absence (blue) of α -GalCer. NKT cells capable of producing IFN- γ persisted in the tumor region rather than in peripheral blood.

longer in the responder group (31.9 months) than in the poor responder group (9.7 months). Thus, the increased IFN- γ production by NKT cells upon α -GalCer stimulation was significantly associated with clinical outcome. In the other Phase I/IIa study with prophylactic therapy by administration of DC/Gal to prevent recurrence in operative lung cancer patients, we have analyzed the NKT cells in the tumor infiltrating lymphocytes (TILs) in lung where we detected the higher number of NKT cells capable of producing IFN- γ (Fig.). These phase I/IIa trial results are encouraging and warrant further evaluation of the survival benefit of this immunotherapy.

A critical role of host DCs in immunized mice treated with α -GalCer-loaded tumor cells (A collaboration with Dr. Shimizu, Therapeutic Model Research Unit)

We showed that tumor cells loaded with α -GalCer are effective APCs for driving innate and adaptive immune responses,

which are able to resist tumor metastases *in vivo*. With the strategy of using NKT cell ligand-loaded tumor cells, we initially found that tumor/Gal cells were killed by innate lymphocytes, mainly NKT and NK cells *in vivo*. Subsequently, we have continued to study the mechanisms of activation of adaptive immunity through *in vivo* maturation of DCs and to optimize tumor antigen delivery systems to DCs. When DCs *in situ* were analyzed, neighboring DCs captured dying tumor/Gal cells and were then matured by activated NKT cells via CD40-CD40L interactions. Importantly, when we analyzed the crucial characteristics of DCs *in situ* in the treated mice, we found that DCs can play a role in cross presentation of tumor antigen and glycolipid to T cells and NKT cells, respectively (two types of cross-presentation). After tumor regression occurred, T cells responding to specific tumor antigens, such as Trp2, Tyrp, Dct and gp100, persisted as memory T cells.

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Team Leader

Mariko Okada (Hatakeyama)

Research Scientists : **Takeshi Nagashima**

Yuichi Shiraishi

Yuko Saeki

Technical Staff : **Ryoko Nakayama (until Dec, 2009)**

Student Trainee : **Yoshimi Naruo**

Administrative Staff : **Rie Morita**



Laboratory for Cellular System Modeling

Genome projects have successfully revealed the genetic features of humans, and enormous amounts of omics data have accumulated over recent years, yet, it is unclear how all these parts are connected and function to produce the desired biological outputs. Understanding of network structures and regulatory relationship of molecules, extraction of general design principles from this information and its clinical application have been a long time challenge for biologists. Especially for mammalian cells, such a generalization of design principles was thought to be almost impossible. However, the idea that such universal cellular logic designs and motifs are distributed in living organisms and carried over during an evolutionary process has started to become more widely accepted. Such network structures often shift the cellular state to different, irreversible phenotypes in a dynamic cellular reaction process using limited numbers of genes. The laboratory for Cellular Systems Modeling has been carrying out research to identify and reconstruct biological networks and basic regulatory principles from quantitative data using computational, theoretical and experimental biology approaches. Particularly our laboratory focuses on system analysis of signal transduction networks, which determine cell fate in mammalian cells. Biological findings, mathematical models and computational algorithms developed in our laboratory have been widely used for prediction of drug effects or identification of biomarker in cancer.

The main topic of our current research is quantitative analysis of membrane receptor signaling and its transcriptional control in the cell differentiation process. For this purpose, we take both bottom-up and top-down approaches. For bottom-up studies, kinetic mathematical modeling of pathway and parameter acquisition using computational and experimental approaches are carried out. This approach is quite useful when the components and their interconnections in the reaction network are already known. However, even in the most well-studied cellular signaling pathways, quantitative reaction kinetics and their effect on global cellular changes are not well understood. Our working hypothesis is that small quantitative changes in

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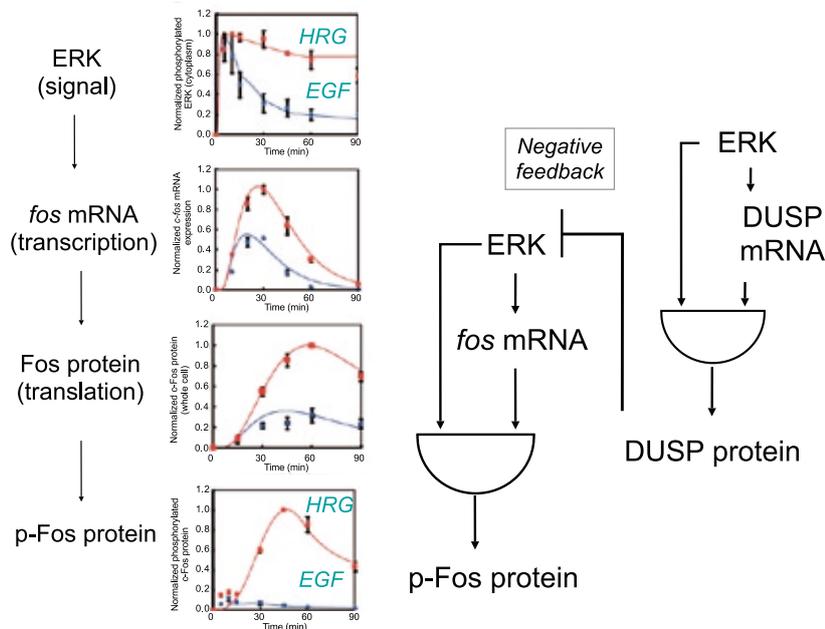


Figure: Cells respond to different growth factors (epidermal growth factor; EGF and heregulin; HRG) and induce distinct ERK activation profiles and binary production of activated Fos proteins. The binary production of Fos is thought to direct cells to different phenotypes (EGF; proliferation and HRG; differentiation). Kinetic modeling (right) with feedforward, negative feedback and AND-gate for p-Fos production only in the differentiation process stimulated by HRG.

signaling pathways eventually cause a switch-like response in the cell decision-making process. The question then becomes where, when and what molecules are most responsible for these events. To answer these questions, we take time-course quantitative experimental data using cultured cell line (MCF-7 breast cancer cell as a cell model) that undergoes proliferation and differentiation in response to different growth hormones. Based on the data and the literature, we constructed ordinary differential equation (ODE)-based kinetic models. For the past few years, we have worked on the model to explain digital (all-or-none) outputs of c-Fos transcription factor activation (Cell, 2010). The model can explain that a structure with co-existence of a signal-dependent feedforward loop of *de novo* c-fos transcription and c-Fos stabilization by the same signal (an AND-gate structure) can generate the digital output of c-Fos. This model is also valid for production of transcription factors in B-cell development and neurite elongation of PC12 cells, therefore it is most likely that the model captures general network principles of signal network during cellular differentiation process. Once a computational model is established, we determine critical kinetic parameters by experiments for validation and generation of new hypotheses. We collaborate closely with the single molecular imaging laboratory (Y. Sako, RIKEN ASI) and obtain association constants and diffusion constants of proteins in living MCF-7 cells. We believe that membrane receptors may determine the majority of cellular kinetics, therefore we focus on the

receptor and effector proteins with SH2 domains. We also predict binding kinetics of protein-protein interactions in signaling pathways using molecular dynamics (MD) simulation.

For the top-down approach, we perform transcriptome and proteome analyses to determine overall systems properties and unknown molecular regulations. Microarray and ChIP-chip transcriptional analysis have been done for the same MCF-7 cell platform. We are interested in the signal (context)-dependent activation of transcription factors and rewiring of transcriptional regulation. Therefore we performed phospho-tyrosine and phospho-serine/threonine proteome analysis using LC mass spectrometry in collaboration with Tokyo Univ. Integrated analysis of transcriptome and phosphoproteome data generated several hypotheses that explain drug-resistance mechanisms in breast cancer cells. We also developed in-house database and statistical models for analysis of these omics data.

Our mathematical model of membrane receptor signaling pathway (Biochem J. 2003 and Mol. Syst. Biol. 2007) has been referenced and modified for prediction of drug effects by a group at MIT (Mol Syst Biol 2009) and used for a clinical study to identify biomarkers in drug-resistant breast cancer by an Edinburgh group (Cancer Res 2009). Since systems biology studies are not accomplished without close collaboration with authorities in broad areas of science, we have established variety of international and domestic collaborations to resolve our biological problems.

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Group Director
Osamu Ohara

Senior Research Scientist : **Hiroshi Kitamura**

Research Scientist : **Yayoi Kimura** (~May)

Research Associates : **Atsushi Hijikata**
Koichi Oshima (~August),
Mai Yamagishi
Takuya Kitamoto (~May)

Technical Staff : **Masatoshi Ito, Miho Izawa,**
Chisato Kikuguchi (~December),
Aoi Ozawa

Temporary Employees : **Seiko Watanabe, Nobutake Suzuki,**
Kayoko Nagata (~May),
Atsuo Kobayashi,
Tomoko Hasegawa,
Saori Hayashi, Ryosuke Yashi,
Eri Yamamoto, Yoshie Kawase,
Kyoko Toyama (October~),
Hideaki Takaku (October~)

Students Trainees : **Nanako Shimura, Noriko Nakagawa**

Administrative Staff : **Kazuyo Nomura**



Laboratory for Immunogenomics

It has been our important and basic mission to function as a “Gateway” to genomics for immunologists. Thus, we have taken a three pronged approach to our research activities since our group launched: (1) central support activities; (2) strategic and collaborative research activities; and (3) exploratory research activities aimed at new technology development. During 2009, as in the past, we have made a significant effort to enhance strategic and collaborative research activities. In addition, we have initiated a program to provide a cytokine/chemokine profiling service as part of the central support activities, and our RefDIC database has been enhanced to accommodate these data. Furthermore, as an important component of the bioinformatic infrastructure for the Primary Immunodeficiency Disease project, we have developed new functions on a newly developed browser of human mutations. This enables us to integrate many lines of information about known mutations in human gene and to detect likely disease-causative mutation(s) in newly determined human gene sequences. Besides these activities, we have also made great effort in research category (3). As an example, the recent results of the macrophages secretome analysis are described below. Another emphasis in research category (3) is on development of technology for single-cell analyses at the mRNA and protein levels. Based on our experiences so far, we anticipate that our three-pronged approach will synergistically activate and expedite the development of immunogenomics in RCAI. Described below are two main results of our research activities in 2009.

Secretome analysis of macrophages

To maintain a dynamic and integrated immune system, immune cells closely communicate with each other by means of humoral factors and cell surface molecules. Thus, information about membrane and secretory molecules is especially important for the immunological field. Using conventional transcriptomics, we can obtain a wealth of expression data about membrane/secretory molecules. However, this type of analysis has only provided us with information about the abundance of transcripts, and this does not necessarily correlate with their actual protein levels. On the other hand, although proteomics is expected to provide

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2. Ohara O. From transcriptome analysis to immunogenomics: current status and future direction. *FEBS Lett* 583, 1662-1667 (2009)

more direct information about the protein profile, there are some limitations in sensitivity and throughput. Thus, a bridging method between transcriptomics and proteomics would serve as a key step in immunogenomics. To this end, we performed DNA microarray analysis of membrane-bound polysomes, in which membrane/secretory proteins are synthesized. This strategy enables us to obtain genome-wide expression data at the translation level for membrane/secretory proteins with high sensitivity. In the mouse macrophage-like J774.1 cells, transcripts isolated in this way and detected by ~3,000 probe sets on a Mouse 430 GeneChip preferentially encode proteins that localize to membrane-bound polysomes rather than to cytoskeletal-bound and/or free polysomes. Bacterial LPS affected the expression of 422 transcripts, of which 136 transcripts were not modulated either in a total RNA pool or cytoskeleton-bound/free polysomes, but in the membrane-bound polysomes. In the genes thus identified, 25 genes encode functionally uncharacterized membrane/secretory proteins whose expression changed in peritoneal macrophages as well as in J774.1 cells after LPS stimulation. For example, *LAM27* is on this gene list and is known as an uncharacterized membrane protein kinase. In agreement with the change in the mRNA level in membrane-bound polysomes, *LAM27* protein was temporally decreased in the membrane fraction. Overexpression of this protein attenuated induction of more than 100 proinflammatory genes in macrophages upon LPS stimulation. This is a nice demonstration that our “*SECRETOME*” approach can uncover novel regulatory molecules in activated macrophages.

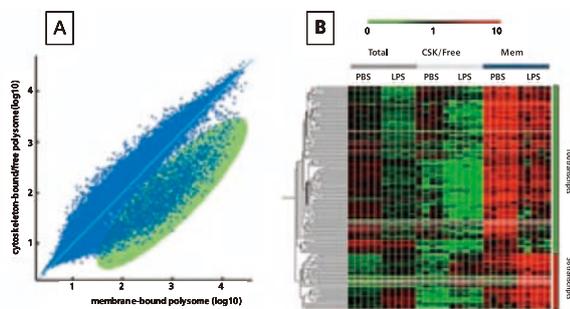


Figure 1: Enhanced mRNA profile viewer in RefDIC. (Panel A) Genome-wide detection of transcripts encoding secretory/membrane proteins I. A comparison of expression data between membrane-bound polysomes and cytoskeleton-bound/free polysomes in macrophage-like J774.1 cells is shown. The green oval shows ~3,000 probe sets detecting transcripts that preferentially localize in membrane-bound polysomes. (Panel B) Identification of LPS-affected transcripts encoding secretory/ membrane proteins. Mouse macrophage-like J774.1 cells were stimulated with LPS or PBS for 4hr. RNA in membrane-bound polysomes (Mem), cytoskeleton-bound/free polysomes (CSK/Free), and a total RNA pool (total) were subjected to microarray analysis using a Mouse Genome 430 2.0 GeneChip. Changes in the mRNA level of 136 total genes were detected only in membrane-bound polysomes.

Expansion of Mutation@A Glance for human genetic diseases

As we described in last year's annual report, we have developed a new bioinformatics tool, called Mutation@A Glance, which enables us to analyze the effects of genetic variations in genes known to be responsible for primary immunodeficiency diseases (PIDs). Mutation@A Glance has served as a useful informational platform for DNA diagnosis in PIDs. However, because of recent progress in the technology to identify vast numbers of genetic variations, we started to consider whether this informational platform should be expanded to cover all the human genes. We have thus begun to incorporate more comprehensive information regarding all known human genes into Mutation@A Glance to serve as a general informational platform for human mutation analyses. As a result, genetic variation data for over 2,400 known disease-associated genes other than PID genes have been incorporated into this platform and most protein-encoding human genes can now be subjected to mutation analysis using this “One Stop” platform.

One of the important issues in mutation analysis is to distinguish bona-fide disease causing non-synonymous (ns) substitutions (one amino acid in a protein replaced by another due to single nucleotide substitution in the DNA sequence) from the vast numbers of naturally occurring non-synonymous single nucleotide polymorphisms (nsSNPs). Although several methods to predict the effect of ns substitution are already available, none is solely sufficient to accurately predict the effects of all of the known disease-associated mutations. Thus, we felt that inclusion of other types of information would improve prediction accuracy. As a new perspective, we have included a great deal of information about molecular interactions in Mutation@A Glance. Using a statistical analysis, we found that the frequency of disease-associated ns substitutions located at the sites of molecular interactions is significantly higher than that of non-disease associated nsSNPs, which offers a rationale for the introduction of this kind of information into Mutation@A Glance. As a typical example, spatial localization of disease-causing mutations and nsSNPs in STAT3 are shown in Fig. 2.

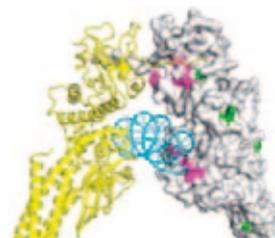


Figure 2: Display of molecular interactions of a PID-causing gene, STAT3.

Two STAT3 subunits (a white space-filling model and a yellow ribbon model are depicted) form a homo-dimer and bind to double-stranded DNA (cyan) in this X-ray crystal structure. The disease-causing mutations and non disease-associated nsSNP sites in STAT3 are colored magenta and green, respectively.

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Unit Leader

S. Sujatha Mohan

Research Associate : **Suresh Kumar Ramadoss**



Research Unit for Immunoinformatics

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 genomic, transcriptomic, 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.

RAPID: Resource of Asian Primary Immunodeficiency Diseases – “An Integrated Informational Platform”

PIDs comprise more than 150 different disorders that affect the development, function, or both of the immune system. Patients with these intrinsic defects may have increased susceptibility to recurrent and persistent infections and, in some cases, elevated susceptibility to autoimmune diseases and cancer. Our main aim is to bring together the clinicians and basic researchers in Asia who are interested in PID. We have recently developed an open access database on PID designated as “Resource of Asian Primary Immunodeficiency Diseases – RAPID”, a web-based compendium of molecular alterations and gene expression at the mRNA and protein levels of all PID genes reported from PID patients. The database also includes other pertinent information about protein-protein interactions, mouse studies and microarray gene expression profiles in various organs and cells of the immune system and it can be accessed at <http://rapid.rcai.riken.jp/> (Fig. 1).

RAPID architecture

RAPID is an object-oriented database. We used Zope (<http://www.zope.org>) for the development of RAPID. Zope is a leading open source web application server and is built using the programming language Python (<http://www.python.org>). MySQL is used as a backend data storage system.

RAPID data content

Sequence-variations in PID genes identified in patients are manually curated from published literature and mapped to NCBI RefSeq genomic, cDNA and protein sequences as per the recommendations of the Human Genome Variations Society (HGVS). Each mutation entry in the database is also linked to “Mutation@A glance” a web-based graphical user

Recent publications

1. Kandasamy, K.*, Mohan, S.S*, Raju, R., Keerthikumar, S. et al. NetPath: A Public Resource of Curated Signal Transduction Pathways. *Genome Biology* (2010) (in press). *These authors contributed equally
2. Kandasamy, K., Keerthikumar, S., Rajesh, R., Keshava Prasad, T. S., Ramachandra, Y. L., Mohan, S. and Pandey, A. PathBuilder – Open Source Software for Annotating and Developing Pathway Resources. *Bioinformatics*, 25(21): 2860-2 (2009).
3. Shivakumar Keerthikumar, Sahely Bhadra, Kumaran Kandasamy, Rajesh

interface (GUI) enabled tool which allows users to visualize the mutation position both at the level of DNA and protein sequences as well as homology based three-dimensional structures with various types of information such as SNP, protein domains and functional sites. RAPID also contains protein-protein interaction information from the Human Protein Reference Database, expression profiles from Gene Expression Omnibus and RefDIC, and corresponding mouse phenotypes from the Mouse genome database (MGI).

RAPID statistics

At present, RAPID comprises a total of 188 PID genes, out of which 171 genes are reported with over 4500 unique disease-causing mutation data obtained from more than 1400 citations as of January, 2010. Detailed statistics of annotated PID mutation data are depicted in Table.

The candidate PID genes prediction program

Currently, much attention has been focused on identifying novel candidate PID genes through various in-silico approaches. In this context, with the information available in RAPID, we have used Support Vector Machine (SVM) based parameter classification to predict candidate PID genes by scanning genes in the human genome. The genes prioritized in this way will be further tested and the most probable candidates will be experimentally validated by biomedical investigators (Published in DNA Research, 2009). Among the predicted candidate PID genes, there are 226 signal transducers, 224 transcriptional regulators, 128 proteins involved in immune response, 80 cell cycle regulators, 78 apoptotic proteins and 61 inflammatory responsive proteins. Some of the predicted genes in our catalog have recently

been implicated as PID genes, while others represent attractive candidates to be analyzed in patients where the disease etiology cannot be ascribed to any of the known PID genes.

RAPID project outlook

Our next focus will be on the analysis of annotated PID data such as correlation of mutation types and effects, influence of mutation occurrence in its functional domains, identification of hotspot mutations, and disease-causing mutation distribution and frequency with reference to ethnic groups. Subsequent interpretation of all this analyzed information should lead to a greater PID knowledgebase.

The semantic web, machine-interpretable descriptions, will be initiated in RAPID for all annotated entries using Resource Description Framework (RDF) and Web Ontology Language (OWL) file formats. The salient feature of this technology is being exploited in automation of web-based information and also facilitates data exchange and sharing of PID data among other interested research groups from all over the world.

Collaborations & Funding

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), Japan".

Table: Highlighted list of annotated PIDgenes mutation data

	No. of genes	No. of entries
Missense	124	2703
Frameshift	114	1219
Nonsense	102	1094
Splice anomalies	79	778
Amino acid deletion	46	132
Mutation type		
Substitution	155	4476
Deletion	114	1164
Duplication	48	241
Insertion	43	162
Sequence features		
Exon	162	5320
Intron	79	707
5 Prime UTR	4	19
Promoter	3	22
3 Prime UTR	3	3



Figure: Home page of RAPID showing various menus, data content and organization, PID network, news and collaboration

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Ranganathan, P., Ramabadrans S., Chaerkady, R. and Pandey, A. Human Protein Reference Database - 2009 update. *Nucleic Acids Research*, 37 (Database issue): D767-72 (2009).

Unit Leader

Takaharu Okada

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

Technical Staff : **Noriko Takahashi**

Administrative Staff : **Mari Kurosaki**

Research Unit for Immunodynamics

The focus of our research is to understand the mechanisms regulating dynamic cellular interactions in the tissues that shape immune responses. For this purpose we use real time imaging, in particular multi-photon microscopy, to analyze *in vivo* cellular migration and interactions. Multi-photon microscopy, which was introduced only recently to the field of immunology, has been revealing striking dynamics of immune cells in the lymphoid organs, underscoring the importance of this approach for resolving the complexity of the immune system. Utilizing this technique, along with other methods, we are currently working on two projects. The first one is to understand the regulation of B cell-helper T cell interactions that determine the fate of B cells in the antibody response. Antigen-engaged B cells undergo proliferation, selection, and multidirectional differentiation to achieve both immediate and long-term humoral immunity. T cell help plays a pivotal role in most of these processes, but it is not known how these processes are differentially regulated by B cell-helper T cell interactions. We tackle this problem by tracking individual B cell interactions with helper T cells and their proliferation and differentiation. This lab also studies the interactions between immune cells and tumor cells in the tumor-draining lymph node. We aim to mechanistically understand the impact of lymph node tumor metastasis on the activation and differentiation of tumor specific T cells.

Roles for stable conjugate formation between B cells and T cells in B cell proliferation and differentiation.

Our previous studies showed that cognate antigen-engaged B and T cells form stable conjugates early after immunization. Stable B-T conjugates are motile, with the B cells migrating ahead of conjugated T cells, possibly allowing the exchange of partners among conjugates to generate the optimal responses. We also recently found that B-T conjugates migrate along the stromal cell network, which has been reported to serve as a migration scaffold for naïve B and T cells (Fig. 1). To study the role of the conjugation in B cell proliferation in the T-dependent antibody response, we

Recent publications

1. Ebisuno Y., Katagiri K., Katakai T., Ueda Y., Nemoto T., Inada H., Nabekura J., Okada T., Kannagi R., Tanaka T., Miyasaka M., Hogg N., Kinashi T. Rap1 controls lymphocyte adhesion cascades and interstitial migration within lymph nodes in RAPL-dependent and -independent manners. *Blood*. Epub ahead of print.
2. Katagiri K., Katakai T., Ebisuno Y., Ueda Y., Okada T., Kinashi T. Mst1 controls lymphocyte trafficking and interstitial motility within lymph nodes. *EMBO J.* 28, 1319-1331 (2009)



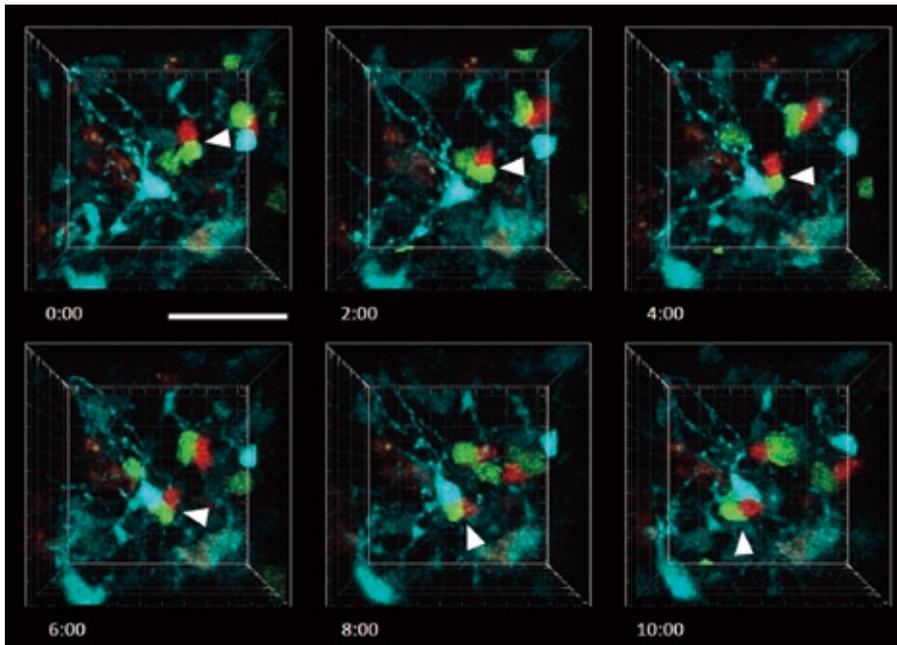


Figure 1 : Migration of a B cell-T cell conjugate on stromal cells in the lymph node. The time-lapse images show dynamics of cognate antigen-specific B cells (green) and helper T cells (red). In cyan are primarily irradiation-resistant stromal cells. The mouse lymph node was imaged by two-photon laser microscopy 36 hr after immunization with the cognate antigen. Arrowheads indicate the same B-T conjugate. Elapsed time is shown as minutes:seconds. Scale bar, 30 μm . The images are presented as 60 μm z-projections.

developed the imaging system to visualize the cell cycle of antigen-engaged B cells *in vivo* in collaboration with Dr. Miyawaki's group (Laboratory for Cell Function Dynamics, BSI, RIKEN) and Dr. Kurosaki's group (Laboratory for Lymphocyte Differentiation, RCAL, RIKEN). We are also collaborating with Dr. Midorikawa's group (Laser Technology Laboratory, ASI, RIKEN) to optimize the two-photon excitation for multi-probe cell cycle imaging. Our initial imaging results have begun to reveal the relationship between B-T conjugation and cell cycle progression at various phases of the immune response. After several cycles of proliferation, antigen-engaged B cells form germinal centers, which are important for long term humoral immunity. As evidence suggests that prolonged B-T conjugation is critical for germinal center formation, we are attempting to develop imaging systems to track differentiation of germinal center B cells in parallel with B-T interactions.

Interactions between tumor cells and tumor-specific T cells in the lymph node

Cytotoxic T cells have the capacity to induce tumor regression. Although several studies have analyzed dynamics of tumor-infiltrating CTLs and their interaction with tumor cells, these studies used exogenous model antigens to target

CTLs to tumor cells. On the other hand, most authentic tumor-associated antigens are self antigens. Thus, the dynamics of CTLs reactive to 'self' tumor-associated antigens in the tumor environment is not known. In addition, interactions of CTLs with tumor cells that have metastasized into the lymph node have not been dynamically analyzed. We investigated the dynamic behavior of melanocyte antigen-reactive CD8 T cells and their interactions with melanoma cells in the lymph node in collaboration with Dr. Shimizu (Research Unit for Therapeutic Model) and Dr. Fujii (Research Unit for Cellular Immunotherapy). We found that after vaccination with specific peptides these self-reactive CD8 T cells became sessile, most likely due to stable conjugation with DCs, proliferated robustly, and produced interferon- γ just like exogenous antigen-engaged CD8 T cells in the lymph node. However, these self-reactive CTLs were unable to form stable conjugates with melanoma cells that had metastasized into the lymph node parenchyma. This is in contrast to the efficient conjugation observed between model antigen-specific CTLs and tumor cells exogenously expressing the model antigen. Our further study of this immune ignorance will help understand the mechanism of tumor escape from the immune system.

3. Grigorova I.L., Schwab S.R., Phan T.G., Pham T.H., Okada T., Cyster J.G. Cortical sinus probing, S1P1-dependent entry and flow-based capture of egressing T cells. *Nat. Immunol.* 10, 58-65 (2009)

Unit Leader

Makio Tokunaga

Student Trainees : Akira Okada
Yuma Itou
Jun Takimoto



Research Unit for Molecular Systems Immunology

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 and Molecular Quantification in Cells

We have demonstrated that clear visualization of single molecules in cells enables their molecular quantification. Clear single-molecule visualization was achieved using TIRF and HILO microscopy. We have been involved in the development of total internal reflection fluorescence (TIRF) microscopy, a light-microscopic technique. TIRF has become a widespread technique for single-molecule imaging at surfaces, but cannot be used for molecular imaging inside cells.

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

To evaluate the HILO microscopy technique, we reconstructed three-dimensional images of the nuclear pore complexes (NPCs) in cells from z-scanned serial images (Fig. 1b). Clear, point-like images of NPCs were obtained without the need for deconvolution to remove out-of-focus

Recent publications

1. Fukagawa, A., Hiroshima, M., Sakane, I., Tokunaga M.: Stochastic emergence of multiple intermediates detected by single-molecule quasi-static mechanical unfolding of protein. *BIOPHYSICS*, 5, 25-35 (2009).
2. Miletic AV., Graham DB. Sakata-Sogawa K., Hiroshima M., Hamann H.J., Cemerski S., Kloeppel T., Billadeau DD., Kanagawa O., Tokunaga M. and Swat, W: Vav Links the T Cell Antigen Receptor to the Actin Cytoskeleton and T Cell Activation Independently of Intrinsic Guanine Nucleotide Exchange Activity. *PLoS One*. 4 (8), e6599 (2009).

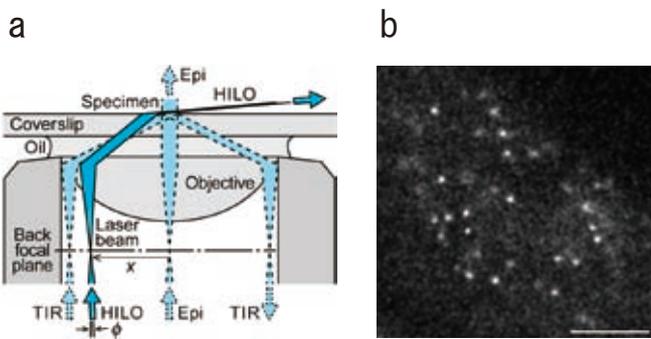


Figure 1: Molecular imaging enables one to visualize and quantify molecular dynamics, interactions, and kinetics in cells for molecular systems biology. (a) HILO microscopy for molecular imaging in cells. Illumination by a highly-inclined and thin beam increases image intensity and decreases background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

haze. The fluorescence intensity of the point images of NPCs followed the theory of image formation quite well. Further, there was much less photobleaching than in conventional confocal microscopy because of the lower intensity and non-focused nature of the illumination.

We examined the thickness of the illumination beam and obtained illumination intensity profiles in the z-direction. We decreased the illumination thickness by the reduction of the illumination diameter. Notably, the full width at half maximum of the profile, i.e., the illumination thickness was less than 7 μm at the diameter below 20 μm .

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

To explore potential new uses of this technology, we performed quantitative analysis on nuclear import to demonstrate its application to kinetic studies. We could visualize single molecules of GFP-importin β mediating the import of

cargo through nuclear pores in cells as bright spots on the nuclear envelope. Molecular interactions with the assembled NPC were quantified by single molecule analysis. Retention times, the number of associated molecules, the dissociation constant, and stoichiometry of import were all determined.

“In silico” Modeling and Simulation

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

We are now expanding the simulation studies into whole-cell simulation of single lymphocytes based on single molecule imaging and quantification (Fig. 2). Direct comparison with molecular imaging is indispensable for the simulation, since the values of parameters have a huge number of degrees of freedom. We demonstrated that clear visualization of single molecules in cells enabled the quantification. The combination of single molecule quantification and “in silico” reconstructions of cell functions opens new approaches for developing molecular systems biology.

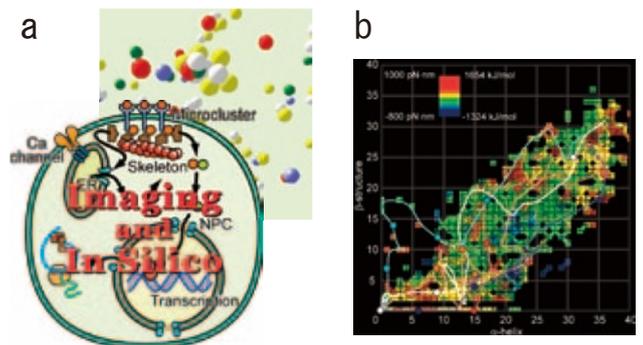


Figure 2: The combination of single molecule quantification and “in silico” modeling opens new approaches for developing molecular systems biology. (a) Aiming at understanding immune cells as molecular systems, we are going to construct “in silico” cell models based on single-molecule quantification. 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. (b) Two-dimensional ‘topographic’ energy landscape of single-molecule protein unfolding by MD simulations. Three representative trajectories are shown as lines. Multiple transition states were detected, and individual trajectories took highly heterogeneous pathways. Thus, single-molecule measurement has achieved notable success in detecting stochastic features of protein conformations. We are now expanding this type of study from molecules into cells.

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Unit Leader
Willem van Ewijk

Research Scientist : **Katrin Ishii-Schrade**



Research unit for Thymic Microenvironments

In the thymic environment, lympho-stromal interaction forms the basis for both lymphoid and stromal cell development (Fig.1). Thus, on the one hand, thymic epithelial cells (TECs) promote maturation of T lymphocytes, while in turn, developing thymocytes induce maturation and functional stability of the thymic stroma, a phenomenon earlier designated as “thymic crosstalk” (1-3).

Notably, in the absence of developing thymocytes, the thymic environment is perturbed and unexpected epithelial cells appear such as ciliated cells, goblet cells and absorptive cells, forming cysts (Fig. 2). Importantly, this abnormal stromal thymic phenotype can be quickly repaired, once developing thymocytes are added back to the thymic environment, highlighting both the impressive plasticity of the thymic microenvironment, and the instructive role of developing T lymphocytes in TEC development (3,4).

The immunodeficient thymus

In 2009, we have studied the development of the thymic environment in the absence of developing thymocytes, specifically focusing on the origin and generation of the aberrant epithelial cell types localized in thymic cysts (Fig.2). It can be argued that these cells may have an extra-thymic origin, and in that case these cells should not express thymic epithelial specific transcription factors. However, in studies using FoxN1 reporter mice, we have shown that the cyst lining epithelial cells have passed through a Foxn1-dependant developmental pathway, univocally demonstrating that they have originated from normal TECs. We also found that cysts are not caused by downregulation of Foxn1 at the protein level. Also in normal TEC's FoxN1 is downregulated with prolonged age, questioning the role of Foxn1 in the *maintenance* of thymic environments in general.

We then analyzed the thymic origin of cyst lining epithelial cells in an *in vitro* experimental system, where cortical and medullary TECs were tested for their potential to create thymic cysts under the influence of deoxyguanosine, a potent blocking agent for T cell development. We showed that medullary, rather than cortical TECs had the capacity to form cysts.

In a separate study, we have analyzed the molecular nature of factors involved in thymic crosstalk. We showed

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2. Holländer GA, Wang B, Nichogiannopoulou A, Platenburg PP, Ewijk W van, Burakoff SJ, Gutierrez-Ramos J-C, Terhorst C. *Developmental control point in induction of thymic cortex regulated by a subpopulation of prothymocytes*. *Nature* 1995, 373:350-353.

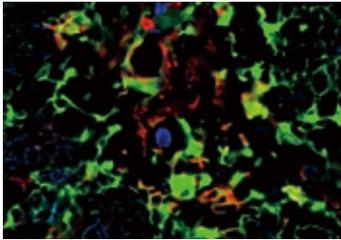


Figure 1: Foxn-1 (green) expression in subpopulations of professional thymic epithelial cells in 23 wk old Foxn-1 EGFP mice, stained by antibodies directed to cytokeratin 5 (red; medullary epithelial cells) and cytokeratin 8 (blue; cortical epithelial cells)

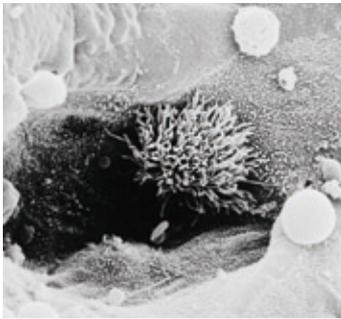


Figure 2: Scanning electron micrograph of a thymic cyst.

that Notch-1 activation on TECs by DLL-1 is important in the generation of the professional 3-D phenotype of TEC's (5). Thus, the Notch-1-DLL-1 interaction is a prime molecular example of thymic crosstalk, because both types of molecules are reciprocally expressed on TECs and developing T lymphocytes, and this molecular interaction drives both TEC development as well as T cell development. This latter aspect is now being employed to generate an artificial thymic environment.

The artificial thymus

Importantly, Notch signaling in developing hemato-progenitor cells induces restriction of these cells along the T-lineage (5). We have set-up an *in vitro* system to generate T lymphocytes, based on Notch signaling. By infecting the fibroblast cell line TsT with a DLL-1 containing retrovirus, hematopoietic progenitor cells added to the monolayer culture are committed into the T cell lineage, generating CD4⁺8⁺ thymocytes. However, CD4 and CD8 T cells did not develop. To promote the final steps in T cell development we have added MHC expressing cells to the TsT4/DLL1 culture. Thus, bone marrow dendritic cells (BMDCs) were generated *in vitro* from Rag 1^{-/-} mice, using GM-CSF and LPS. CD11c⁺ MHCII⁺ BMDCs were sorted by FACS and added with T cell precursors to TsT4/DLL1 monolayers. Such isolated BMDC's nicely develop as MHC II expressing cells within the TsT4/DLL1 monolayer, but so far these cells have been unable to induce the final steps in T cell maturation. We then questioned whether viral expression of the cortical TEC-specific proteosomal subunit β5T on BMDC's could promote positive selection of CD8 T cells. So far, the results of this study are not yet conclusive. We are now analyzing the interaction

of β5T-pulsed DC's and pre-selection DP thymocytes, in a more precise way (where we reduce cell heterogeneity).

An alternative approach to generate an artificial thymus includes studies on the trans-differentiation capacity of non-thymic epithelial cells into thymic epithelial cells. Using our *in vivo* approach we have shown that intra-thymic transfer of epithelial cells derived from the intestine results in integration of these extra-thymic cells into the thymic environment. Interestingly, these cells develop morphologically into 3-D organized TEC's, expressing keratin (Fig. 3). A minor subpopulation of these newly formed TEC's also expresses MHCII, making these cells candidate "teachers" in T cell selection.

Using an *in vitro* approach, where we have implanted tissue fragments or cell suspensions of other organs into fetal thymi, we have confirmed the induction capacity of the thymic environment. We found that epithelial cells derived both from the fetal intestine and lung can trans-differentiate into TEC's. In these domains, host-derived thymocytes integrate into the new epithelium (Fig. 4), supporting the notion that these newly formed environments support T cell development.

The construction of an artificial thymic environment generating a balanced T cell repertoire is not a simple task. We have established the first steps on the way to define minimal requirements for the development of T lymphocytes outside the body. In our *in vitro* approach we need to search for molecules and cell types completing the final steps in T cell maturation. Our *in vivo* approach will concentrate both on the plasticity of thymus-induced epithelial cell differentiation as well as the complete functional maturation of intra-thymic implanted epithelial cells.

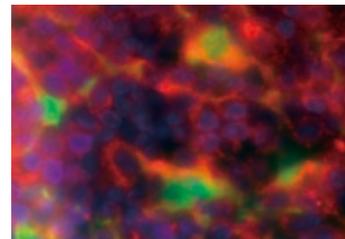


Figure 3: Keratin expression in 3-D GFP⁺ intestine derived epithelial cells, 4 weeks after intra-thymic injection in the mouse thymus. GFP:green; DAPI:blue; keratin:red.

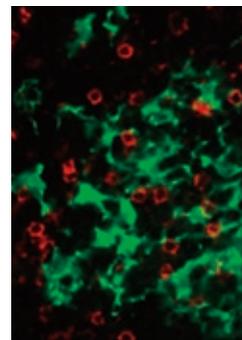


Figure 4: CD 45⁺ thymocytes (red) reside amongst GFP⁺ intestine derived epithelial cells (green) in fetal thymic organ culture.

3. Ewijk W van, Holländer G, Terhorst C, Wang B. *Stepwise development of thymic microenvironments in vivo is regulated by thymic subsets. Development* 2000, 127: 1583-1591.

4. Germeraad, W.T.V., Kawamoto, H., Itoi, M., Jiang, Y., Amagai, T., Katsura, Y., van Ewijk, W. *Development of thymic microenvironments in vitro is Oxygen dependent and requires permanent presence of T cell progenitors. J. Histochem. Cytochem.* 2003, 51:1225-1235.

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Laboratory Head
Miguel Vidal

Research Scientists : **Kaori Hisada**

Technical staff : **Asako Shibano**

Research Unit for Immunoepigenetics

Epigenetic regulation by the Polycomb group (PcG) of genes silences gene expression through mechanisms not fully understood. PcG activity is essential for self-renewal of adult stem cells and the maintenance of their developmental potential, as well as for the stable repression of genes in imprinted domains or in the inactive X-chromosome. Distinctive PcG complexes bear either histone H3K27 methyltransferase or histone H2A monoubiquitin ligase activities, that sometimes act coordinately. PcG targets are cell type and differentiation context dependent. Recruiting of PcG complexes to their targets is thought to occur through DNA binding proteins and/or non-coding RNAs. Some time ago, we identified a protein that interacts with the Polycomb E3 monoubiquitin ligases Ring1A and Ring1B, and also with the DNA binding protein YY1. The properties of the so-called RYBP protein (Ring1 and YY1 binding protein) made it a candidate for PcG recruiting and, indeed, recently it has been shown that it contributes to PcG-dependent repression of regions of the HoxD cluster with YY1 binding sites. In addition, RYBP is an essential gene during embryonic development and it has a function in adult B-cell homeostasis (our unpublished observations).

Genetic analysis of RYBP function in an embryonic stem cell model

Biochemical analysis by other groups has identified RYBP as a member of the pluripotency network of transcriptional regulators of embryonic stem (ES) cells. To study RYBP function we are using a conditional loss-of-function model in embryonic stem (ES) cells. These ES cells carry both a floxed RYBP allele and a Cre-ER fusion protein which is activated in the presence of hydroxytamoxifen. RYBP gene inactivation, as with that of other PcG products, has little effect on ES cell self-renewal. In contrast, genes derepressed in RYBP-deficient ES cells are rather unrelated to the set of developmentally relevant genes that are maintained repressed by PcG products. However, many of these PcG targets are bound by RYBP in ES cells, suggesting that either the paralog Yaf2 substitutes functionally for the absence of RYBP in mutant cells, or that its contribution

Recent publications

1. Calés, C., Román-Trufero, M., Pavón, L., Serrano, I., Melgar, T., Endoh, M., Pérez, C., Koseki, H. and Vidal, M. (2008) Inactivation of the Polycomb group protein Ring1B unveils an antiproliferative role in hematopoietic cell expansion and cooperation with tumorigenesis associated to Ink4a deletion. *Mol. Cell. Biol.* 28:1018-28
2. Román-Trufero, M., Méndez-Gómez, H., Pérez, C., Hijikata, A., Fujimura, Y., Endo, T., Koseki, H., Vicario-Abejón, C. and Vidal, M. (2009) Maintenance of undifferentiated state and self-renewal of embryonic neural stem cells by Polycomb protein Ring1B. *Stem Cells* 27: 1559-1570



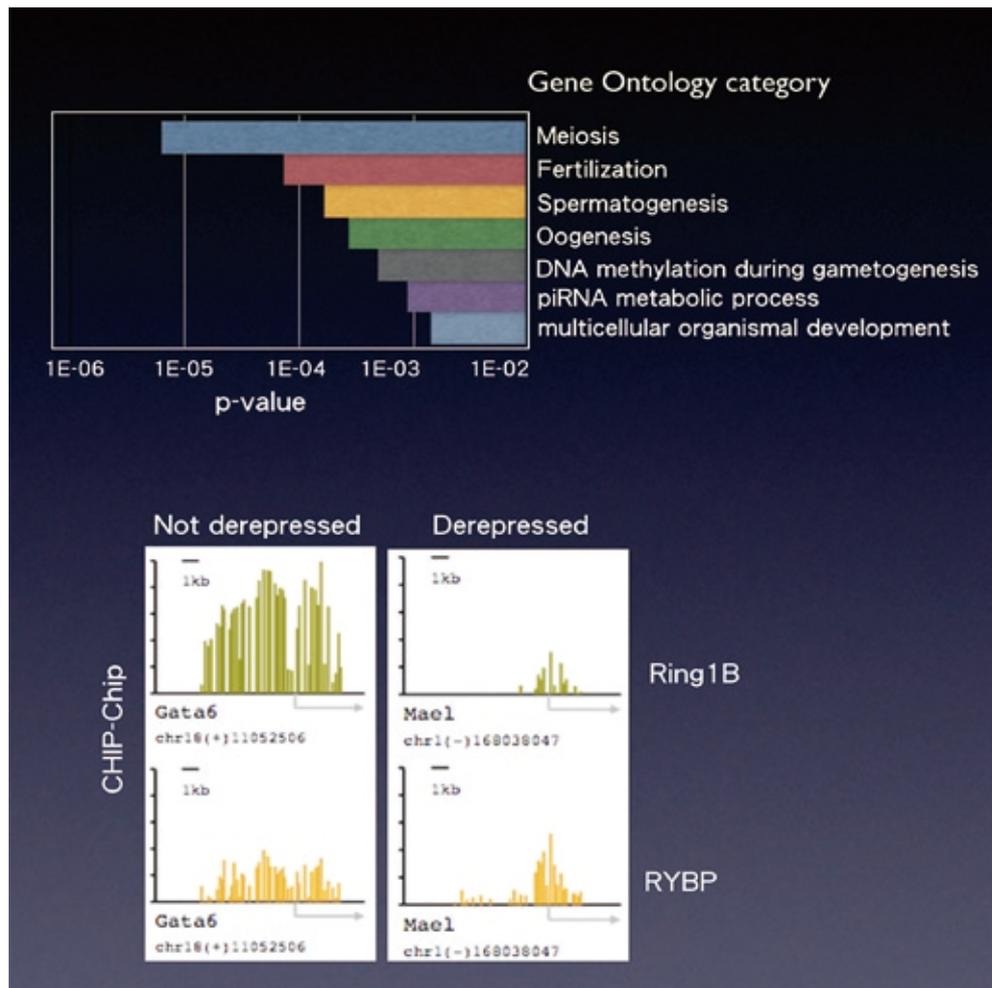


Figure: RYBP gene control in ES cells. (Top Panel) Gene Ontology (GO) analysis of probes derepressed in ES cells carrying a floxed RYBP four days after addition of 4-hydroxytamoxifen. (Bottom Panel) two examples of targets bound by RYBP: Mae1 (right) is derepressed in mutant ES cells, whereas expression of Gata6 (left) is unaffected by RYBP inactivation. The panels show the different binding patterns of Ring1B, a Polycomb component that interacts with RYBP.

to the repression of PcG targets in ES cells is not essential. Interestingly, though, among derepressed probes in RYBP mutant cells we found some corresponding to a subset of germline specific genes and other genes that are also derepressed in ES cells lacking components of the DNA methylation machinery. We are currently exploring the

possibility that among RYBP functions in early embryogenesis, one is in the stable silencing (involving DNA methylation) of specific genes in somatic cells. Also, to evaluate functional redundancy, we are generating mice carrying conditional alleles of RYBP and its only paralog Yaf2.

3. Vidal, M. (2009) Role of Polycomb proteins Ring1A and Ring1B in the epigenetic regulation of gene expression *Int. J. Dev. Biol.* 53:355-370.

4. Hirabayashi and, Y., Suzuki, N., Tsuboi, M., Endo, T.A., Toyoda, T., Shinga, J., Koseki, H., Vidal, M. Gotoh, Y. (2009) Polycomb limits the neurogenic competence of neural precursor cells to promote astrogenic fate transition. *Neuron* 63: 600-13

iPS Project Team

Core Members :

Haruhiko Koseki (Group Director)

Kyoko Masuda (Research Scientist)

Momoko Okoshi-Sato (Technical Staff)

Chieko Tezuka (Technical Staff)

Adjunct Members :

Hiroshi Kawamoto (Team Leader)

Fumihiko Ishikawa (Unit Leader)

Osamu Ohara (Group Director)

Hiroshi Watarai (Senior Research Scientist)

Daisuke Yamada (Research Scientist)

Genta Kitahara (Technical Staff)

Masako Fujita (Technical Staff)

Sakura Sakata (Technical Staff)

Yuko Nagata (Technical Staff)

Mariko Tomizawa (Technical Staff)

Yuri Suzuki (Technical staff)

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

Identification of genes that resist against reprogramming (Yamada et al., in progress)

Reprogramming to generate iPS cells (iPSCs) is a less efficient process when using mature lymphocytes compared to fibroblasts. We hypothesized the presence of genes that are difficult to fully reprogram in lymphocytes. To test this, we established 20 and 5 independent iPSC clones from mature B- and NKT cells, respectively. We have investigated gene expression profiles of 6 B cell-derived and 2 NKT cell-derived NKT cells and compared them with those of ESCs and identified a group of genes which reproducibly failed to be reprogrammed in lymphoid cells.

Reprogramming of natural killer T (NKT) cells to iPS cells and their development into functional NKT cells *in vitro*. (Watarai et al., submitted)

Although iPSCs hold tremendous potential for cell-replacement therapy, the efficacy of cells with desired functions differentiated *in vitro* from iPSCs has not been rigorously assessed. We established iPSCs derived from natural killer T (NKT) cell cloned mice (NKT mice), which harbor functional NKT cell-specific rearranged T cell receptor loci in the germline. These iPSCs could be efficiently differentiated into NKT cells *in vitro*. Moreover, adoptive transfer of iPSC-derived NKT cells into NKT cell-deficient

Recent publications

1. Schwermann J, Rathinam C, Schubert M, Schumacher S, Noyan F, Koseki H, Kotlyarov A, Klein C, Gaestel M. MAPKAP kinase MK2 maintains self-renewal capacity of haematopoietic stem cells. *EMBO J.* 28:1392-1406. (2009).
2. Román-Trufero M, Méndez-Gómez HR, Pérez C, Hijikata A, Fujimura Y, Endo T, Koseki H, Vicario-Abejón C, Vidal M. Maintenance of undifferentiated state and self-renewal of embryonic neural stem cells by Polycomb protein Ring1B. *Stem Cells.* 27:1559-1570. (2009).



Figure : Chimeric mice generated from NKT cell-derived iPSCs are shown in the left panel. Black pups represent germline transmission of NKT cell-derived iPSCs (right).

mice recapitulated the known adjuvant effects of natural NKT cells and suppressed tumor growth *in vivo*. Our study shows for the first time that fully functional immune cells can be generated from iPSCs and also demonstrates the feasibility of expanding functionally competent NKT cells via an iPSC phase in a mouse model, thereby, illustrating the clinical potential of this approach for NKT cell-targeted adjuvant therapy in humans.

Development of human iPSC-derived hematopoietic stem & progenitor cells (Ishikawa et al., in progress)

We are attempting to dissect the developmental program and pathway from human ES/ iPSC cells to the human hematopoietic lineage. When human iPSC cells were cultured with SCF/TPO/FL/TGFβ1 under feeder-free conditions for 7-14 days, the expression level of SSEA4 was decreased and there was reciprocal up-regulation of SSEA1 expression. The cultured cells did not retain the morphological features characteristic of iPSC colonies, and formed embryoid bodies. However, the hematopoietic induction was not sufficient, as demonstrated by the lack of expression of the pan-leukocyte antigen, CD34, or Flk-1 on the surface of the cultured human iPSC-derived cells. We then set up co-cultures of human iPSC cells and OP9 cells. The stromal support with or without Notch signal via delta-like ligand 1 (DLL1) successfully induced the development of the mesodermal lineage, as evidenced by the appearance of hCD45⁻hCD34⁺ cells and subsequent development of hCD45⁺hCD34⁺ cells *in vitro*. We are currently investigating the differentiation potential of these candidate mesodermal or hematopoietic progenitor cells *in vivo* and *in vitro*.

Functional differences between ES and iPSC cells revealed by B cell induction in an *in vitro* culture system (Kawamoto et al., in progress)

We investigated whether iPSC cells have the same features as ES cells. We examined ES and iPSC cells in terms of their developmental potential to produce hematopoietic cells. We tested a total of 20 iPSC clones including those derived from mature B cells, from NKT cells and from mouse embryonic fibroblasts (MEF), in comparison with several ES lines. All ES cell clones produced all types of hematopoietic cells in the *in vitro* culture systems, including erythrocytes, myeloid lineage cells, T cells and B cells. In contrast, eight out of nine iPSC cell clones did not produce B cells, although the other types of hematopoietic cells were generated. This can not be due to insufficient reprogramming, because the same observations were seen in several iPSC cell clones derived from mature B cells. These results clearly show that iPSC cells are not identical to ES cells in terms of their ability to produce B cells.

A novel technology for cloning of human mAb using by using iPSC cells (Kawamoto et al., in progress)

We are using iPSC cell technology to develop a new method to make human antibody for therapeutic use. We plan to i) immunize humanized mice with NP-antigen, ii) make iPSC cells from antigen-specific human B cells, iii) collect mAb from B cells induced from iPSC cells. To establish the above system, we performed a pilot study. Antigen-specific B cells were isolated from immunized normal mice, and we successfully induced iPSC cells from these B cells. From these iPSC cells, we produced chimeric mice, and confirmed that antigen-specific B cells were generated.

3. Watarai H, Rybouchkin A, Hongo N, Nagata Y, Sakata S, Sekine E, Dashtsoodol N, Tashiro T, Fujii SI, Shimizu K, Mori K, Masuda K, Kawamoto H, Koseki H, and Taniguchi M. Generation of functional NKT cells *in vitro* from embryonic stem cells bearing rearranged invariant Vα14-Jα18 TCRα gene. *Blood*, 14, 115(2) 230-237 (2010)

4. Saito Y, Uchida N, Tanaka S, Suzuki N, Tomizawa-Murasawa M, Sone A, Najima Y, Takagi S, Aoki Y, Wake A, Taniguchi S, Shultz L.D, Ishikawa F. Cell cycle entry potentiates elimination of quiescent chemotherapy-resistant human leukemia stem cells. *Nature Biotechnology* 28(3), 275-280 (2010)

Central Facilities

Central Facilities in RCAI provide all researchers in the Center with access to the most advanced equipment and technologies. The Central Facilities consist of five sections; the Genomics Laboratory managed by Osamu Ohara (Lab. for Immunogenomics), the Animal Facility managed by Haruhiko Koseki (Lab. for Developmental Genetics), and the Confocal, Monoclonal Antibody, and FACS Laboratories managed by Takashi Saito (Lab. for Cell Signaling).

Genomics Lab.

Senior Research Scientist: **Hiroshi Kitamura**
 Research Scientist: **Yayoi Kimura**
 Research Associate: **Mai Yamagishi, Atsushi Hijikata**
 Technical Staff: **Tomoko Hasegawa, Saori Hayashi, Ryosuke Yashi, Nobutake Suzuki, Akio Kobayashi**

The Genomics Lab provides various services to the members of the Center: proteomics analysis, *in vitro* translation, multiplex suspension array, DNA microarray, DNA sequencing, and cDNA/Genomic clone distribution (Table). In 2009, we started a new program to distribute primer/labeled probe sets for qRT-PCR analysis of immune cells.

The lab organized a RIKEN Technical Support Workshop on Oct. 8-9, 2009, and exchanged views with 70 staff members from other RIKEN Centers, Omics Science Center, Brain Science Institute, Center for Developmental Biology, Bioinformatics And Systems Engineering Division, and Advanced Science Institute. The goal was to improve the technologies of the Central Facilities by sharing information with other RIKEN's Centers. As part of this process, a new website in RIKEN SciNES was constructed by the Genomics Lab. (Fig.).

Table: Services provided by the Genomics Lab in 2009

Proteomics	# of samples	# of teams
Two-dimensional electrophoresis	15	1
Mass Spectrometry Analysis	393	1
<i>In vitro</i> translation (recombinant protein synthesis)	# of samples	# of teams
	3	1
Multiplex suspension array	# of samples	# of teams
	2,723	11
Affymetrix GeneChip (Exon array, Gene array)	# of arrays	# of teams
Human	223	4
Mouse	376	21
Total	599	23
DNA sequencing	# of samples	# of teams
36cm capillary	15,563	14
50cm capillary	12,507	11
Total	28,070	17
cDNA clone delivery	# of clones	# of teams
	58	8



Figure : A top page of a new website for information exchange among RIKEN technical assistance groups on RIKEN SciNES

Animal Facility

Senior Technical Scientist: **Takanori Hasegawa**
 Technical Scientist: **Shinobu Mochizuki**
 Technical Staff: **Masashi Matsuda, Tomoyuki Ishikura, Isamu Hisanaga, Yusuke Iizuka, Hiromi Tatsumi, Yurie Kawamoto**
 Administrative Staff: **Hiroko Iwamoto**
 Animal care takers (outsourced) : 22 people

The animal facility at RCAI provides a high-standard facility for animal experiments for RIKEN Yokohama Institute. The facility also supports the generation of knock-out, transgenic and humanized mice (See *Research Highlights* section)

1. Maintenance, generation and cryopreservation of genetic resources

In 2009, over 51,200 mice and 5 rats were maintained in the SPF area and 500 mice in an isolated area. 502 mouse lines were introduced into the SPF area by a combination of *in vitro* fertilization (IVF) and embryo transfer. Cryostocks of 424 lines were also generated. The facility also maintained relatively large colonies of several commonly used strains such as NOD/SCID/C γ KO, Rag1KO and various Cre recombinase deleters and provided them to users on demand. The facility provided technical assistance to generate chimeras (169 lines) and transgenic mice (14 lines) and to establish and maintain ES cells. In addition, they generated a new internally available database for genetic resources.

2. Introduction of human BAC clones into NOD/SCID/C γ KO mice

In collaboration with the Lab. for Immunogenomics, the Lab. for Developmental Genetics and the Res. Unit for Human Disease Model, the facility has launched an activity to improve the efficacy of transplantation of human hematopoietic stem cells into NOD/SCID/C γ KO mice by “humanizing” the host strain. For this purpose, we have introduced large genomic fragments containing human genes encoding MHC, cytokines, adhesion molecules, virus receptors and others into NOD/SCID/C γ KO mice. To date, they have established 6 BAC transgenics and confirmed the expression of human genes on a C57BL6 background and begun back-crossing these mice onto the NOD/SCID/C γ KO background using the speed-congenic method.



Photo : Animal Facility won RCAI Outstanding Contribution of the Year 2009. (From left, Chie Yoshida, Hiroko Iwamoto, Tomoyuki Ishikura, Takanori Hasegawa, Shinobu Mochizuki, Isamu Hisanaga, Yusuke Iizuka and Hiromi Tatsumi)

Confocal Lab.

Collaborative Researcher: Yasutaka Wakabayashi (Leica Microsystems K.K.)

The Confocal Lab provides imaging equipment and technical support in collaboration with Leica Microsystems. Currently, six confocal microscope systems are available:

1. Inverted system with visual and multiphoton MP laser that is suitable for time-lapse imaging of living cells and organs.
2. Inverted system with a 405 nm laser that is suitable for time-lapse imaging of living cells in a controlled environment (CO₂, temperature, and humidity).
3. Inverted system with visual and UV lasers that can be used for calcium detection.
4. Upright system with visual and UV lasers that is suitable for standard fixed specimen observation.
5. Intravital upright system with visual and MP lasers that can be used for *in vivo* imaging of various tissues such as lymph nodes.
6. Intravital upright system with a single visual laser, double MP lasers and a high speed scanner that can be used for *in vivo* imaging and for other specialized applications such as light stimulation.

During 2009, the Lab. provided a training course for 21 investigators. The total running time of the microscopes was more than 2302 hours (Fig.).

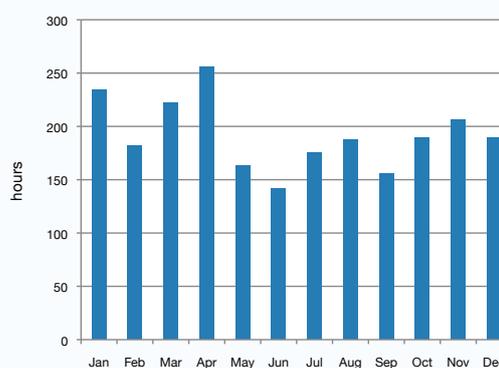


Figure : Running time of the microscopes in 2009

Monoclonal Antibody Lab.

Technical Staff: Tomomi Aoyama, Mayuko Matsuda

The Monoclonal Antibody (mAb) Lab restarted its activity in April 2009 after a period of inactivity during 2008. This new activity is partially supported by Becton Dickinson, which will continue its support in 2010. Two technical staff members, Tomomi Aoyama and Mayuko Matsuda, are primarily engaged in the generation of mAbs at the request of individual RCAI investigators. The mAb Lab has produced mAbs against 20 different antigens, which were requested by 7 laboratories, including mAb against a Zn transporter and a surface marker on regulatory dendritic cells. The lab will continue to produce mAbs that meet the needs of RCAI researchers, and will also focus on development of mAb directed to strategic proteins such as allergy related molecules and several G protein-coupled receptors, which are important but difficult targets.

FACS Laboratory

Technical Scientist: Hanae Fujimoto

Technical Staff: Yukiko Hachiman

The FACS Lab provides a range of support for flow cytometry and cell sorting, procedures that are essential for nearly all immunological experiments. In 2009, two new FACS machines, Aria II and Canto II were installed (Table). In addition, the capacity to sort single cells onto glass microscope slides was added (Photo). The laboratory's activities are divided into three main parts: technical support and training for FACS users, cell sorting operation services, and management/maintenance of the FACS equipment.

Table: FACS instruments in the central facilities

	FACS	no of machines
Analyzer	Calibur	6
	LSR	1
	Canto II	1
Sorter	Aria I	2
	Aria II	1
	Vantage	2
	Diva (digital vantage)	1

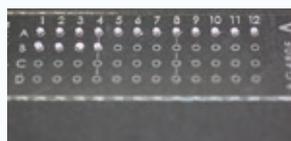


Photo: Sorting single cells onto glass microscope slides

- **Technical support and training**

In 2009, the facility offered 15 technical courses (10 for cell sorting and 5 for cell analysis). Courses were held at 7 different levels, Calibur basic, Calibur option, Canto II, Vantage basic, Vantage option, Aria basic and Aria option. A total of 52 RCAI researchers participated in these courses.

- **Cell sorting operation service**

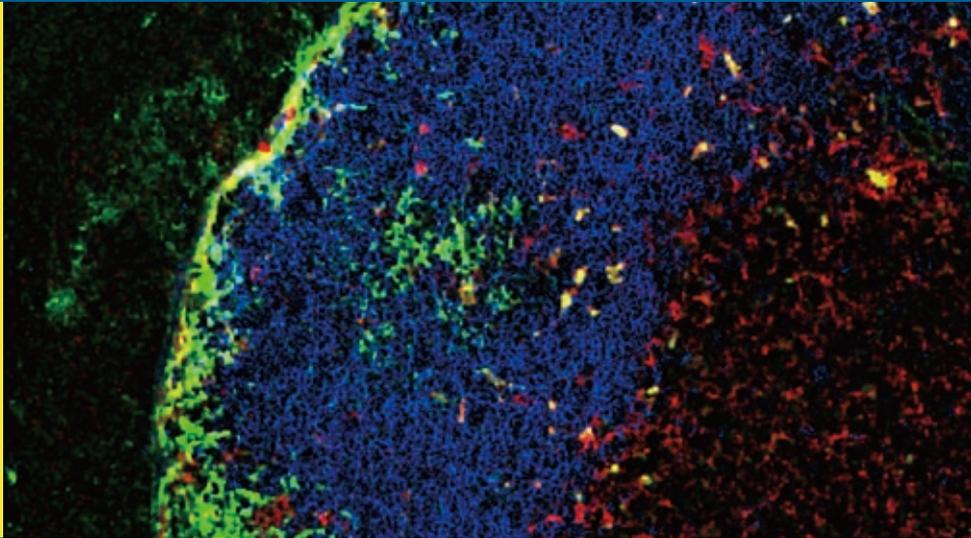
The FACS Lab provides a cell sorting operation service. Our experienced operators perform the actual cell sorting after consultation on experimental design with RCAI researchers. In 2009, 343 sorting experiments were performed by our FACS operators.

- **Management/maintenance of FACS equipment**

FACS instruments 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.

2009

Collaborative Networks



RCAI and Torii Collaborate for the Allergy Vaccine

In 2009, RCAI established a program for bridging research (translational research) platform. To fill the critical gap between basic research and later stage drug development, the program offers an 'exchange zone' where RCAI, universities, hospitals and pharmaceutical companies work together for the drug development.

The process from the discovery of a new molecule to its marketing is long, expensive and very uncertain. Developing a drug intended to treat a rare disease does not allow the recovery of the capital invested for its research. Thus, sponsors are reluctant to develop these 'orphan drugs' and to take the high-risks for early stage research for innovative drug discovery.

The new program aims to support the development of orphan drugs or innovative drugs that private companies would hesitate to develop by themselves. The program is comprised of two steps; 1) RIKEN, in collaboration with universities, will analyze the mechanisms, pharmacological effects, efficacy and safety. 2) Based on the results, companies will develop the drug and conduct clinical trials. (Fig.)

"This is like an exchange zone in athletic relays. The sprinters run together in the exchange zone to give/receive the baton firmly and smoothly. The key is how smoothly the baton is taken over from one runner to the next. RCAI creates the baton (innovation) and passes firmly to the next runner," said Director Taniguchi.

In December, 2009, RIKEN and Torii Pharmaceutical Co., Ltd. agreed to collaborate for the development of cedar pollen allergy vaccine under this program.

Cedar pollen allergy afflicts an estimated more than 20 % of the Japanese population. The new vaccine involves polyethylene glycol bound to a recombinant fusion protein of two cedar (*cryptomeria japonica*) pollen antigens (Cry j1 / Cry j2). Through animal trials, RCAI has found the vaccine effective and it reduces the likelihood of anaphylactic shock. Using Torii's technology for making chemical compounds, they will develop a vaccine for human use.

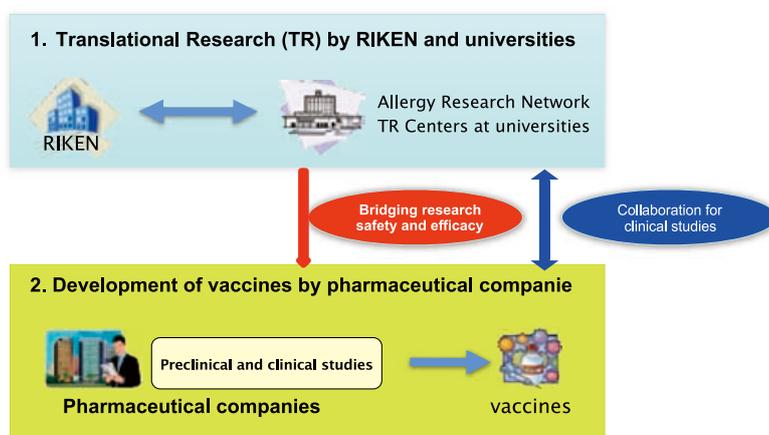


Figure: Two-step scheme for the bridging research

Open Laboratory for Allergy Research

RCAI launched an open laboratory for allergy research where researchers from external institutes, universities or hospitals can have access to the Center's resources and conduct collaborative projects on allergy research. In FY2009, three projects were launched under this program.

Dr. Kenji Matsumoto (National Center for Child Health and Development) and Dr. Naoki Shimojo (Chiba Univ.) started an allergy cohort study combined with the generation of humanized allergy mouse model. Dr. Noriko Tsuji (Advanced Industrial Science and Technology) will analyze the mechanisms of oral tolerance and its application for food allergy. Dr. Masato Kubo (Tokyo Univ. of Science) will analyze IL-4 and the class switch of B cells to IgE producing plasma cells.

Table: Members of Open Laboratory for Allergy Research

Kenji Matsumoto	National Center for Child Health and Development
Naoki Shimojo	Chiba University
Noriko Tsuji	Advanced Industrial Science and Technology
Masato Kubo	Tokyo University of Science

Multidisciplinary Research Projects

With the aim of developing new paradigms in immunology, RCAI launched a program to support a limited number of multidisciplinary collaborative projects lead by RCAI researchers. The program provides 5-10 million

JPY/year to each collaborative research projects for up to five years.

In FY2009, five projects were selected for the support.

Table: Awardees of RCAI Multidisciplinary Research Projects 2009

RCAI Project Leader	Project Title	Collaborators
Hiroshi Ohno	Genomic, transcriptomic and metabolomic analysis of the human ulcerous colitis	Mamoru Watanabe (Tokyo Medical and Dental Univ.) Masahira Hattori (The Univ. of Tokyo) Jun Kikuchi (RIKEN Plant Science Center)
Satoshi Ishido	Modeling of antigen presentation	Yuji Sugita (RIKEN Advanced Science Institute)
Ichiro Taniuchi	Regulation of gene expressions for thymic development	Atsushi Mochizuki (RIKEN Advanced Science Institute)
Hiroshi Kawamoto Haruhiko Koseki Fumihiko Ishikawa Masato Tanaka Ichiro Taniuchi Osamu Ohara Toshitada Takemori Masaru Taniguchi	Creation of the artificial immune cells	Harukazu Suzuki (RIKEN Omics Science Center) Jun Kawai (RIKEN Omics Science Center) Yoshihide Hayashizaki (RIKEN Omics Science Center) Carsten O. Daub (RIKEN Omics Science Center) Piero Carninci (RIKEN Omics Science Center)
Hisaaki Shinohara	Modeling of NF- κ B activation signals	Mariko Hatakeyama (RIKEN Advanced Science Institute)

RCAI and the Univ. of Palermo Sign a Cooperative Agreement

On September 11, 2009, RCAI and the Univ. of Palermo agreed to cooperate for scientific and educational exchanges. Dr. Taniguchi, Director of RCAI, and Professor Lagalla, the rector of the Univ. of Palermo, signed the agreement at the ceremony held at the Univ. of Palermo. The agreement includes a) the exchange of professors and researchers for conferences or seminars, b) the exchange of scientific informative materials, and c) the co-organization of advanced study courses, seminars and research activities.

Palermo Univ. is located in north-western Sicily. The university was officially founded in 1806, but its earliest history dates back to the 15th century. A number of important scientific personalities did their work at Palermo University, including the astronomer Giuseppe Piazzi, the chemist Stanislao Cannizzaro, the architect Giuseppe Venanzio Marvuglia, and the physicist Emilio Segre.



Photo: Agreement and a medal given by the Univ. of Palermo

Harvard Summer School Program at RCAI

RCAI provides an undergraduate student internship program in collaboration with Harvard University's Summer School. From this year, the participants earn university credits in the Harvard Summer School Program at RCAI.

In 2009, RCAI accepted three students, Ms. Denise Ye, Ms. Kelly Brock and Mr. Andrew Hardigan from June 8 to August 16. They conducted a research project at Lab. for Lymphocyte Development, Lab. for Immunogenomics, and Lab. for Cell Signaling respectively. They also participated in a series of basic immunology lectures, RCAI's International Summer Program (RISP), the RCAI-JSI International Symposium on Immunology and basic Japanese classes. Besides these studies, they enjoyed the cultural exchange with students of Yokohama Science Frontier High School (photos), and excursions to Kamakura and the Nasu mountains.



Photo: Japanese culture visit at Yokohama Science Frontier High School. Harvard Summer School students learned Kendo (*left*) and Sado (*right*) from Japanese high school students.



RIKEN Joint Graduate School Program International Program Associate

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

The IPA students studying at RCAI in 2009 were

Li Yingqian (Nanjing University, China) studied at Lab. for Immune Diversity (01)

Sebastian Nieke (Tübingen Univ, Germany) studied at Lab. for Immune Transcription (02)

Li Shuyin (China Agriculture University, China) studied at Lab. for Immune Diversity (03)



The 2nd Symposium for PID in Asia



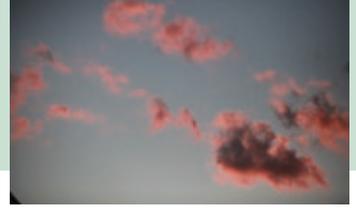
The second Symposium for Primary Immunodeficiency Diseases (PID) in Asia was held on February 4-5 at the Kazusa Academia Hall, Chiba, Japan. This event was organized by Dr. Michio Oishi (01), Director, Kazusa DNA Research Institute and Dr. Toshio Hara (Leader, PID Study Group in Japan, Ministry of Health, Labour and Welfare). It was well attended by many prominent PID physicians not only from Japan but also from other parts of the world including USA, France, Finland, China, India, Hong Kong, Korea, Thailand, and Vietnam.

PID are a genetically heterogeneous group of disorders that mainly affect distinct components of the innate and adaptive immune systems, such as neutrophils, macrophages, dendritic cells, natural killer cells, and T and B lymphocytes. The major focus of this meeting was to discuss the prevailing issues and challenges encountered in PID diagnosis and treatment. In addition to establishing an active Asian PID network among interested groups, it was hoped that this type of interaction would facilitate mutual exchange of relevant information required for timely PID diagnosis and therapeutic intervention to save many precious innocent lives.

The meeting proceedings began with a "PID network" session, chaired by Dr. Shigeru Tsuchiya, in which the status of PID in Japan was discussed by Dr. Toshiro Hara and subsequently, Dr. Kosuke Imai highlighted significant developments in the Primary Immunodeficiency Database in Japan (PIDJ) since its inception a couple of years ago. Next there was a presentation by Dr. Capucine Picard, a well-known PID expert from the French national PID reference center, Necker Hospital, Paris. She vividly explained her clinical experiences in identifying PID patients who have been susceptible to both invasive and non-invasive bacterial infections along with early/severe/transient clinical phenotypes. These PIDs affect key signaling molecules, IRAK-4 and MyD88 and Dr. Picard also delineated the functional role in

IL1-beta and Toll-like receptor (TLR) dependent pathways. This session was followed by talks describing recent developments in basic and clinical PID research, including effective introduction of gene therapy protocols for the treatment of various PIDs – Wiskott Aldrich syndrome (WAS) and chronic granulomatous disease (CGD). This later topic was lucidly presented by two speakers, the first Dr. Anne Galy (02), INSERM Research Director at Genethon, a French not-for profit organization for biotherapies, who has been a pioneer in establishing clinical gene therapy programmes for various human diseases particularly for WAS, and now has a phase I/II gene therapy clinical trial underway, using the ex-vivo gene transfer approach. The second speaker on this topic was Dr. Joong Gon Kim, Seoul National University, Korea, who has been involved in designing gene therapy protocols using retroviral vectors for treating X-CGD patients. Dr. John Ziegler, a PID physician from Sydney Children's Hospital, Australia then discussed the working network system of ASCIA - Primary Immunodeficiency Diseases Register of Australia and New Zealand - and explained the current status of veno-occlusive disease in these regions.

The second day of the symposium commenced with topics related to pathophysiology of PIDs and the challenges in establishing PID diagnosis. Dr. Mei W. Baker (03), Science Advisor, Newborn Screening Program, University of Wisconsin-Madison, USA, described the workflow of newborn screening for SCID, in which two screening systems are used: T cell receptor excision circles (TRECs) by a real-time PCR system or flowcytometric analyses of lymphocyte subpopulations. She further described patients who have been successfully treated by hematopoietic stem cell transplantation. Next, Dr. Yu-lung Lau (04), a well known PID physician from the Department of Paediatrics and Adolescent Medicine, The University of Hong Kong, China, explained how PID care can be successfully established among PID physicians in Asia using an e-consultation



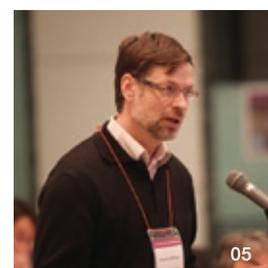
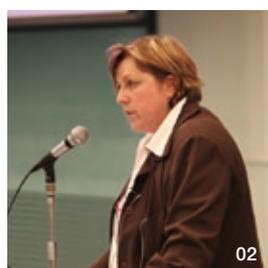
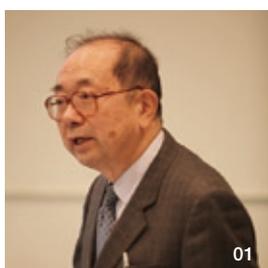
approach thereby ensuring an efficient working group for PID diagnosis. This approach would save a great deal of time and energy, and also promote free genetic diagnosis, as has already been clearly demonstrated by the e-consultation system that is now being practiced under his supervision. Dr. Mauno Vihinen (05), from the Institute of Medical Technology, University of Tampere, Finland, next presented an overview of novel systematic classifications of PID based on clinical, pathological and laboratory parameters using advanced computational tools for clustering and network analysis. This method should help users to understand the intricacies of genotype and phenotype correlations that could be further applied to any systems biology-based human disease classification. During this

session, all participants had a nice opportunity to observe the high through-put sequencing facility that has been set up at the Department of Human Genome Research, Kazusa DNA Research Institute (KDRI), Japan under the directorship of Dr. Osamu Ohara. The final session dealt with the current status of PID clinical experiences observed among developing countries in South East Asia and included discussions about available economical therapeutic modalities to control PIDs.

At its conclusion, all of the participants agreed that this kind of meeting would provide the right ambience for initiating global collaborations through strategic research programs, thereby sharing PID information, infrastructure and laboratory facilities among the PID community.

Table: Program

4-Feb	
1. PID network	
Toshiro Hara (Kyushu University, JAPAN)	PID in Japan : Past, present and future
Kosuke Imai (National Defense Medical College Hospital, Japan)	PIDJ (primary immunodeficiency database in Japan), 2008 - 2009
Capucine Picard (Necker Hospital, INSERM, Paris Descartes University, France)	Clinical features and outcome of patients with IRAK-4 and MyD88 deficiency
2. Recent Developments in Clinical PID Researches	
Xiaochuan Wang (Children's Hospital of Fudan University, China)	Promoting the recognition of primary immunodeficiency in clinical practice
John Ziegler (Sydney Children's Hospital, Australia)	Veno-occlusive disease and immune deficiency : an update
Anne Galy (INSERM, France)	Gene therapy for Wiskott Aldrich syndrome
JoongGon Kim (Seoul National University, Korea)	Gene therapy for CGD : Korean experience
3. Recent Developments in Basic PID Researches	
Yoji Sasahara (Tohoku University, Japan)	The role of WIP in Wiskott-Aldrich syndrome
Yoshiyuki Minogishi (Tokyo Medical and Dental University, Japan)	Molecular origins and mechanisms of hyper-IgE syndrome
Fumihiko Ishikawa (RIKEN RCAI, Japan)	Humanized PID mouse model for basic and translational immunology
Osamu Ohara (RIKEN RCAI; Kazusa DNA Research Institute, Japan)	Exploration of PID causative mutations: An Japan model and the future direction
5-Feb	
1. Pathogenesis/pathophysiology of PID	
Taizo Wada (Kanazawa University, Japan)	Revertant somatic mosaicism in primary immunodeficiency
Satoshi Okada (Hiroshima University, Japan)	Severe congenital neutropenia in Japan
Toshio Heike (Kyoto University, Japan)	Pitfall for diagnosis of autoinflammatory disorders
2. Diagnosis: Present and Future	
Tomohiro Morio (Tokyo Medical and Dental University, Japan)	Common variable immunodeficiency (CVID): Molecular basis of immune dysfunction
Mei W. Baker (University of Wisconsin-Madison, USA)	Newborn screening for SCID: What we've learned so far
Yu-lung Lau (The University of Hong Kong, PR China)	Promoting PID care in Asia through e-consultation and free genetic diagnosis
3. Bioinformatic/Genomic Researches for PID	
Mauno Vihinen (University of Tampere, Finland)	Novel systematic classification of PIDs
Sujatha Mohan (RIKEN RCAI, Japan)	Resource of Asian Primary Immunodeficiency Diseases : RAPID : A road ahead



International Research Collaboration Award

The RCAI International Research Collaboration Award is a unique program supporting researchers outside of Japan in setting up semi-independent research units within the laboratory of their collaboration partner at the Center. The program provides up to 10 million JPY/year to each collaborative research projects for up to three years.

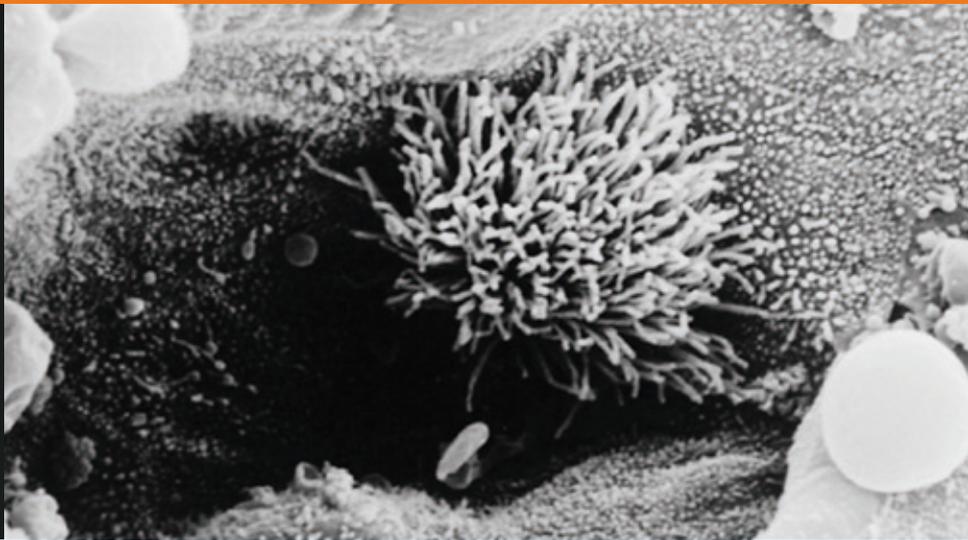
Since the program started in 2004, 14 projects have been funded (Table). In FY2009, Drs. Bix and Kubo published their work on *Mina*, an *Il4* repressor, that controls T helper type 2 bias (Okamoto et al. *Nature Immunology*, 10, 872-879 (2009))

and Drs. Osato, Ito and Taniuchi's work on Runx1 deficiency resulted in successful publication (Jacob et al. *Blood* 115, 1610-1620 (2010)). Drs. Ellmeier and Taniuchi's paper on transcription factor network that control CD4/CD8 cell fate decision of DP thymocytes is now in press (Sakaguchi et al, *Nature Immunology*, in press). Two collaborative projects took place in FY2009, and contributed not only to scientific discoveries but also for international exchange of people and international visibility.

Table: Awardees of RCAI International Collaboration Award Program

Year	Awardee	Title of Research	Host Lab.
2004-2006	Michael Dustin New York University School of Medicine	Analysis of dynamism and function of immunological 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 allergic inflammation	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
2006-2008	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 Surveillance
2008-	Stefano Casola IFOM-The FIRC Institute of Molecular Oncology Foundation	Nuclear reprogramming of terminally differentiated plasma cells to study the specific role of IgA in mucosal and systemic immunity and B cell development	Sidonia Fagarasan Laboratory for Mucosal Immunity
2008-	Yoshiaki Ito and Dr. Motomi Osato Institute of Molecular and Cell, National University of Singapore	Understanding of tumor suppressive mechanism of Runx complexes against leukemia and gastrointestinal cancer	Ichiro Taniuchi Laboratory for Transcriptional Regulation

2009
Events



RCAI International Summer Program 2009



The fourth RCAI International Summer Program (RISP 2009) was held in Yokohama July 3-10. Forty-two graduate students and postdoctoral fellows from sixteen countries participated in RISP 2009. The first part of the program was held at RCAI and featured 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/Japan Society of Immunology meeting, "Cellular and Genetic View on Autoimmunity" held at the Pacifico Conference Center in Yokohama. Four of the RISP participants also stayed on for a month-long laboratory internship at RCAI.

The focus of the lectures at RCAI was on regulation of immune responses, in particular by regulatory T cells and TH17 cells, but other topics included lymphocyte development/differentiation, immunity to infection, mucosal immunity, and innate immunity. These lectures provided a broad overview of the immune system, with approaches ranging from biochemical to whole animal analyses and human clinical trials. The invited speakers incorporated introductory material as well as recent highlights from their own research into their talks. The research interests of the participants were similarly varied, making for a unique opportunity for cross-fertilization among immunology subdisciplines during the oral and poster presentations as well as in more informal settings. The question periods following talks by the invited lecturers and participants were spirited and stimulating.

Several of the invited lecturers also gave talks at the RCAI/JSI meeting, where they described further new developments in their research programs. There were also many new speakers and topics, thus further increasing the breadth of the immunology coverage for the RISP participants. Awards for best RISP posters were presented at a reception during the RCAI/JSI meeting. All of the participants gathered to express their appreciation to the organizers for inviting them to RISP 2009, an exceptional experience from both scientific and cultural perspectives. In a survey completed after the RISP, the participants all agreed that they would recommend the program to colleagues, and more than half indicated that they would consider a postdoctoral position at RIKEN. The success of this unique program is due to the efforts of the Organizing Committee, chaired by Dr. Tanaka and Dr. Ohno, and the RISP Secretariat, Ms. Aki, who kept the entire operation running smoothly, as well as to the efforts of the outstanding participants. Planning is already underway for RISP 2010, scheduled for August, just prior to the 14th International Congress of Immunology in Kobe.



Table 1 : Lectures

Lecturer	Title
Hiroshi Kawamoto, RIKEN RCAI, Japan	Lineage Restriction Pathway in Hematopoiesis: revision of the classical concept of myeloid-lymphoid dichotomy
Clifford Lowell, University of California, USA	The STIM1 calcium sensor is required for activation of the phagocyte oxidase during inflammation and host defense
Hiroshi Nakajima, Chiba University, Japan	Th2 cells, Th17 cells, and allergic airway inflammation
Takashi Tokuhisa, Chiba University, Japan	Role for Bcl6 in development and maintenance of germinal center B cells
Sidonia Fagarasan, RIKEN RCAI, Japan	T cell-independent and T cell-dependent f IgA synthesis in gut
Ellen Rothenberg, California Institute of Technology, USA	Gene regulatory guidance mechanisms for early lymphocyte development
Masaru Taniguchi, RIKEN RCAI, Japan	NKT cell-mediated adjuvant cell therapy for cancer patients
Diane Mathis, Harvard Medical School, USA	Central tolerance
Alexander Rudensky, Memorial-Sloan Kettering Cancer Center, USA	Class struggle and regulatory T-cells
Thomas Tedder, Duke University Medical Center, USA	Regulatory B cells and B10 cells during immune responses, inflammation, autoimmunity and cancer
Vijay Kuchroo, Harvard Medical School, USA	Differentiation of Th17 cells
Yong-Jun Liu, M.D. Anderson Cancer Center, USA	Negative feedback regulation of type 1 IFN response by pDC specific receptor ILT7 and ligand
Takaharu Okada, RIKEN RCAI, Japan	Lymphocyte migration and interactions during the antibody response
Christian Münz, University Hospital of Zürich, Switzerland	Macroautophagy in innate and adaptive immunity

Table 2 : Participants

Name (country)			
John Altin (Australia)	Susan Johnson (Australia)	Jing Pan (China)	Thomas Tiller (Germany)
Carrie Arnold (USA)	Marko Knoll (Germany)	Hemanth Ramaprakash (USA)	Damon Tumes (Australia)
Rosa Maria Barreira da Silva (Portugal)	Meghan Koch (USA)	Andrea Reboldi (Italy)	Marian Turner (Australia)
Niklas Björkström (Sweden)	Andreia Lino (Portugal)	Joseph Reynolds (USA)	Aaron Tyznik (USA)
Joaquim Carreras (Spain)	Cindy Ma (Australia)	Dipayan Rudra (India)	Yvonne Vercoulen (Netherlands)
Aranzazu Cruz-Adalia (Spain)	Ramon Mayoral (Spain)	James Scott-Browne (USA)	Gabriel Victora (Brazil)
Priya Dedhia (USA)	Jaclyn McAlees (USA)	Doo-Hee Shim (Korea)	Jennifer Walker (UK)
Jessy Deshane (USA)	Matthew Meredith (USA)	Sunita Singh (India)	Ricardo Weinlich (Brazil)
Swantje Hammerschmidt (Germany)	Jessica Moffat (Australia)	Heidi Snider (USA)	Hakim Yadi (UK)
Mark Headley (USA)	Yingting Mok (Singapore)	Todd Suscovich (USA)	
Juandy Jo (India)	Diego Mourao Sa (Brazil)	Duncan Sutherland (Australia)	



RCAI-JSI International Symposium on Immunology 2009

Cellular and Genetic View on Autoimmunity



The fifth RCAI-JSI International Symposium on Immunology, hosted by the RIKEN Research Center for Allergy and Immunology (RCAI) in conjunction with the Japanese Society for Immunology (JSI), was held on July 9-10 in Yokohama. Organized jointly by RCAI Director Masaru Taniguchi and JSI President Kayo Inaba, the symposium is part of a series of annual conferences aimed at providing a forum for discussions of cutting-edge immunological research. This year's event proved a great success, drawing more than 350 participants from around the world with 21 internationally recognized speakers describing their research on the topic of "Cellular and Genetic View on Autoimmunity".

The symposium consisted of four sections focusing on different aspects of autoimmunity: "Negative Regulation of Autoimmunity", "Cellular and Molecular Basis of Autoimmunity", "Genetics in Autoimmunity", and "Inflammation and Autoimmunity".

In the session on Cellular and Molecular Basis of Autoimmunity, Professor Kiyoshi Takeda of Osaka University described his research on lamina propria dendritic cells (DC). Findings from his group indicate that ATP, the molecular unit of currency in intracellular energy transfer, also acts as a bacterial mediator in the development of TH17 cells, a helper T cell subset thought to play a key role both in defending against certain pathogens and, conversely, in driving autoimmune diseases. This discovery is an important example of the interplay between intestinal microbiota and the host immune system.

In a later section on Inflammation and Autoimmunity, Dr. Toshio Hirano of Osaka University and RCAI described an "IL-6 amplifier" which appears to be involved in the etiology of autoimmune and inflammatory diseases. Dr. Josef Penninger of the Austrian Academy of Sciences outlined his group's research on the RANK/RANK ligand (RANKL), which plays an important role in bone metabolism. In experiments using a knockout strategy, Dr. Penninger found that mutant mice without RANKL can no longer mount a febrile response to a variety of stimuli, and that humans with RANK deficiency, like the mice, also suffer from both life-threatening osteopetrosis and absence of the physiologically important febrile response.

In bringing together cutting-edge immunological researchers, this year's RCAI-JSI symposium provided participants a unique opportunity to share ideas and learn about the latest discoveries. Next year's symposium promises further advances in this rapidly evolving field.



Table : Program

Session I : Negative regulation of autoimmunity	
Shimon Sakaguchi, Kyoto University, Japan	T cell signaling, regulatory T cells, and autoimmunity
Alexander Rudensky, Memorial Sloan-Kettering Cancer Center and HHMI, USA	Plasticity of regulatory T cell suppression program
Christopher A. Hunter, University of Pennsylvania, USA	Role of IL-27 in regulation of inflammation
Vijay K. Kuchroo, Harvard Medical School, USA	Differentiation of Th17 cells
Christophe Benoist, Harvard Medical School, USA	Genomics of tolerance
Session II : Cellular and molecular basis of autoimmunity	
Thomas F. Tedder, Duke University Medical Center, USA	Regulatory B cells and B10 cells during immune responses, inflammation, autoimmunity and cancer
Diane Mathis, Harvard Medical School, USA	Initiation of arthritis by commensal microbes
Jun-ichiro Inoue, The University of Tokyo, Japan	TRAF6 in controlling autoimmunity
Hiroki Yoshida, Saga University, Japan	Interleukin 27 signaling pathways in regulation of immune and autoimmune responses
Fiona Powrie, University of Oxford, UK	Intestinal homeostasis: a balancing act between effector and regulatory T cells
Kiyoshi Takeda, Osaka University, Japan	Commensal bacteria-derived ATP mediates development of intestinal Th17 cells
Session III : Genetics in autoimmunity	
Henri-Jean Garchon, Cochin Institute and Inserm U567, France	Shaping the genetic risk for autoimmunity at the major histocompatibility complex
Jacek Majewski, McGill University, Canada	Genome-wide analysis of transcript isoform variation in humans
Kazuhiko Yamamoto, The University of Tokyo and RIKEN CGM, Japan	Genome-wide association studies of rheumatoid arthritis and the ethnic differences
Session IV : Inflammation and autoimmunity	
Carola G. Vinuesa, The Australian National University, Australia	Autoimmunity as a consequence of aberrant positive selection of germinal center B cells by T _{FH} cells
David A. Hafler, Harvard Medical School, USA	Natural human CD4 ⁺ CD25 ^{high} T _{regs} and Th17 effector cells
Yoichiro Iwakura, The University of Tokyo, Japan	The role of DCIR in the development of autoimmune arthritis
Toshio Hirano, Osaka University and RIKEN RCI, Japan	"IL-6 amplifier": a key player in autoimmune and inflammatory diseases
Josef M. Penninger, Austrian Academy of Sciences, Austria	Learning from SARS – common injury pathways in lung infections?
Luigina Romani, University of Perugia, Italy	IOD as a key to balancing inflammation and tolerance to microbiota
Michel Gilliet, The University of Texas, USA	Antimicrobial peptides in plasmacytoid dendritic cell-driven autoimmunity



US-Japan International Cancer Systems Biology Meeting

The US-Japan International Cancer Systems Biology Meeting was held October 28-29 at the RIKEN Yokohama Institute. The event was hosted by the US National Cancer Institute, The Systems Biology Institute, RIKEN RCAI, and the Japanese Foundation for Cancer Research. The goals of the meeting were threefold: 1) to bring together leading experts in systems biology and cancer to exchange current state-of-the-art research, 2) to identify challenges and barriers that may be inhibiting progress in the field, in particular in terms of bringing current knowledge into the clinic, and 3) to identify opportunities for interactions and collaborations at the individual and institutional levels.

Cancer is an ideal, albeit difficult, target for the systems biology approach. It has long been known that cancer is a complex disease and it is becoming more appreciated that there is considerable heterogeneity even in an individual patient, both within the tumor and over time. The talks at the meeting were very diverse, but there were common themes and issues. Much of the deep analysis of cancer as a system uses cell lines rather than primary tumor cells. The potential pitfalls of this approach have long been recognized by reductionist biologists and are appreciated by systems biologists as well. Nonetheless, cell lines are invaluable as a starting point; knowledge gained from cell line studies can provide the foundation for subsequent *ex vivo* analyses of primary cells. A point made by several speakers is the importance of having a common repository of cancer cell lines so that all researchers in the field are applying systems biology tools to as uniform a starting population as possible. Such cell line panels are currently maintained in the US by the NCI (NCI60) and in Japan by the Japanese Foundation for Cancer Research (JFCR39). However, the participants were in agreement that having one central source of single-cell-derived, well-characterized, clonal cell lines would be highly desirable.

One of the goals of systems biology as it relates to cancer is to develop personalized cancer therapy. It is now possible to screen patients for mutations/amplifications in known oncogenes and tumor suppressor genes, and this information can sometimes provide an initial rational approach to appropriate therapy. However, as pointed out by several speakers, typically only a fraction of patients categorized in this way will respond to a given therapy. The genetic complexity of individual tumors and the existence of multiple different additional mutations clearly play a role in treatment failures, and ferreting out this type of higher order information is within the realm of the systems biologist. Although not yet possible, the goal is to be able to provide patients with a profile of their cancer that will allow for effective therapy. The development of tools to accomplish this is a major challenge for systems biology.

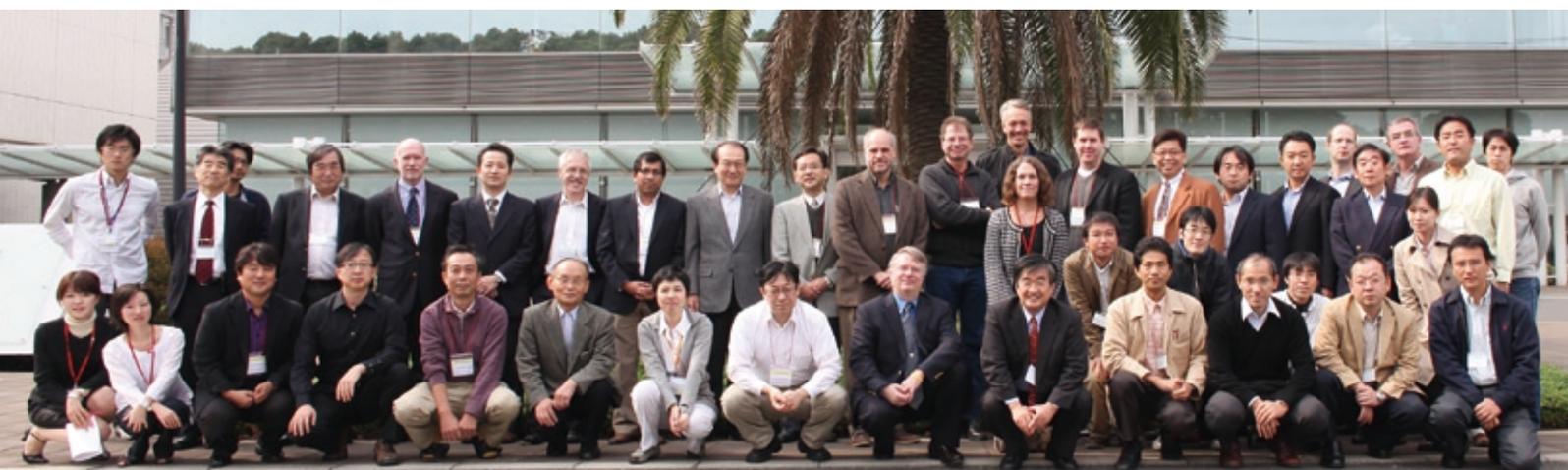
One presentation that generated considerable discussion was by Stephen Friend of Sage Bio-networks. He predicted that as the tools now in use develop, there will be a ten-fold increase in data generation, and that the systems biology community is unprepared to handle this data explosion. He pointed out that taking individual network approaches, e.g. transcriptome, metabolome, etc., even as complex as these each are, is insufficient for making progress against cancer and other diseases, and that a mechanism is needed to link all data sets together. Although there seemed to be general agreement on the value of this approach, many issues still remain, for example patient privacy regulations and the lack of electronic medical records in many hospitals, making any “omics” data incomplete and sometimes useless if it cannot be tracked back to clinical outcomes. Other concerns include intellectual property rights, which might preclude some investigators, often at the insistence of their institutional guidelines, from submitting data to such a repository, as well as a more general problem, allocation of credit to individuals in an area that is of necessity big Team Science.

Clearly systems biology has great potential yet is experiencing some growing pains. The Yokohama participants agreed that meetings such as this one were very fruitful, as scientists wrestle with complex and sometimes thorny issues.



Table : Program

Oct. 28	
Hiroaki Kitano (Sony CSL, Inc., The Systems Biology Institute, Japan)	Cancer systems biology: a road ahead
Roger Brent (Fred Hutchinson Cancer Research Center, USA)	For cell signaling systems-- what is the signal and what information does it carry?
Alexander Hoffman (University of California, San Diego, USA)	Signaling dynamics and the cellular response
Takao Yamori (Japanese Foundation for Cancer Research, Japan)	Drug discovery of molecular targeted drugs by an information-rich cancer cell line panel JFCR39
Edison Liu (Genome Institute of Singapore, Singapore)	Transcriptional regulation on a genomic scale
Ju-Seog Lee (M. D. Anderson Cancer Center, USA)	Decoding cancer signatures: systems biology approaches
Harukazu Suzuki (RIKEN Omics Science Center, Japan)	The transcriptional network in a human acute myeloid leukemia cell line
Mariko Okada-Hatakeyama (RIKEN RCAI, Japan)	Network design of cell differentiation
Kevin White (University of Chicago, USA)	Defining genomic networks in breast cancer
Samik Ghosh (The Systems Biology Institute, Japan)	A systems biology approach towards targeted cancer therapeutics: a study in the MAPK pathway
Oct. 29	
Yosef Yarden (The Weizmann Institute, Israel)	Defective feedback regulation of signal transduction networks in tumors
Kohei Miyazono (University of Tokyo, Japan)	Regulation of cancer progression by TGF-beta signaling
Stephen Friend (Sage Bionetworks, USA)	Sage: Together building probabilistic causal disease models via an open access contributor network
Osamu Ohara (RIKEN RCAI, Kazusa DNA Research Institute, Japan)	From transcriptome analysis to immunogenomics
Joe Nevins (Duke University Medical Center, USA)	Dissecting the complexity and heterogeneity of human cancer
Bonghee Lee (Gachon University of Medicine and Science, Korea)	A proteome-wide map of protein translocation identifies an essential protein complex for human glioma progression
Michiyuki Matsuda (Kyoto University, Japan)	The exhaustive modeling approach: Collection of missing parameters of EGF-Ras-ERK signaling cascade
Gordon Mills (M. D. Anderson Cancer Center, USA)	Systems approach to personalized medicine



Briefing session for the RCAI adjunct graduate school programs May 23, 2009



RCAI accepts graduate students from various universities through the adjunct professorship mechanism. There were several new adjunct appointments in 2009. Dr. Tsuneyasu Kaisho (Team Leader, Laboratory for Host Defense) became a new visiting professor at Yokohama City University. Furthermore, the Research Institute of Biological Sciences at Tokyo University of Science agreed to collaborate with RCAI for research education. Dr. Osamu Ohara (Group Director, Lab. for Immunogenomics) and Dr. Shohei Hori (Unit Leader, Res. Unit for Immune Homeostasis) were appointed as visiting professors, and Dr. Tadashi Yokosuka (Senior Researcher, Lab. for Cell Signaling) became a visiting associate professor of the university. RCAI will start accepting graduate students from Tokyo University of Science in 2011. The total number of adjunct/associate professors in RCAI is now 22, and the Center collaborates with six university graduate schools (Table).

On May 23, RCAI held a briefing session on adjunct graduate school programs. The aim of this session was to introduce this RCAI program to students who are interested in studying immunology. Twenty participants gathered from Aomori, Tottori, Nagano, Kyoto, Hyogo, Saitama, Kanagawa and Tokyo. Dr. Saito, Deputy Director, first briefly summarized the adjunct graduate school programs, and then representatives from seven laboratories introduced their research topics. After the oral session, twelve laboratories had poster presentations and then each of the participants had the opportunity to visit the laboratories of his/her interest.

The session provided an opportunity for students to visit and talk directly with lab leaders, and to consider their future directions. "This briefing was very fruitful for me, because I could talk directly with researchers," a participant commented.

Table : RCAI adjunct graduate school programs

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)
	Shin-ichiro Fujii (Visiting associate professor)
	Yasuyuki Ishii (Visiting associate professor)
School of Biomedical Science, Tokyo Medical and Dental University	Fumihiko Ishikawa (Visiting associate professor)
	Takashi Saito (Visiting professor)
	Tomohiro Kurosaki (Visiting professor)
	Masato Kubo (Visiting professor)
International Graduate School of Arts and Sciences, Yokohama City University	Sidonia Fagarasan (Visiting associate professor)
	Hiroshi Ohno (Visiting professor)
	Tsuneyasu Kaisho (Visiting professor)
Research Institute of Biological Sciences, Tokyo University of Science	Satoshi Ishido (Visiting associate professor)
	Osamu Ohara (Visiting professor)
	Shohei Hori (Visiting professor)
	Tadashi Yokosuka (Visiting associate professor)



RIKEN Yokohama Open Campus July 4, 2009

The RIKEN Yokohama Institute Open Campus was held on July 4, 2009. The number of visitors to this event increased for the fifth consecutive year, this time numbering 2,614, including 841 children and 1,773 adults, a 26% increase compared to last year. Twenty-one RCAI laboratories participated in this event and set up exhibits together in the recreation hall of the main building of the Yokohama Institute (photo above). Using posters, movies or experimental samples, the RCAI scientists explained their research topics to the visitors in a way easy to understand. One of the new RCAI exhibits this year was a cell culture of cardiac myocytes differentiated from murine iPS cells. Visitors were surprised to see the synchronized regular beats of the cultured cells. Another new exhibit was constructed in part to honor Dr. Osamu Shimomura, who received the 2008 Nobel Prize "for the discov-

ery and development of the green fluorescent protein, GFP". This fluorescent protein is now used in a wide variety of biological research fields. A tissue sample from a GFP transgenic mouse enthralled the visitors, who also saw how the fluorescence could be excited by ultraviolet light (photo below). Many children at the Open Campus were especially attracted to exhibits of experimental animals such as xenopus, planaria and zebrafish. The visitors, ranging in age from kindergarten to retirees, enthusiastically asked questions of the researchers and shared their common interests in science.



RCAI Retreat Aug. 31- Sep. 1, 2009

The aim of the RCAI Retreat is to improve the research activities of the Center by gathering the members at a quiet place with few distractions. This setting provides the RCAI scientists with the opportunity for in depth discussion and critiques of ongoing research projects, and this open communication promotes the development of new interactions and collaborations. At the 2009 RCAI Retreat, all in attendance including researchers and staff members (technical, and assistant) were encour-

aged to exchange information and initiate new collaborations. This year, Dr. John F. Foley, the editor of *Science Signaling*, attended the meeting at the Nihon Aerobics Center in Chiba Prefecture together with 124 RCAI members.

On August 31, two poster presentation sessions were held. There were 90 posters in total, and both new unpublished data and ongoing projects were introduced to the retreat participants. The top nine posters were selected by vote of the laboratory



The dedicated efforts of the organizers contributed to the success of the retreat. Drs. Sato and Wang were in charge of the general organization of the meeting. Ms. Yamamoto, Ms. Uchimura and Ms. Fukui cooperated to coordinate travel, food and accommodation, and ensured that the program went very smoothly. Several researchers commented that the quality of the retreat continues to rise every year.

heads and these received the Excellent Poster Awards from the Director (See Research Highlights). Oral presentations were held on September 1. Dr. Foley introduced science related journals, *Science Signaling* and *Science Translational Medicine*, which at that time was planned for almost immediate launch. Ten selected young postdoctoral fellows then introduced their projects, which stimulated active discussions from the floor.



Super Science High School

July 31, 2009



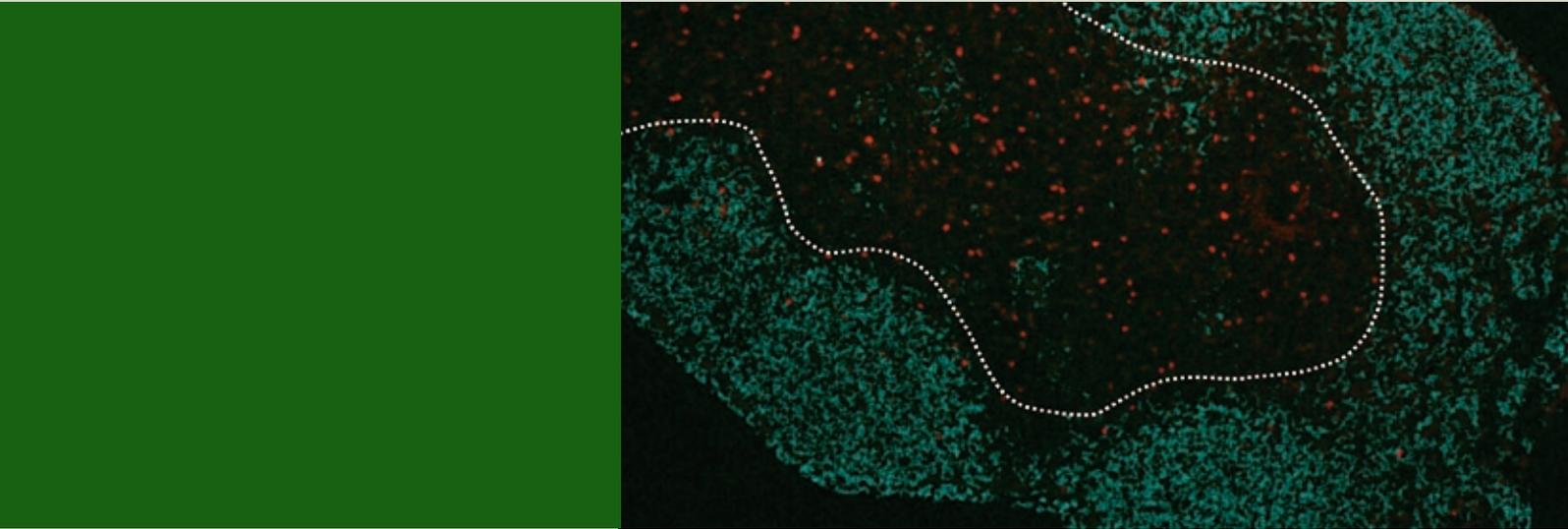
“Super Science High Schools” are schools designated by the Ministry of Education, Culture, Sports, Science and Technology to strengthen and improve scientific education. RCAI holds a workshop every year for students from the Super Science High Schools in Kanagawa prefecture. This year, 22 students and 6 teachers from Hakuyoh, Seisho, Yokosuka and Kanagawa Sohgo High Schools were invited. After special lectures on “Immunological Organs” and “AIDS Virus and Immunity” by Drs. Hisahiro Yoshida and Tomohiro Kurosaki, the students had the opportunity for informal discussions with the lecturers over lunch. In the afternoon, the students got hands-on experience with mouse dissection and histological staining of the immunological organs. In the final session, students were divided into groups for discussion and presentations. This year’s theme for discussion was “Development of a specific antibody for the

new influenza and the possible problems”. “The topic was timely but difficult. I learned how hard it is to develop logical ideas and then present them well. I’ll have to be tough,” one student said. “I was worried about the dissection, but now I am interested in anatomy,” another said. Through this experience, the students had a chance to look ahead to their future career paths.

In addition to this workshop, RCAI invited students from Jisyukann Junior High School in August and Takasaki High School in September. Dr. Yasuyuki Ishii explained to them the basics of allergy and answered their questions about asthma and hay fever.

2009

Data and Statistics



Publications (Apr. 2009-Mar. 2010)

Table : RCAI Publidations (Apr. 2009-Mar. 2010)

Journal Title	IF(2008)	FY2009
Nature	31.4	2
Nature Genetics	30.3	1
Nature Immunology	25.1	5
Nature Biotechnology	22.3	1
Immunity	20.6	2
Nature Cell Biology	17.8	1
Journal of Clinical Investigation	16.6	3
Journal of Experimental Medicine	15.5	4
Neuron	14.2	1
Immunol Rev	11.8	2
Current Opinion in Immunology	10.5	1
Blood	10.4	6
Trends in Immunology	9.9	3
J Allergy Clin Immun	9.8	1
Am J Respir Crit Care Med	9.8	1
PNAS	9.4	1
Journal of Cell Biology	9.1	1
EMBO Journal	8.3	2
Stem Cells	7.7	2
Clin Cancer Res	6.5	1
J Cell Sci	6.2	3
Genome Biol	6.2	1
Journal of Immunology	6.0	13
Molecular and Cellular Biology	5.9	2
J Med Genet	5.7	1
Am J Pathol	5.7	1
Journal of Biological Chemistry	5.5	1
Curr Top Microbiol Immunol	5.1	1
DNA Repair	5.1	1
International Immunology	3.2	8
Other Journals		31
Total		104

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Invited Presentations

Speaker	Title	Meeting	Month-Year
Fagarasan, S.	Dynamic interactions between bacteria and B cells in GALT	Tolerance and Development, Fondation des Treilles, France	Apr-09
Tokunaga, M.	Single molecule imaging in living cells	4th Global COE International Symposium, Tokyo, Japan	May-09
Tokunaga, M.	Clear visualization of single molecules living cells	Extreme Photonics Symposium of RIKEN, Wako, Japan	May-09
Mohan, S.	RAPID: Resource of Asian Primary Immunodeficiency Diseases - An Integrated Informational Platform	Keystone Symposia Conference, Human Immunology and Immunodeficiencies, Beijing, China	May-09
Hirano, T.	"Interleukin 6 Amplifier", a key player in inflammatory diseases ---How extensive a view from the top of the mountain is!	Symposium of The Crafoord Prize, Stockholm, Sweden	May-09
Hatakeyama, M.	Binary response of ErbB signaling to determine cell fate	The 2nd RIKEN - University of Edinburgh Joint Workshop for Computational and Systems Biology, Tokyo, Japan	May-09
Saito, T.	Spatiotemporal regulation of lymphocyte activation	The 2nd RIKEN - University of Edinburgh Joint Workshop for Computational and Systems Biology, Tokyo, Japan	May-09
Fukada, T.	A model mouse with mutated zinc transporter	The 2nd Tokyo Anti-aging Academy, Tokyo, Japan	May-09
Fukada, T.	Zinc and zinc transporter: involvement in cellular functions and inherited pathogenesis	The 9th Scientific Meeting of The Japanese Society of Anti-Aging Medicine, Tokyo, Japan	May-09
Kurosaki, T.	Erk signaling in B lymphocytes	FASEB Summer Research Conferences, Snowmass Village, U.S.A.	Jun-09
Saito, T.	Sensing self and non-self danger by ITAM-coupled innate receptor Mincle	FASEB Summer Research Conferences, Snowmass Village, U.S.A.	Jun-09
Tanaka, M.	Mechanism of tolerance induction to cell-associated antigens by apoptotic cell clearance	Gordon Research Conferences, Apoptotic Cell Recognition & Clearance, New London, USA	Jun-09
Kubo, M.	Control of skin inflammation by cytokine signaling	Immuno-Cardiology Conference, Tokyo, Japan	Jun-09
Kurosaki, T.	PLC γ /calcium signaling in immune cells	Protein Coupling in Cellular Networks, Freiburg, Germany	Jun-09
Taniuchi, I.	Transcription factors network that regulates thymocyte differentiation	Seminar at Chiba University, Chiba, Japan	Jun-09
Kurosaki, T.	Erk signaling in B lymphocytes	Singapore-Osaka, 1st Joint SigN-IFReC Meeting, Singapore	Jun-09
Saito, T.	Visualization of T cell activation and co-stimulation	Singapore-Osaka, 1st Joint SigN-IFReC Meeting, Singapore	Jun-09
Hirano, T.	"IL-6 amplifier": a key player in autoimmune and inflammatory diseases	Singapore-Osaka, 1st Joint SigN-IFReC Meeting, Singapore	Jun-09
Ishii, Y.	Immunotherapy for cedar pollinosis by new vaccine technologies	The 21st Spring Meeting of Japanese Society of Allergology, Gifu, Japan	Jun-09
Kaisho, T.	Molecular mechanisms on dendritic cell activation by nucleic acid adjuvants	The 21st Spring Meeting of Japanese Society of Allergology, Gifu, Japan	Jun-09
Ishikawa, F.	Reconstitution of human immune system in mice	The 5th International Workshop of Kyoto T Cell Conference 2009, Kyoto, Japan	Jun-09
Kawamoto, H.	Retention and termination of myeloid potential in T cell progenitors	The 5th International Workshop of Kyoto T Cell Conference 2009, Kyoto, Japan	Jun-09
Kubo, M.	Runx/CBF- β is a critical gatekeeper that directs the Th1 and Th17 differentiation	The 5th International Workshop of Kyoto T Cell Conference 2009, Kyoto, Japan	Jun-09
Taniuchi, I.	Transcriptional control of CD4/CD8 lineage choice	The 5th International Workshop of Kyoto T Cell Conference 2009, Kyoto, Japan	Jun-09
van Ewijk, W.	The thymus....follow me!	The 5th International Workshop of Kyoto T Cell Conference 2009, Kyoto, Japan	Jun-09
Saito, T.	Dynamic assembly of signaling complex for positive and negative costimulation of T cell activation	The 5th International Workshop of Kyoto T Cell Conference 2009, Kyoto, Japan	Jun-09
Kurosaki, T.	Erk signaling in B lymphocytes	The 5th Leukocyte Signal Transduction Workshop, Heraklion, Greek	Jun-09
Hirano, T.	Interleukin 6 hunting	The 74th Japanese Society of Interferon and Cytokine Research, Kyoto, Japan	Jun-09
Fujii, S.	NKT cell-dendritic cell (DC) cross-talk.	The 74th Japanese Society of Interferon and Cytokine Research, Kyoto, Japan	Jun-09
Fukuda, S.	Multiple Omics Identified Microbial Metabolites to Protect Host from Enterohemorrhagic Escherichia coli O157:H7 Lethal Infection	The 8th International Workshop on Advanced Genomics, Tokyo, Japan	Jun-09
Kawamoto, H.	Making iPS cells from lymphocyte	Japanese Association for Animal Cell Technology 2009, Tsukuba, Japan	Jul-09
Hirano, T.	"IL-6 amplifier": a key player in autoimmune and inflammatory diseases.	RCAl-JSI International Symposium on Immunology 2009, Kanagawa, Japan	Jul-09
Taniuchi, I.	Transcriptional control of helper versus cytotoxic lineage choice	Seminar at MD Anderson Cancer Center, Houston, USA	Jul-09
Fukada, T.	The role of zinc transporter Slc39a13/Zip13 in connective tissue development of mouse and human	The 20th Annual Scientific Meeting of the Japanese Society for Biomedical Research on Trace Element, Tokyo, Japan	Jul-09
Ishikawa, F.	The role of AML stem cell niche	The 27th Annual Meeting of the Japanese Society for Bone and Mineral Research, Osaka, Japan	Jul-09
Hirano, T.	Is Dysregulation of IL-6 Amplifier a Key Event of Rheumatoid Arthritis?	The 9th World Congress on Inflammation, Industry Sponsored Symposium 2, Tokyo, Japan	Jul-09
Saito, T.	Dynamic regulation of T cell recognition and activation.	The JSI Summer School, Hyogo, Japan	Jul-09
Hirano, T.	"A view from the summit"-- Lessons from mountain climbing-- three decades with Interleukin 6	The JSI Summer School, Hyogo, Japan	Jul-09
Tanaka, M.	Strategy of tolerance induction by apoptotic cell clearance	4th Medical Biotech Forum, Dalian, China	Aug-09
Sakata-Sogawa, K. & Tokunaga, M.	Single molecule imaging and quantitative analysis of T cell signaling initiation	CREST Symposium, Tokyo, Japan	Aug-09
Taniuchi, I.	Essential requirement of Cbfb2 variant for Smad4-mediated TGFB signals and for development and homeostasis of immune system	EMBO Workshop on RUNX Transcription Factors, Oxford, UK	Aug-09
Kawamoto, H.	Insight into the phylogenetic origin of lymphocytes	Japanese Association for Developmental and Comparative Immunology, Annual Meeting, Fujisawa, Japan	Aug-09
Saito, T.	Spatiotemporal regulation of T cell activation	Meeting for the MEXT Priority Research on the Molecular Mechanisms and function of Transportsome, Aso, Japan	Aug-09
Sakata-Sogawa, K. & Tokunaga, M.	Single molecule imaging analysis of T cell signaling initiation	Scientific Research of Priority Areas Symposium, Tokyo, Japan	Aug-09
Fukuda, S.	Construction of Gut Environment Assessment System Based on Multiple Omics	19th Annual Meeting of the Japanese Society for Mathematical Biology, Tokyo, Japan	Sep-09
Taniguchi, M.	A novel subset of mouse NKT cells bearing the IL-17 receptor B responds to IL-25 and contributes to airway hyperreactivity	2nd European Congress of Immunology, Berlin, Germany	Sep-09
Ohno, H.	Molecular mechanism for the formation of tunneling nanotube connecting distant cells	Bioscience Seminar, Chiba University, School of Medicine, Chiba, Japan	Sep-09

Wang, J-Y.	How can AID induce a high frequency of A:T mutations in the germinal center B cells?	College of Biological Sciences, China Agricultural University, Beijing, China	Sep-09
Kurosaki, T.	Role of PLC γ 2 in humoral memory	EMBO Conference Signaling in the Immune System, Siena, Italy	Sep-09
Kawamoto, H.	How are cell fate decisions made during hematopoiesis?	Japanese Society of Mathematical Biology, Annual Meeting, Tokyo, Japan	Sep-09
Yamada, D. Koseki, H.	Application of iPS technology for NKT cell-mediated adjuvant therapy in mice	Joint Cold Spring Harbor Laboratory/ Wellcome Trust Conference, Hinxton, UK	Sep-09
Taniuchi, I.	Transcription factors network that governs cell fate determination by thymocyte	JSMB 2009, Tokyo, Japan	Sep-09
Kawamoto, H.	Induction of self-renewal program in developing hematopoietic progenitors by inhibiting their differentiation	JST-CIRM Workshop, Kyoto, Japan	Sep-09
Fagarasan, S.	T-dependent and T-independent IgA responses in the gut	Microanatomy of the Immune Responses in Health and Disease, University of Birmingham, UK	Sep-09
van Ewijk, W.	The thymus, classrooms, teachers and students	Southwestern Medical University, Dallas, USA	Sep-09
Udono, H.	A role of immunochaperones in host-defense mechanism	Symposium of Immunity on mammals and plants, Tokyo, Japan	Sep-09
Kaisho, T.	Vaccination strategy based on plasmacytoid dendritic cell biology	The 13th annual meeting of The Japanese Society for Vaccinology, Hokkaido, Japan	Sep-09
Tokunaga, M. & Sakata-Sogawa, K.	Single molecule imaging in living cells	The 58th Discussion Meeting of Japan Polymer Science Society, Kumamoto, Japan	Sep-09
Sato, K.	Control of immune response by regulatory dendritic cells	The 7th Seminar for New Animal Breeding & Genetics, Japanese Society of Animal Breeding and Genetic, Tsukuba, Japan	Sep-09
Fukada, T.	The zinc transporter Zip13 is required for mammalian tooth development; its implication in dental physiology and pathogenesis	The Satellite Symposium of the 51st Annual Meeting of the Japanese Society for Oral Biology, Niigata, Japan	Sep-09
Taniguchi, M.	NKT cells bridging innate and acquired immunity	University of Palermo, Palermo, Italy	Sep-09
Taniguchi, M.	IL-17RB ⁺ NKT subset responsible for development of AHR	University of Palermo, Palermo, Italy	Sep-09
Taniguchi, M.	Clinical application of NKT cell-targeted therapy and its future direction	University of Palermo, Palermo, Italy	Sep-09
Ohno, H.	GP-2-mediated bacterial uptake by M cells and intestinal immune response	52nd Frontier Bioscience Colloquium, Osaka University, Japan	Oct-09
Ishido, S.	E3 ubiquitin ligase for MHC molecules	82nd annual meeting of the Japanese biochemical society, Kobe, Japan	Oct-09
van Ewijk, W.	The thymus, classrooms and students	City of Hope, Pasadena, USA	Oct-09
Kawamoto, H.	The myeloid-based model: revised scheme for hematopoiesis	Kanazawa University, special seminar, Kanazawa, Japan	Oct-09
Sato, K.	Immunotherapy with regulatory dendritic cells against allergic asthma	The 121th Annual Meeting of Kanto Area of Japanese Pharmacological Society, Tokyo, Japan	Oct-09
Hirano, T.	Zinc, immunity, allergy and inflammation: Zinc is a signaling molecule.	The 16th Hiroshi Kobayashi Memorial Lecture at The 415th Osaka Dermatological Association, Osaka, Japan	Oct-09
Udono, H.	Unique features of HSP-Ag fusion protein as a cancer vaccine	The 4th International Congress on Stress Response in Biology and Medicine, Sapporo, Japan	Oct-09
Nishida, K.	Role of zinc/zinc transporter in mast cell-mediated allergic reaction	The 59th Annual Meeting of Japanese Society of Allergology, Akita, Japan	Oct-09
Sato, K.	Immunotherapy with regulatory dendritic cells	The 59th Annual Meeting of Japanese Society of Allergology, Akita, Japan	Oct-09
Koseki, H.	Application of iPS technology for NKT cell-mediated adjuvant therapy in mice	The 71st Annual Meeting of the Japanese Society of Hematology, Kyoto, Japan	Oct-09
Fukada, T.	The role of zinc transporter Slc39a13/Zip13 in mammalian health and disease	The 82nd Annual Meeting of the Japanese Biochemical Society, Kobe, Japan	Oct-09
Kubo, M.	RUNX is a critical gatekeeper that directs the Th1 and Th17 differentiation	The 82nd Annual Meeting of the Japanese Biochemical Society, Kobe, Japan	Oct-09
Nishida, K.	Role of zinc in mast cell-mediated allergic reaction	The 82nd Annual Meeting of the Japanese Biochemical Society, Kobe, Japan	Oct-09
Sakata-Sogawa, K. & Tokunaga, M.	Single molecule imaging and quantitative analysis of T cell signaling initiation	The 82nd Annual Meeting of the Japanese Biochemical Society, Kobe, Japan	Oct-09
Ohara, O.	From transcriptome analysis to immunogenomics	US-Japan International Cancer Systems Biology Meeting, Yokohama, Japan	Oct-09
Okada-Hatakeyama, M.	Network design of cell differentiation	US-Japan International Cancer Systems Biology Meeting, Yokohama, Japan	Oct-09
Hirano, T.	What does 'self' mean for the immune system? The plasticity of self.	2009 Tekijuku Memorial Lectures, Osaka, Japan	Nov-09
Saito, T.	CARD9 and innate immunity	Center for the Study of Inflammatory Bowel Disease 19th Annual Workshop, Boston, USA	Nov-09
Kawamoto, H.	The myeloid-based model: insight into original stage of mixed type leukemia	Japanese Society of Pediatric Hematology, Annual Meeting, Chiba, Japan	Nov-09
Wang, J-Y.	Learning from the immune system	Kwansei Gakuin University, Hyogo, Japan	Nov-09
Tokunaga, M.	Clear visualization of single molecules living cells	Seminar of Graduate School of Life Science, Hokkaido University, Sapporo, Japan	Nov-09
Ishikawa, F.	In vivo model for Human Leukemia	The 16th RIKEN President's Discretionary Fund workshop, Gifu, Japan	Nov-09
Fukada, T.	The zinc transporter Zip13: its roles in skeletogenesis and inherited connective tissue disorders	The 1st Orthopedic Research Club, Kisarazu, Japan	Nov-09
Tanaka, T.	PDLIM2, a nuclear ubiquitin E3 ligase, negatively regulates inflammatory responses	The 2009 Fall Conference of The Korean Association of Immunologists, Seoul, Republic of Korea	Nov-09
Fukada, T.	The zinc transporter SLC39A13/ZIP13 is required for bone, tooth and connective tissue development in mouse and human; its involvement in BMP/TGF- β Signaling Pathways.	The 26th Naito Conference of Osteobiology, Hyogo, Japan	Nov-09
Ishikawa, F.	In vivo investigation of human diseases using humanized mice	The 37th Annual Meeting of the Japanese Society of Clinical Immunology, Tokyo, Japan	Nov-09
Ishikawa, F.	Translational medicine using humanized mouse	The 39th Annual Meeting of the Japanese Society for Immunology, Osaka, Japan	Nov-09
Ishikawa, F.	Translational medicine using humanized mouse	The 39th annual meeting of The Japanese Society for Immunology, Osaka, Japan	Nov-09
Ishikawa, F.	Humanized mouse for biomedical research	The 3rd Chiba Global COE Symposium, Chiba, Japan	Nov-09
Naito, T.	Ikaros-mediated epigenetic regulation of Notch signaling in T cell development and leukemogenesis	The 3rd Chiba Global COE Symposium, Chiba, Japan	Nov-09
Suzuki, K.	T-independent IgA responses in the gut	The First CSI/JSI/KAI Joint Symposium on Immunology, Shanghai, China	Nov-09

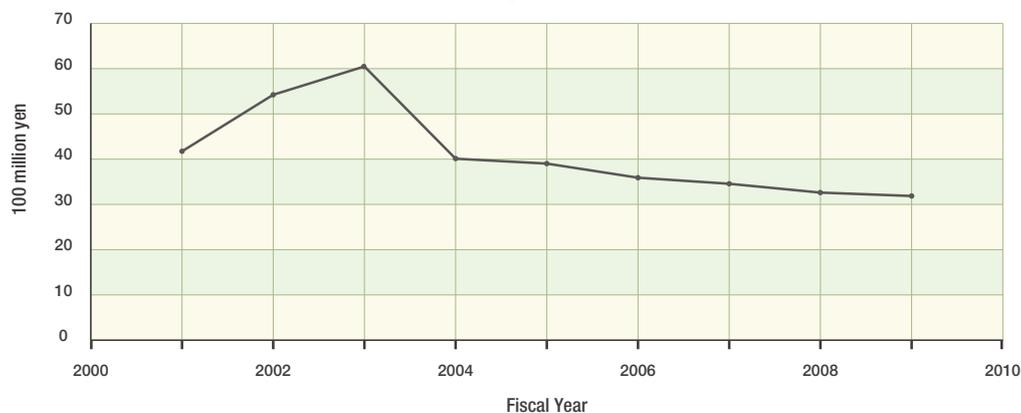
Ishikawa, F.	Humanized mouse for biomedical research	The First CSI/KAI Joint Symposium on Immunology, Shanghai, China	Nov-09
Fukada, T.	The zinc transporter ZIP13 is required for bone, tooth and connective tissue development in mouse and human; its involvement in BMP/TGF- β signaling pathways	2009 International Society for Zinc Biology Meeting, Jerusalem, Israel	Dec-09
Fagarasan, S.	T-dependent and T-independent IgA responses in the gut	Annual Meeting of the Japanese Society for Immunology, Osaka, Japan	Dec-09
Kawamoto, H.	Insight into original stage of mixed type leukemia based on the myeloid-based model	Japanese Society of Hematology, Kinki-area Meeting, Kyoto, Japan	Dec-09
Kawamoto, H.	T cell lineage determination: termination of myeloid potential in T cell progenitors	Japanese Society of Immunology, Annual Meeting, Symposium, Osaka, Japan	Dec-09
Taniuchi, I.	Transcriptional control of helper versus cytotoxic lineage choice in thymus	Seminar at MPI, Berlin, Germany	Dec-09
Okada, T.	Real-time imaging of cell-cell interactions during acquired immune responses	The 32th Annual meeting of Japan Molecular Biology Society, Yokohama, Japan	Dec-09
Sakata-Sogawa, K. & Tokunaga, M.	Single molecule imaging and quantitative analysis of living cells	The 32th Annual meeting of Japan Molecular Biology Society, Yokohama, Japan	Dec-09
Tokunaga, M. & Sakata-Sogawa, K.	Future of Quantitative Biology: Single molecule imaging and quantification of living cells	The 32th Annual meeting of Japan Molecular Biology Society, Yokohama, Japan	Dec-09
Ishido, S.	Novel immune regulation by ubiquitination	The 32th Annual meeting of Japan Molecular Biology Society, Yokohama, Japan	Dec-09
Yokosuka, T.	Dynamic regulation of T-cell costimulation by TCR microclusters and the c-SMAC	The 38th Annual Meeting of the Japanese Society for Immunology, Osaka, Japan	Dec-09
Sato, K.	Crucial roles of B7-H1 and B7-DC expressed on mesenteric dendritic cells in oral tolerance	The 38th Annual Meeting of the Japanese Society for Immunology, Osaka, Japan	Dec-09
Aiba, Y.	Preferential localization of IgG memory B cells adjacent to waned germinal centers	The 38th Annual Meeting of the Japanese Society for Immunology, Osaka, Japan	Dec-09
Baba, Y.	Store-operated calcium entry in B cells	The 38th Annual Meeting of the Japanese Society for Immunology, Osaka, Japan	Dec-09
Hori, S.	Robustness and plasticity in regulatory T cell differentiation	The 38th Annual Meeting of the Japanese Society for Immunology, Osaka, Japan	Dec-09
Kaisho, T.	Lecture for The Japanese Society for Immunology Award	The 38th Annual Meeting of the Japanese Society for Immunology, Osaka, Japan	Dec-09
Haruhiko Koseki,	HP1g links histone H3K9 methylation to homologous chromosome pairing during meiotic prophase	The Epigenetics 2009 Australian Scientific Conference at Epigenetics and Development, Melbourne, Australia	Dec-09
Taniuchi, I.	Roles of Runx proteins during T cell development	TWINCORE Seminar, Hanover, Germany	Dec-09
Kubo, M.	Immuno-epigenetics of allergic diseases	XXI World Allergy Congress (WAC) 2009, Buenos Aires, Argentine	Dec-09
Ohara, O.	Functional genomics focused on immune systems	XXI World Allergy Congress (WAC) 2009, Buenos Aires, Argentine	Dec-09
Ohno, H.	Uptake via Glycoprotein 2 by M cells is important for mucosal immunosurveillance of FimH ⁺ Bacteria	Adaptive and Innate Immune Responses to Neglected Tropical Diseases Conference, US-Japan Cooperative Medical Science Program, San Diego, CA, USA	Jan-10
Taniuchi, I.	Transcriptional regulations of T lymphocyte development	Seminar at Kumamoto Univ., Japan	Jan-10
Sakata-Sogawa, K. & Tokunaga, M.	Single molecule microscopy for immunological cell signaling	Sweden-Japan joint colloquium, Stockholm, Sweden	Jan-10
Yokosuka, T.	Dynamic regulation of T-cell costimulation by TCR microclusters and the c-SMAC	The 13th International Membrane Research Forum /The 6th iCeMS International Symposium, Featuring Membrane Meso Mechanisms, Kyoto, Japan	Jan-10
Hase, K.	GP2-dependent antigen transcytosis by M cells initiates mucosal immune response.	The 4th Stage Surface Barrier Immunology Study Group (SBARIS). 2nd Meeting, Tokyo, Japan	Jan-10
Saito, T.	Dual specificity receptor for damaged cells and pathogen	US-Japan Joint Immunology Board & Parasitic Disease Panel Conference, San Diego, USA	Jan-10
Kawamoto, H.	The myeloid-based model: revised scheme for hematopoiesis	Yamaguchi University, Immunology Seminar, Kyoto, Japan	Jan-10
Ohno, H.	Glycoprotein 2 and M-Sec, genes specifically expressed in M cells, specialized intestinal epithelial cells	21st International Training Program Seminar, Okayama University, Japan	Feb-10
Kawamoto, H.	Lineage restriction pathway in hematopoiesis:Revision of the classical concept of myeloid-lymphoid dichotomy	Harvard Medical School, Wednesday Immunology Seminar Series, Boston, USA	Feb-10
Saito, T.	Molecular dynamics for T cell activation and costimulation	Keystone Symposia, Lymphocyte Activation and Gene Expression, Breckenbridge, USA	Feb-10
Hori, S.	Stability and plasticity in regulatory T cell differentiation	Keystone Symposia, Tolerance and Autoimmunity, Taos, New Mexico, USA	Feb-10
Ohara, O.	Exploration of PID-causative mutations: Japan model and the future direction	The 2nd Symposium of PID in Asia, Chiba, Japan	Feb-10
Fukada, T.	Zinc and zinc transporter: their roles in osteogenesis and bone disease	The 4th Bone Research Seminar, Tokyo, Japan	Feb-10
Tokunaga, M.	TIRF principle and applications with a focus on biological samples	DKFZ/EMBL Practical Course, Heidelberg, Germany	Mar-10
Ohno, H.	Comprehensive analysis of the protective mechanism of enterohaemorrhagic Escherichia coli O157:H7 infection by probiotic Bifidobacteria via multi-omics approach	Symposium of the Azabu Academic Frontier Project-Matching Fund Subsidy for Private Universities, Tokyo, Japan	Mar-10
Fukada, T.	Involvement of zinc transporter Slc39a13/Zip13 in hereditary connective tissue disease	The 130th Annual Meeting of the Pharmaceutical Society of Japan, Okayama, Japan	Mar-10
Hirano, T.	Zinc signaling in immunity, allergy and inflammation	The 130th Annual Meeting of the Pharmaceutical Society of Japan, Okayama, Japan	Mar-10
Nishida, K.	Role of zinc transporter in allergic reaction	The 130th Annual Meeting of the Pharmaceutical Society of Japan, Okayama, Japan	Mar-10
Fukuda, S.	Elucidation of the effectiveness of probiotics against lethal infection of Enterohaemorrhagic Escherichia coli O157:H7	The 83rd Annual Meeting of Japanese Society for Bacteriology, Yokohama, Japan	Mar-10
Fukada, T.	Zinc transporter Zip13: its involvement in dental development and disorders	The 8th Oral Medical Science Frontier, Fukuoka, Japan	Mar-10
Fukada, T.	The Zinc transporter Zip13: its involvement in skeletogenesis and pathogenesis in mouse and human	The Satellite Symposium on Bone of the 14th International Congress of Endocrinology, Osaka, Japan	Mar-10

Date	Title	Lecturer
11-May-09	Expression dynamics of endogenous antisense / non-coding RNA in mammals: Expression analysis by genomic science approach	Dr. Hidenori Kiyosawa (RIKEN BRC)
26-May-09	Mutant mice analysis reveals roles of Kif7 in Hedgehog pathway and Pericentrin in interneuron migration	Dr. Setsu Endoh-Yamagami (Genentech, Inc. / University of California, San Francisco, USA)
1-Jun-09	An ENU screen for modifiers of epigenetic reprogramming	Dr. Emma Whitelaw (NHMRC Australia Fellow, Queensland Institute of Medical Research, Australia)
2-Jun-09	Anti-IgE therapy for severe asthma and allergy: rationale and drug actions	Dr. Tse Wen Chang (Distinguished Research Fellow, Genomics Research Center Academia Sinica, Taipei, Taiwan)
8-Jun-09	Subversion of PKCalpha: impact on during B cell lineage commitment, development and leukaemogenesis	Dr. Alison Michie (Senior Lecturer of Molecular Lymphopoiesis, University of Glasgow, UK)
16-Jun-09	The mammalian transcriptome	Dr. Piero Carninci (RIKEN OSC)
30-Jun-09	Reprogramming the cardiac cell fate and remodeling the heart formation	Dr. Jun K. Takeuchi (Cardiovascular Research, Global Edge Institute, Tokyo Institute of Technology)
2-Jul-09	Host defence system of larval asteroid	Dr. Ryouhei Furukawa (University of Keio)
23-Jul-09	Interactive T-like and B-like lymphocytes in lampreys: Evolutionary implications	Dr. Masayuki Hirano (Emory Vaccine Center and Department of Pathology and Laboratory Medicine, Emory University, USA)
30-Jul-09	A voyage of hematopoietic stem cell and her pilot	Dr. Yoshio Katayama (Kobe University Graduate School of Medicine)
30-Jul-09	Nuclear export factor hCAS/CSE1L associates with chromatin and regulates expression of selective p53 target genes	Dr. Tomoaki Tanaka (Chiba University Hospital, Department of Clinical Cell Biology,)
19-Aug-09	Identification of a human CD4 ⁺ T-cell clone that recognizes and kills renal cell carcinoma cells through alpha/beta TCR-mediated recognition of the soluble TRAIL/DR4 complex	Dr. Ken-ichi Hanada (National Cancer Institute, NIH, USA)
14-Oct-09	Epigenetic regulators supporting nuclear receptor function in gene regulations	Dr. Shigeaki Kato (Institute of Molecular and Cellular Biosciences, The University of Tokyo)

28-Oct-09	A novel function for RANKL: Switching on M cell differentiation in small intestinal epithelial cells	Dr. Ifor Williams (Department of Pathology Emory University School of Medicine, USA)
2-Nov-09	Unc93B1 biases Toll-like receptor responses to nucleic acid in dendritic cells towards DNA- but against RNA-sensing	Dr. Kensuke Miyake (Division of Infectious Genetics, The Institute of Medical Science, The University of Tokyo)
5-Nov-09	Polycomb function in B-cell development and adaptive immunity	Dr. Stefano Casola (IFOM, the FIRC Institute of Molecular Oncology Foundation, Milano, Italy)
19-Nov-09	Kinetic Monte Carlo simulations of EGFR clustering in heterogeneous cell membranes	Dr. Michail Stamatakis (Department of Chemical Engineering, University of Delaware, USA)
30-Nov-09	Gut homing T cells, retinoic acid and intestinal DCs	Dr. William Agace (Lund University, Sweden)
8-Dec-09	The selective pressures on becoming a T cell	Dr. Eric Huseby (University of Massachusetts Medical School, USA)
8-Dec-09	Ubiquitin-mediated pathways that regulate T cell tolerance and effector function	Dr. Paula Oliver (University of Pennsylvania, USA)
8-Dec-09	Summary of XFEL	Dr. Tetsuya Ishikawa (RIKEN RSC)
9-Dec-09	The transcriptional interactome; Spatial clustering of co-regulated genes at transcription factories	Dr. Peter Fraser (The Babraham Institute, Babraham Research Campus, Cambridge, UK)
14-Dec-09	Live cell imaging of Xic homologous pairing in mouse ES cells: implications for the initiation of X chromosome inactivation	Dr. Osamu Masui (Mammalian Developmental Epigenetics Group, Curie Institute, France)
10-Jan-10	bZIP transcription factor Batf regulates germinal center formation and class switch recombination	Dr. Wataru Ise (Washington University School of Medicine, USA)
1-Feb-10	Role of the composition of the commensal microbiota in Th17 cell differentiation and function	Dr. Ivaylo I. Ivanov (New York University School of Medicine, USA)
22-Feb-10	Visualizing antigen-specific helper T cell immunity: how, where, and when	Dr. James McLachlan (Tulane University School of Medicine, USA)
16-Mar-10	Control of immunity by SAP family molecules	Dr. Andre Veillete (Clinical Research Institute of Montreal, Canada)

Budget, Personnel and Patents

RCAI Budget (JPY 100 Million)

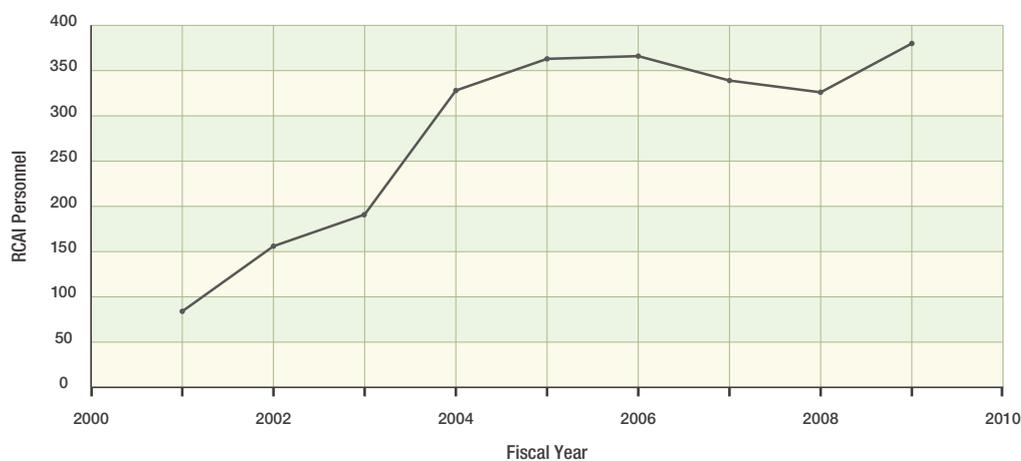


RCAI Budget FY2001-2009 (JPY 100 Million)

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

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

RCAI Personnel



RCAI Personnel

2001	84
2002	156
2003	191
2004	328
2005	363
2006	366
2007	339
2008	326
2009	380

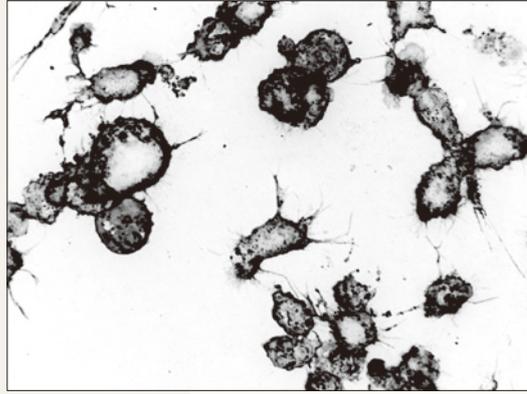
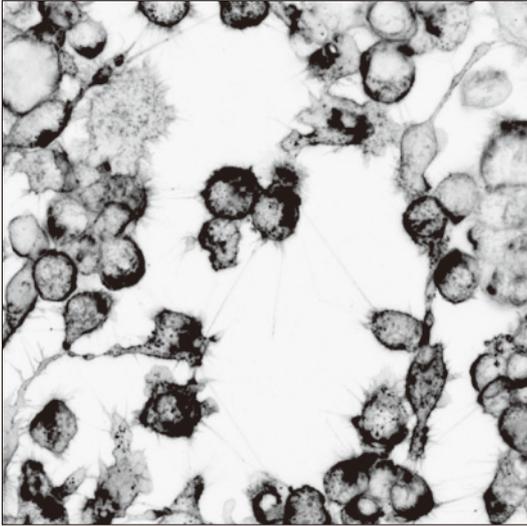
RCAI Staff Composition (as of Mar. 2010)

Category	Number
Director	1
Senior Advisor	1
Group Director	7
Team Leader	14
Unit Leader	12
Coordinator	1
Senior Scientist	8
Research Scientist	40
Postdoctoral Researcher	18
Research Associate	7
Student Trainee	51
Intern	1
Research Consultant	2
Research Fellow	3
Visiting Scientist	63
Senior Technical Scientist	1
Technical Scientist	7
Technical Staff I	26
Technical Staff II	53
Assistant	20
Part-time Staff	2
Temporary Employment	42
Total	380

RCAI Patents FY 2009 (as of Mar. 2010)

There were 28 patents filed from April 2009 - March 2010.

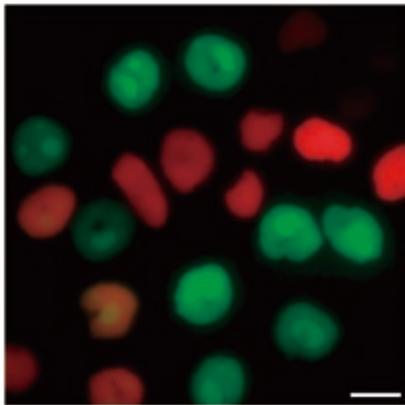
Original Photos of the Cover and Front Pages



Front cover

The formation of tunneling nanotubes (TNTs). The formation of TNTs connecting mouse macrophage cells (*left*) is blocked when M-Sec is depleted by knockdown of gene expression (*right*). (See *Research Highlights* section)

Image courtesy of Laboratory for Epithelial Immunology



Front page of Research Highlights section

Control of protein expression levels by judicious selection of promoter sequences. CHO cells expressing a Fucci: fluorescent ubiquitination-based cell cycle indicator (combination of mKO2-Cdt1 and mAG1-Geminin) in the nucleus.

For quantitative analysis of the interaction of signaling proteins, it is important to observe multiple molecules simultaneously. To this end, the Research Unit for Single Molecule Imaging and the Research Unit for Molecular Systems Biology established methods to obtain isogenetic cell lines expressing two fluorescence tagged proteins at a homogenous and low, but detectable level. An expression vector was designed to contain two cassettes of promoter and fluorescence-tagged protein sequences in tandem. To optimize the expression level of the fluorescence proteins for single molecule imaging, the investigators selected suitable promoter sequences, and inserted insulator sequences if necessary. This method allows to the precise analysis of interactions between two different molecules. (See *Laboratory Activities* section)

Image courtesy of the Research Unit for Single Molecule Imaging and the Research Unit for Molecular Systems Biology

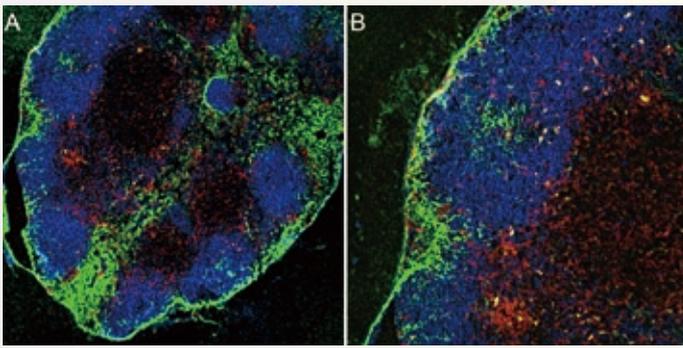


Front page of Laboratory Activities section

In vitro images of follicular dendritic cells (FDCs). A phase-contrast micrograph of FDCs cultured for two weeks on a collagen coated plate in the presence of an LT- β R agonist antibody and TNF- α .

Follicular dendritic cells (FDCs) are radio-resistant cells that were identified by their ability to retain immune complexes. More recently it was found that FDCs secrete several cytokines and chemokines involved in the migration and aggregation of lymphocytes, especially B cells into follicles. The Laboratory for Mucosal Immunity aims to characterize FDCs *ex vivo* in order to understand their functional compartmentalization and to determine if they play roles not only in somatic hypermutation required for affinity maturation but also for class switch recombination in B cells. (See *Laboratory Activities* section)

Image courtesy of Laboratory for Mucosal Immunity

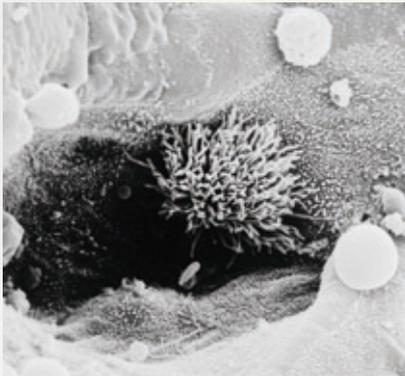


Front page of Collaborative Network section

Localization of CD169-positive sinus macrophages in lymph nodes. A cryosection of lymph nodes was stained for CD169 (Green), CD11c (Red) and B220 (Blue) and observed by confocal microscopy. Original magnification; x40 (A), x200 (B).

CD169-positive sinus macrophages are localized in the sinus of the brachial lymph nodes, CD11c-positive dendritic cells are localized in the T cell area, and B220-positive B cells are localized in follicles. The Laboratory for Innate Cellular Immunity has previously established transgenic mice in which CD169-positive cells can be transiently depleted by diphtheria toxin (DT) administration (CD169-DTR mice). Using this mouse, they study the role of sinus macrophages in the presentation of dead cell-associated antigens (See *Laboratory Activities* section)

Image courtesy of Laboratory for Innate Cellular Immunity

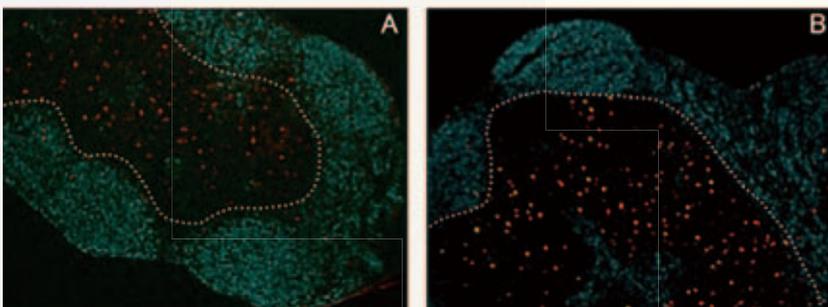


Front page of Events section

Scanning electron micrograph of a thymic cyst.

The Research Unit for Thymic Microenvironments studies development of the thymic environment, a.o. specifically focusing on the origin and generation of the aberrant epithelial cell types localized in thymic cysts. In the absence of developing thymocytes, the thymic environment is perturbed and unusual epithelial cells appear, such as ciliated cells, goblet cells and absorptive cells, forming cystic structures. Importantly, this abnormal stromal thymic phenotype can be quickly repaired, once developing thymocytes are added back to the thymic environment, highlighting both the impressive plasticity of the thymic microenvironment, and the instructive role of developing T lymphocytes in creating intact functional thymic environments. (See *Laboratory Activities* section)

Image courtesy of Research unit for Thymic Microenvironments



Front page of Data and Statistics section

Antigen-specific *de novo* conversion of CD4⁺Foxp3⁻T cells into CD4⁺Foxp3⁺iT_{regs} in mesenteric lymph nodes during oral tolerance. KJ1-26⁺Foxp3^{EGFP}-T cells were transferred into BALB/c mice, and the animals were subsequently fed PBS or OVA protein one day later. Cryosections from mesenteric lymph nodes on day 11 after the adoptive transfer were stained for KJ1-26 (red) and B220 (blue). Foxp3 was detected by green fluorescence from the Foxp3^{EGFP} knock-in allele.

Oral tolerance is a key feature of intestinal immunity, generating systemic tolerance to ingested antigens. Antigen-specific conversion of CD4⁺T cells into CD4⁺Foxp3⁺inducible regulatory T cells (iT_{regs}) occurred in mesenteric lymph nodes (MLNs) during oral tolerance induction. The Laboratory for Dendritic Cell Immunobiology studies the role of the B7 family of costimulatory molecules in this conversion. (See *Laboratory Activities* section)

Image courtesy of Laboratory for Dendritic Cell Immunobiology



RIKEN Research Center for Allergy and Immunology

RIKEN Yokohama Institute

1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama City, Kanagawa, 230-0045, Japan

Tel : 045-503-9111

Fax : 045-503-9113

Email : yokohama@riken.jp

<http://www.rcai.riken.jp/indexE.html>